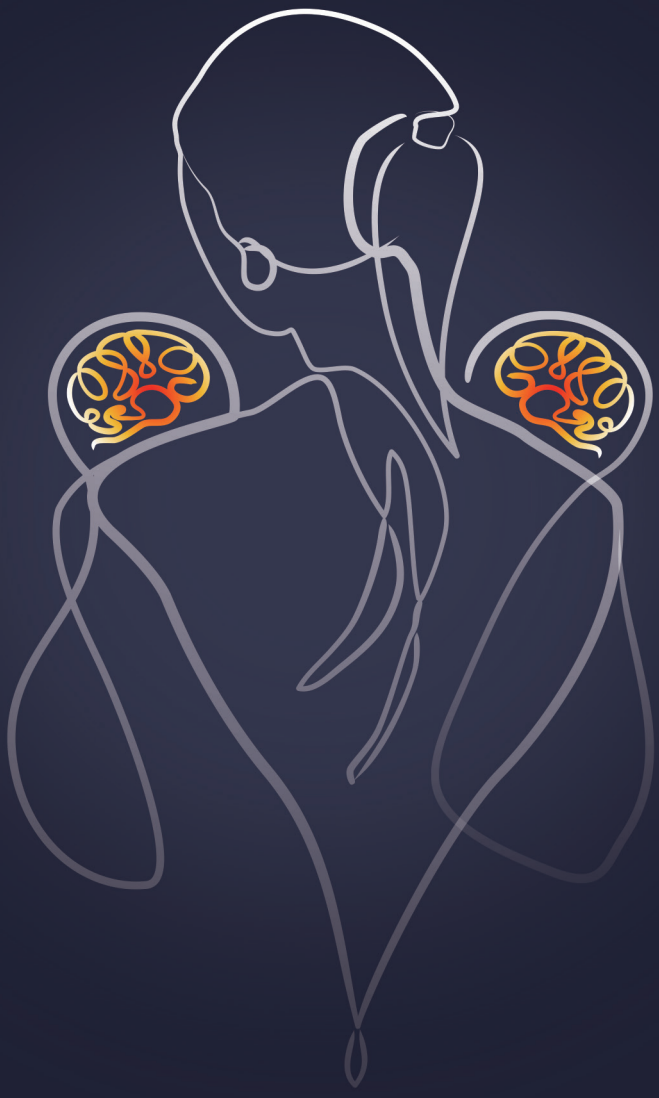
Brain matters in twin-twin transfusion syndrome

Spruijt, M.S.

BRAIN MATTERS IN TWIN-TWIN TRANSFUSION SYNDROME



Marjolijn Spruijt

Brain matters in twin-twin transfusion syndrome

The research presented in this thesis was carried out within the departments of Pediatrics and Obstetrics of the Leiden University Medical Center.

ISBN
978-94-93391-92-5

Cover design
Rinske Verberg

Design/lay-out and print
Promotie In Zicht | promotie-inzicht.nl

Financial support for printing of this thesis was kindly provided by the Universitaire Bibliotheken Leiden.

BRAIN MATTERS IN TWIN-TWIN TRANSFUSION SYNDROME

Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir. H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op woensdag 15 januari 2025
klokke 14.30 uur

door

Marjolijn Sophie Spruijt
geboren te Nijmegen

Promotores en promotiecommissie

Promotor
Prof. dr. E. Lopriore

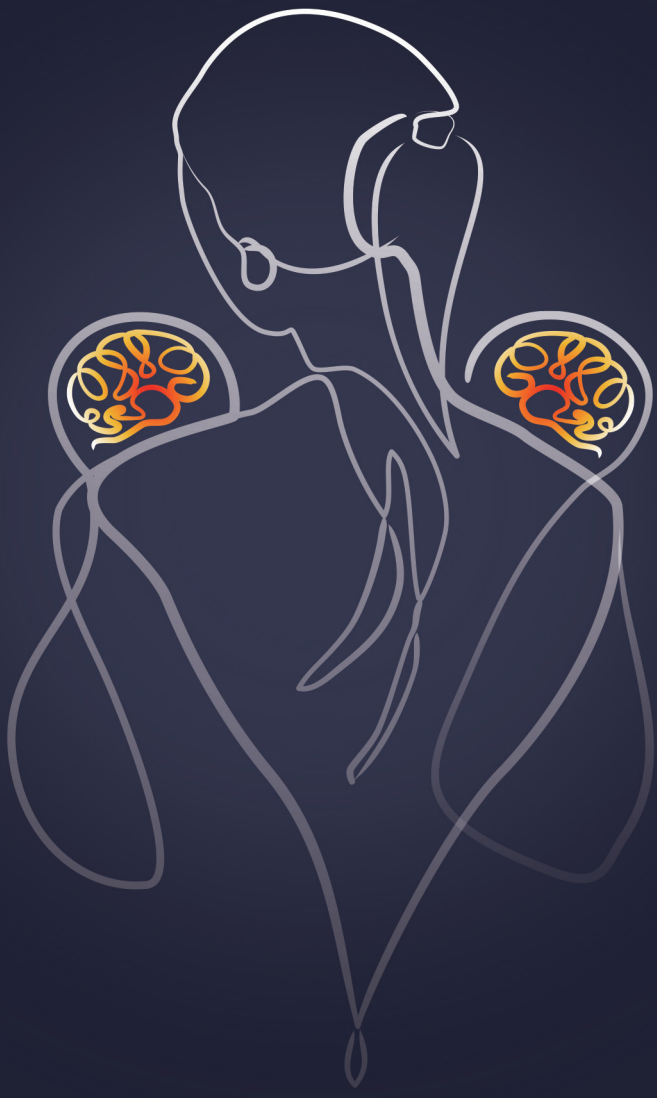
Co-promotores
Dr. J.M.M. van Klink
Dr. S.J. Steggerda

Leden promotiecommissie

Prof. dr. A.B. te Pas	
Prof. dr. L. Lewi	Universitair Ziekenhuis Leuven
Prof. dr. W.P. de Boode	Radboudumc Nijmegen
Dr. J. Dudink	Universitair Medisch Centrum Utrecht

Ik draag dit proefschrift op aan mijn moeder.
Mama, je bent nog altijd bij me.

Table of contents



Part ONE

Introduction and aim

General introduction

The story of Amy and Rosie

Mrs. de Jong was in her first pregnancy and expecting monochorionic twins. At one of her biweekly ultrasound check-ups around 16 weeks' gestation, a discordance in amniotic fluid between the twins was noted. Mrs. De Jong was referred to the Leiden University Medical Center, the national referral center for monochorionic twin complications and fetal therapy in The Netherlands. Our obstetricians confirmed the discordant amniotic fluid volumes, but criteria for twin-twin transfusion syndrome (TTTS) were not met. The future parents were counseled about the chances of progression to TTTS and Mrs. De Jong was followed with regular ultrasound examinations at our center. Indeed, progression to TTTS stage 1 was seen at 19 weeks' gestation, but in the absence of maternal symptoms, the future parents were counseled towards expectant management. After further progression to TTTS stage 2 at 21 weeks' gestation, fetoscopic laser coagulation of the placental anastomoses using the Solomon technique was performed.

Recovery from TTTS was evident in the days following laser surgery and both fetuses appeared to be in good condition. Ultrasonography of the former recipient's brain 9 days after laser surgery showed an asymmetry of the lateral ventricles, without any other clear abnormalities. The intertwin membrane could not be visualized between the twins, suggestive of iatrogenic mono-amnionicity. Reassuringly, although the asymmetry was still present, both the recipient's lateral ventricles remained within normal limits.

At 26 weeks' gestation, Mrs. De Jong's membranes ruptured, and she was admitted to the obstetric ward of our hospital. Antenatal steroids were given, and the couple was counseled about prematurity by one of our neonatologists. When labor started one week later, a primary cesarean section was performed because of monoamnionicity and the risk of cord entanglement. Two daughters were born: former donor Amy, with a birth weight of 930 grams, and former recipient Rosie, weighing 960 grams.

The placenta was examined postnatally, as is our custom, but the chorionic vessels could not be injected with colored dye to check for the presence of residual anastomoses, because the placenta was damaged and torn on the laser line. Histology of the placenta showed equal placental sharing and no signs of fetal thrombosis, nor ischemic changes.

The twins were born in good condition and admitted to our neonatal intensive care unit (NICU), requiring only non-invasive respiratory support. Both girls underwent cranial ultrasonography on the first day of life. Amy's ultrasound was unremarkable and she had an uncomplicated NICU course.

Rosie's antenatal findings were confirmed on her first postnatal cranial ultrasound, which showed a larger left lateral ventricle. Sadly, this was not the only finding, as it was clear that part of the left hemisphere was smaller and subcortical cystic changes as well as an abnormal cortical appearance were seen, especially around the left insular area (see ***Figure 1***, left panel.). Brain magnetic resonance imaging (MRI) was performed around 30 weeks post-menstrual age and showed extensive tissue loss in the territory of the left middle cerebral artery, including loss of volume in the basal ganglia and thalamus, consistent with a previous ischemic stroke.

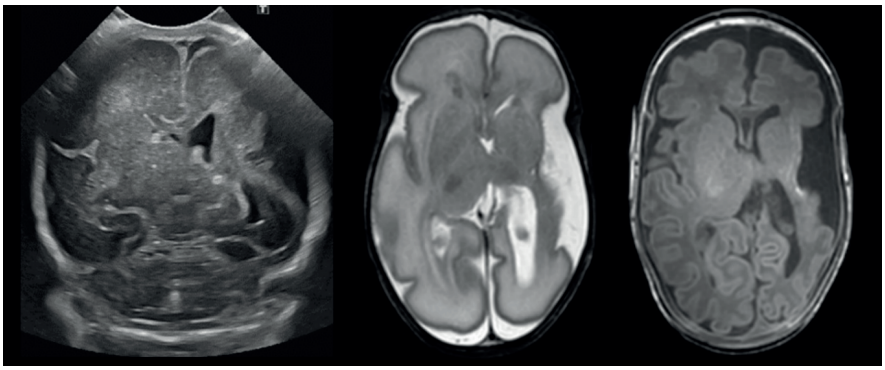


Figure 1. Postnatal neuroimaging of Rosie, the former recipient.

Left: Ultrasound in coronal plane on the first postnatal day, showing asymmetrical ventricles and a smaller left hemisphere (cystic changes were also present, not shown). Middle: MRI at 30 weeks' gestation: T2-weigthed image at the level of the basal ganglia and thalami, showing a smaller left hemisphere, basal ganglia and thalamus as well as compensatory left ventricular dilatation. Right: term-equivalent MRI: T1-weighted image at approximately the same level showing progress of gyrification in all areas of the brain and myelination of the right, but not the left posterior limb of the internal capsule (PLIC).

Rosie's further NICU course was complicated by the need for mechanical ventilation and retinopathy of prematurity. Around 35 weeks postmenstrual age, she was transferred to a local hospital together with her sister. She returned for a second MRI scan around term-equivalent age to assess the evolution of her cerebral injury and the progression of myelination. This term MRI showed a smaller left hemisphere, smaller thalamus and basal ganglia and unilateral ex vacuo ventricular dilatation, gliotic changes in the left insular area, as well as reduced myelination on the left side in the dorsal brainstem, posterior limb of the internal capsule, thalamus, globus pallidus and corona radiata (see ***Figure 1.***, right panel). We explained to Rosie's parents

that we expected Rosie to develop motor problems on the right side of her body, but that it was difficult to predict whether her cognitive and visual development would be affected as well. Therefore, long-term follow-up of her overall development would be very important.

The twins were discharged home on their due date. As expected, Rosie's motor development was progressively asymmetrical, with an early preference for use of her left hand and higher muscle tone of the right arm and leg at a corrected age of 4 months. She is under the care of a rehabilitation specialist, a physiotherapist specialized in developmental support for preterm infants, a pediatric neurologist and a general pediatrician. At the follow-up visit in the LUMC at 12 months corrected age, Rosie's motor development is delayed and asymmetrical, but she is doing well and is showing progress in all areas of development.

Rosie and Amy will return to our outpatient clinic for structured follow-up visits at the (corrected) ages of 2, 5 and 8 years. Starting at the corrected age of 2 years, the follow-up visits will include age-dependent standardized tests for cognitive as well as fine and gross motor development. Parents will be invited to complete questionnaires concerning their children's behavior and psychological well-being. In the meantime, Rosie is followed closely by medical professionals closer to home.

Figure 2. *Top left:* Rosie during her NICU stay, *Middle left:* Amy during her NICU stay, *Top right:* Kangaroo care with mom in the NICU, *Middle right:* The De Jong family: Rosie with mom, Amy with dad, *Bottom:* Amy (front) and Rosie (back) going for a drive.

Names and details about the pregnancy were modified. The parents gave permission for the publication of their story.

Twin-twin transfusion syndrome and fetoscopic laser surgery

Monochorionic twin pregnancies are threatened by several specific complications and TTTS may be the best-known, as well as the most feared one. TTTS develops in about 10% of monochorionic diamniotic twin pregnancies and its natural course is quick and more often than not, devastating. The name of the disease implies that transfusion between twins is pathological in itself. However, this is not the case. In uncomplicated monochorionic pregnancies, intertwin transfusion through deep arterio- venous placental anastomoses occurs equally in both directions or is compensated via superficial arterio-arterial (or rarely, veno-venous) anastomoses. In the case of TTTS, net blood flow from one twin to the other is unidirectional, resulting in volume depletion, oliguria and oligohydramnios in the donor fetus and volume overload with polyuria and polyhydramnios in the recipient.(1) The paradoxical exposure to different vasoactive mediators is thought to contribute to further deterioration of the disease process.(2) The major hemodynamic disturbances in TTTS, if left untreated, will result in previable or preterm delivery, or fetal demise. Because of their poor antenatal condition as well as the preterm brain's vulnerability, survivors of TTTS are at risk of brain injury and severe long-term neurodevelopmental impairment. One of the first management options for TTTS was serial amnioreduction, the repeated removal of large quantities of amniotic fluid from the amniotic sac of the recipient twin. Although this purely symptomatic treatment may be successful in terms of postponing delivery, intertwin transfusion remains unbalanced and the incidences of brain injury and neurodevelopmental consequences after serial amnioreduction remain high. The invention and further development of fetoscopic laser coagulation of placental anastomoses have had a major impact on pregnancy outcomes in TTTS.(3) Fetoscopic laser treatment aims to stop intertwin transfusion by occluding all vascular connections between the two fetuses, thus eliminating the cause of the disease. This treatment is now considered the gold standard for advanced TTTS.

Fetoscopic laser surgery in The Netherlands

Our colleagues from the obstetrics department at the Leiden University Medical Center (LUMC), many of whom contributed to this thesis, introduced fetoscopic laser surgery in our hospital in August 2000.(4) I was trained as a pediatrician and a neonatologist in the LUMC between 2011 and 2019 and

lucky enough to spend most of my time on the neonatal intensive care unit (NICU). It was during this time that I learned all about TTTS and this amazing surgical intervention the fetal surgeons could perform inside the pregnant uterus. As most babies who had undergone laser surgery for TTTS were born preterm, many were admitted to our NICU. Research from the LUMC performed in the early years after the start of the laser surgery program, had shown that short-term neonatal mortality and morbidity were still increased in survivors of TTTS treated with laser surgery, when compared to a control group of uncomplicated monochorionic twins.(5) Specifically, the incidence of cerebral injury was higher (14 versus 6%) and the majority of these injuries occurred antenatally.(6) In the earliest long-term follow-up study of TTTS survivors treated with laser surgery in Leiden between the years 2000 and 2003, the incidence of neurodevelopmental impairment was lower compared to a previous study in a conservatively treated TTTS cohort, but still remained high at 17%.(7, 8) Fortunately, the field of fetal therapy was not sitting still and major developments were ongoing. Monochorionic pregnancies were followed according to a strict national protocol, awareness for TTTS was raised among obstetricians and pediatricians across the country, and our fetal surgeons were performing more and more fetoscopic interventions.

The fetal medicine specialist versus the neonatologist

TTTS research is often performed by fetal medicine specialists, as it should be. They are obstetricians, who know all about twin pregnancies, prenatal imaging, the placenta and delivery. They make the diagnosis, perform fetal treatment, and monitor the pregnancy after laser surgery. Sometimes, they diagnose fetal complications using ultrasound or MRI. During a monochorionic pregnancy, the fetal medicine specialist manages three patients at the same time, three patients whose fates are strongly intertwined. You can imagine that at times, this means they have to make difficult decisions.

I am a neonatologist. We know about babies and a little bit about older children. When things go as planned during a TTTS pregnancy, two of the fetal medicine specialists' patients eventually become our patients. And when these babies experience complications, we make the diagnoses, treat them in the NICU and see them again in the follow-up clinic to evaluate their health and development when they are older. Much of the information that we gather during the NICU stay and follow-up visits of TTTS survivors can be of great value for our fetal medicine specialists. Because the ultimate goal of fetal

therapy in TTTS should be to ensure the healthy long-term survival of the mother and both her twins. How often are we achieving this goal? Can we find out which fetuses are at the highest risk of not achieving it? How common is fetal brain injury in TTTS and what causes it? Can we find ways to prevent it? We need research to answer these questions so that neonatologists may support some of the difficult decisions fetal medicine specialists face during TTTS pregnancies.

Back to Amy and Rosie

Without the developments in the field of fetal therapy, this family's story could have gone quite differently. Without a strict ultrasound screening protocol for monochorionic pregnancy, TTTS may not have been discovered in time to perform fetoscopic laser surgery. Without the possibility of fetoscopic laser surgery, Amy's and Rosie's chances of survival would have been very small. Without well-trained and experienced fetal surgeons, fetoscopic laser surgery may not have been as successful in treating TTTS. This being said, Rosie did not come away unscathed. We had noticed a change on prenatal ultrasound 9 days after laser surgery. Although this had us and the parents worried, we did not proceed to make a fetal MRI scan and we did not know exactly what was going on until we were able to make a postnatal brain MRI.

Amy's and Rosie's story raises many questions. In the current era of fetoscopic laser surgery, what is the incidence of brain injury in TTTS fetuses? What are the risk factors for brain injury? Is the risk of brain injury in TTTS infants decreasing with improving prenatal care and new developments in laser surgery techniques? How often is fetal or neonatal MRI used to detect brain abnormalities and what does MRI add compared to ultrasound? Did Rosie's ischemic brain injury have something to do with the fact that she was the recipient twin, with possible hyperviscosity-polycythemia? Or could it be related to the abrupt interruption of the intertwin transfusion process and sudden hemodynamic changes in brain perfusion after laser surgery? And what is the role of their prematurity? Is Rosie's brain lesion typical for TTTS and what other types of lesions may we come across? Of course, the twins' parents wonder about their future. What are the chances that they will have severe neurodevelopmental impairments (NDI), what risk factors can we identify? Based on Rosie's term-equivalent brain MRI, we predicted that she would develop unilateral cerebral palsy. To what degree does the presence of brain injury predict NDI in TTTS? Are Amy and Rosie at risk for other, milder impairments including behavioral problems?

References

1. Lewi L, Deprest J, Hecher K. The vascular anastomoses in monochorionic twin pregnancies and their clinical consequences. American journal of obstetrics and gynecology. 2013; 208(1):19-30.
2. Fisk NM, Duncombe GJ, Sullivan MH. The basic and clinical science of twin-twin transfusion syndrome. Placenta. 2009;30(5):379-90.
3. De Lia JE, Kuhlmann RS. Twin-to-twin transfusion syndrome--30 years at the front. American journal of perinatology. 2014;31 Suppl 1:S7-12.
4. Middeldorp JM, Klumper FJ, Oepkes D, Lopriore E, Kanhai HH, Vandenbussche FP. [The initial results of fetoscopic laser treatment for severe second trimester twin-to-twin transfusion syndrome in the Netherlands are comparable to international results]. Ned Tijdschr Geneeskd. 2004;148(24): 1203-8.
5. Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Vandenbussche FP, Walther FJ. Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses. J Pediatr. 2005;147(5):597-602.
6. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2006;194(5):1215-20.
7. Lopriore E, Nagel HT, Vandenbussche FP, Walther FJ. Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome. American journal of obstetrics and gynecology. 2003;189(5): 1314-9.
8. Lopriore E, Middeldorp JM, Sueters M, Oepkes D, Vandenbussche FP, Walther FJ. Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2007;196(3):231.e1-4.

Aim and outline of the thesis

The aim of this thesis is to provide insight into the risks of fetal and neonatal brain injury and long-term neurodevelopmental impairment in survivors of TTTS in the era of fetoscopic laser surgery.

This thesis is divided into six parts. You are now reading the first part, consisting of the general introduction and aim and outline of the thesis.

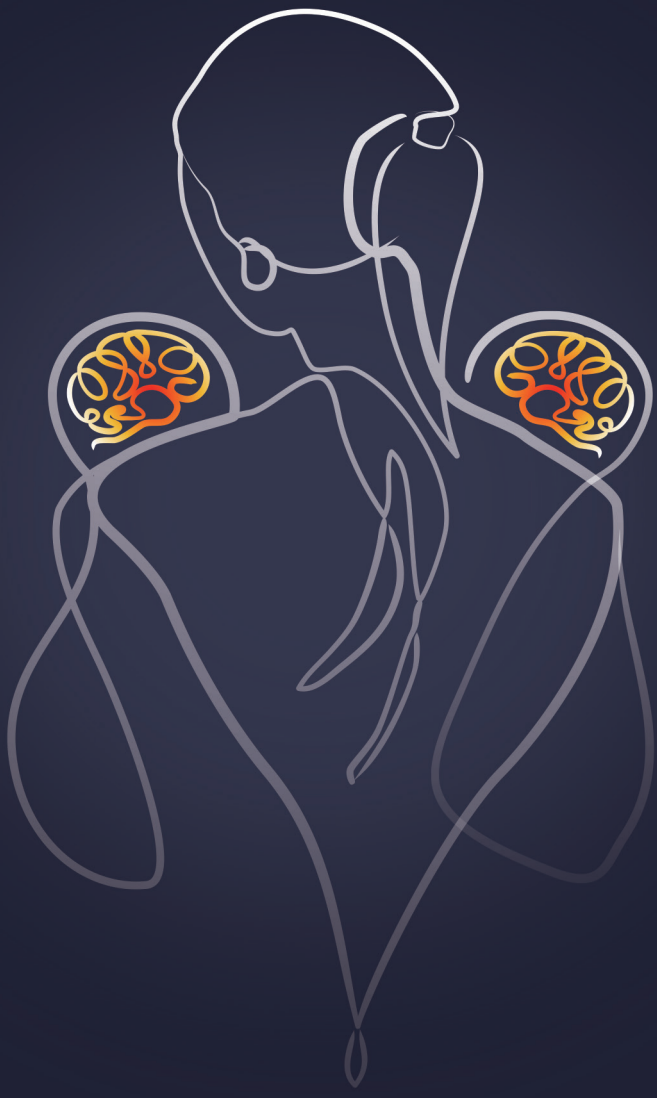
In part two, *chapter 1* provides a review of the literature summarizing the latest insights into TTTS. It covers prenatal management, as well as fetal and neonatal complications, with extra attention for cerebral injury and long-term neurodevelopmental impairment.

The third part of the thesis explores brain injury in TTTS treated with fetoscopic laser surgery. In *chapter 2*, we describe the incidence and risk factors of severe cerebral injury in a large case-control study of TTTS survivors, compared to a control group of dichorionic twins who were matched for gestational age. When we proceed to *chapter 3*, an even larger consecutive cohort is described where we investigate which different types of brain injury occur in fetuses and neonates with TTTS. We also describe the neurodevelopmental outcome of survivors with fetal or neonatal brain injury and review the use of different modalities for neuroimaging in our fetal therapy center.

The focus in the fourth part is on long-term neurodevelopment after TTTS. In *chapter 4*, we compare the neurodevelopmental outcome, measured using a standardized test at the corrected age of two years, between a recent cohort and an earlier cohort of TTTS survivors treated with laser surgery to investigate whether there is still room for improvement. Because studies in recent years have shown a marked decrease in fetal and neonatal mortality and morbidity in TTTS, we introduce mild neurodevelopmental impairment as an outcome measure. We dive deeper into the more subtle impairments in *chapter 5*, which is the description of a study of behavioral outcome after fetoscopic laser surgery for TTTS.

In part five, we explore a possible cause of the sometimes large differences in reported mortality and morbidity rates in TTTS between fetal therapy centers across the world. We do this by investigating the incidence of and reasons for end-of-life decisions in TTTS, which we termed 'intentional fetal or neonatal demise'. This study is described in *chapter 6*.

The sixth and final part of this thesis contains a summary followed by an overall discussion of the results of our studies into brain injury and neurodevelopment after TTTS in relation to the current medical literature. We will conclude the thesis by proposing future research directions in the field of TTTS. Despite significant progress made in recent decades, ongoing research efforts are essential to further improve the lives of affected families, such as Amy's and Rosie's, in the future.



Part TWO

Review

Chapter 1

Twin-twin transfusion syndrome in the era of fetoscopic laser surgery: antenatal management, neonatal outcome and beyond

Marjolijn S. Spruijt
Enrico Lopriore
Sylke J. Steggerda
Femke Slaghekke
Jeanine M.M. van Klink

Expert Review of Hematology 2020;13(3):259-267

Abstract

Introduction

Twin-twin transfusion syndrome (TTTS) is a devastating complication of monochorionic twin pregnancy and remains a major challenge for worldwide fetal medicine specialists. In TTTS, intertwin transfusion through vascular anastomoses in the shared placenta leads to severe hemodynamic imbalance. This review summarizes the current knowledge of TTTS.

Areas covered

The most recent insights concerning the management of TTTS, as well as fetal and neonatal complications are described. Relevant articles were selected based on a Pubmed search using the keywords below. Understanding of the underlying pathophysiology has improved greatly as a result of placental injection studies. Advancements in antenatal management have led to increased perinatal survival and a decreased incidence of neonatal complications, including brain injury and neurodevelopmental impairment.

Expert opinion

Further opportunities for improvement comprise technological innovations in laser procedures and the prevention of preterm rupture of membranes with subsequent prematurity. A noninvasive treatment such as high-intensity focused ultrasound (HIFU) seems to hold promise for the future treatment of TTTS. Fetal MRI studies are important to improve our understanding of fetal brain injury and should relate their findings to long-term neurodevelopment. International collaboration and centralization of care are of paramount importance to ensure the best care for our patients.

Keywords

Laser surgery; neurodevelopment; outcome; monochorionic twins; twin-twin transfusion syndrome

Introduction

Twins are at increased risk of perinatal death and long-term neurologic morbidity, and this risk is highest for monochorionic (MC) twins.(1-3) A large part of the increased risk for MC twins is attributable to twin-twin transfusion syndrome (TTTS). TTTS is one of the most lethal conditions in fetal medicine and remains a major challenge for obstetricians and neonatologists across the world.(4, 5) The implementation of strict protocols for the management of MC twin pregnancies have increased the opportunity for early diagnosis and timely management of TTTS.(6, 7) The management of this devastating disease has advanced considerably since the introduction of fetoscopic laser surgery in the 1990s, leading to increased perinatal survival and more favorable neonatal and long-term outcomes.(8, 9)

TTTS results from an unbalanced blood flow through placental vascular anastomoses connecting the two fetal circulations. These vascular anastomoses in the shared placenta are present in virtually all monochorionic (MC) twin pregnancies, but only in about 10% lead to TTTS. The net transfusion of blood is at the expense of the so-called donor twin, who becomes hypovolemic and anemic, whereas the recipient twin becomes hypervolemic and polycythemic. This leads to the quick development of a significant discordance in amniotic fluid volume between the twins, described as twin polyhydramnios-oligo-hydramnios sequence (TOPS). Hormonal dysregulation has been implicated to play a role in the further development of the syndrome.(10, 11) TOPS can be detected with prenatal ultrasound and is the hallmark of TTTS diagnosis. TTTS is staged using the criteria of Quintero, ranging from stage I disease characterized by TOPS with the donor's bladder still visible, to stage V in which there is fetal demise of one or both twins.(12, 13) The current review aims to summarize the knowledge gained in the last decade, its main focus being on pathophysiology, antenatal management, fetal and neonatal brain injury as well as long-term neurodevelopment after TTTS.

Literature search

Relevant papers were selected based on a Pubmed search of articles published after 2009, using combinations of the following search terms: twin-twin transfusion, laser, outcome, brain injury and neurodevelopment. Relevant literature references of the selected articles were identified and used for historic perspective in a few cases.

Placental injection studies

Although the exact mechanism by which TTTS develops is still poorly understood, placental injection studies using colored dye have much improved our knowledge of MC placentas in the past decade.(14-16) Vascular anastomoses in MC placentas are either arterio-arterial (AA), arterio-venous (AV) or veno-venous (VV) in nature. Injection studies were able to show a relationship between the type, number and size of placental anastomoses and the risk of developing TTTS and other MC pregnancy complications. TTTS placentas have significantly fewer AA anastomoses compared to uncomplicated MC placentas.(15, 17) AA anastomoses allow for bidirectional flow of blood, compensating for any imbalance in inter-twin blood volume caused by AV anastomoses, hereby reducing, but not eliminating, the risk of TTTS.

Placental injection studies have played an important role in the discovery of another form of inter-twin transfusion in MC pregnancy termed twin anemia-polycythemia sequence (TAPS).(18) Like TTTS, TAPS results from unbalanced blood flow through placental anastomoses. However, because TAPS is characterized by the presence of only very small anastomoses, transfusion in TAPS is much slower, allowing time for hemodynamic compensatory mechanisms to take effect and thus preventing the development of hypovolemia in the donor and hypervolemia in the recipient. Therefore, the essential difference between TTTS and TAPS is the absence of oligohydramnios/polyhydramnios in TAPS.(19, 20) TAPS can be diagnosed antenatally by measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) in both twins. In a recent study, it was shown that the difference in MCA-PSV between the donor and recipient is the most accurate predictor of postnatal TAPS with high sensitivity (83%) and specificity (100%). Based on these findings, a new antenatal classification system was proposed using a delta MCA-PSV of > 0.5 multiples of the median (MoM) as criterion for Stage 1 TAPS. Postnatally, TAPS is present when the intertwin hemoglobin difference is > 8 g/dL, combined with either a reticulocyte count ratio > 1.7 or the presence of only very small (< 1 mm) placental anastomoses.(21) TAPS can occur spontaneously (3-5% of MC pregnancies) or after laser for TTTS.(22)

Antenatal management of TTTS

Before the development of fetoscopic laser surgery, antenatal treatment in TTTS was mainly based on serial amnioreduction to reduce polyhydramnios and the consequent risk of preterm delivery.(4) However, short and long-term outcome after serial amnioreduction were poor. Perinatal survival rates usually did not exceed 50% and the risk of neurodevelopmental injury in survivors was high, up to 25%.(23-26)

Fetoscopic laser surgery

Fetoscopic laser surgery is now considered the best available treatment for advanced stages (Quintero stage ≥ 2) TTTS.(24, 27-30) The technique using a fetoscopic laser to disrupt blood flow through the vascular communications was developed in the 1980s and it soon became clear that this therapy would become a 'game changer' in the field of TTTS.(31) In contrast to serial amnioreduction, laser surgery is the only causative treatment, aiming to stop the intertwin fetal transfusion process. The first laser procedures were performed under general anesthesia and required laparotomy and hysterotomy for introduction of the endoscope, followed by laser photocoagulation. Since then, many developments have taken place. Laser technique has evolved from so-called 'non-selective' ablation of all vessels at the chorionic surface close to the intertwin membrane, to the selective technique in which the goal is to only coagulate anastomotic vessels at the vascular equator.(29, 32-35) In TTTS, the vascular equator is at some distance from the intertwin membrane due to the discordant amniotic fluid volumes between the twins. Laser surgery in TTTS is now minimally invasive, usually with local or regional anesthesia and percutaneous fetoscopy under continuous ultrasound guidance, thereby minimizing the risk of maternal complications.(36) Whether laser treatment is the best option for stage I TTTS is uncertain, as different groups have reported different results.(37) A multicenter RCT of stage I disease has recently been carried out and the results will hopefully answer this important question.

Figure 1 illustrates how a laser procedure is performed in a case of TTTS.

Sequential selective laser surgery

In 2007, Quintero and colleagues described a laser technique in which they choose to target the anastomoses in a specific order based on the physiological assumption that further hemodynamic shifts during the laser procedure might contribute to post-laser fetal demise, especially of the donor. They called this technique the sequential selective laser photocoagulation of

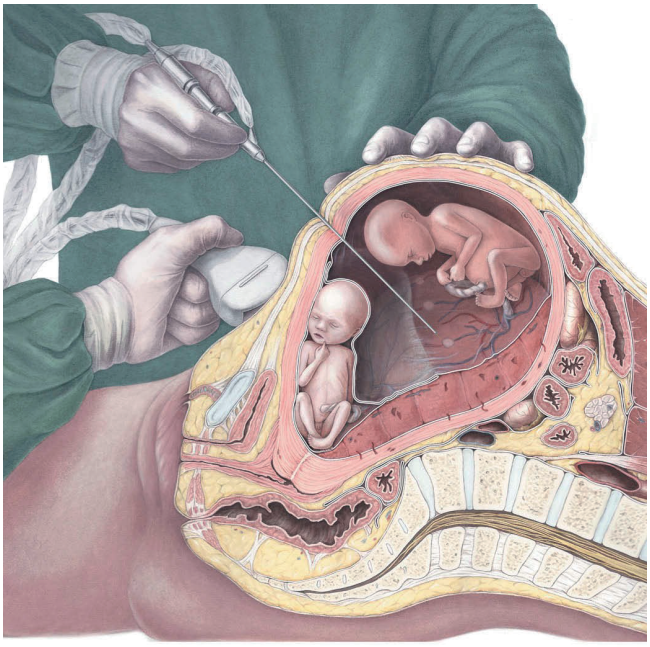


Figure 1. Realistic depiction of a modern-time laser procedure under ultrasound guidance.

Courtesy of Amanda Gautier.

communicating vessels and reported positive results in terms of post-laser IUFD and dual survival rates.(38) A systematic review in 2015 concluded that although data from three cohort studies suggest that dual survival was higher (75% versus 52%) and fetal demise rates were lower after sequential selective laser as compared to standard selective laser surgery, these results must be interpreted with caution, as no randomized controlled trials (RCT) were done and the three included studies had a high risk of bias due to methodological limitations.(39)

Solomon technique

Placental injection studies after laser therapy have revealed that residual anastomoses are present in over 30% of placentas.(40, 41) Residual anastomoses can cause post-laser TAPS or recurrence of TTTS and these complications are linked to adverse fetal, neonatal and long-term outcomes.(42, 43) To minimize the risk of residual anastomoses, a new laser technique was developed, designed

to coagulate the entire vascular equator. This technique was termed the "Solomon technique" after the biblical story of King Solomon who, in order to determine which one of two women was its real mother, suggested to cut a baby in half, after which the true mother begged that the child would be spared and committed to the care of her rival. Obviously, the Solomon technique does not entail splitting a baby in half. It is a modification of the selective laser technique, in which a line is drawn with the laser from one edge of the placenta to the other following the vascular equator, connecting the anastomoses that were first coagulated using the selective technique. The effect is a functional 'dichorionization' of the placenta. The Solomon trial, an RCT comparing the standard selective laser technique to the Solomon technique, showed a significant reduction in post-laser TAPS and recurrence of TTTS from 16% and 7% with the selective technique to 3% and 1% with the Solomon technique, respectively.(44) A secondary analysis showed that the Solomon technique does indeed reduce the risk of residual anastomoses, but they still occur, even when the procedure is recorded to be complete by the fetal therapist. Therefore, Slaghekke and her colleagues conclude that careful follow-up of the pregnancy remains essential also after Solomon laser.(41)

Survival

In 2015, Akkermans and colleagues systematically reviewed all published reports on survival after fetoscopic laser surgery for TTTS over 25 years and found that perinatal survival improved significantly during this time. The mean survival of both twins increased from 31% in reports dating from 1990 to 1995, to 62% in reports published between 2011 and 2014. Survival of at least one twin increased from 70 to 88%.(8) This study also shows a significant improvement in double survival with the more recently developed sequential selective (64%) and Solomon laser technique (71%) compared to the older techniques. The authors argue that improved survival is likely to be multi-factorial and could be the result of evolution in laser technique, a learning curve effect for fetal surgeons, and improvements in referral as well as neonatal care.

Complications of fetoscopic laser surgery

The most important complications after laser surgery are fetal demise and preterm prelabor rupture of the membranes (PPROM).(36, 45) Fetal demise rates are often not reported separately but comprise some combination of fetal demise in utero, miscarriage and selective feticide. In studies that do report the rate of fetal demise after laser, it occurs in 13 to 33% of cases. Some series report higher rates of fetal loss for donors than recipients.(46-49) Although gestational age at birth for TTTS pregnancies has become significantly

higher since the introduction of laser therapy, most series report mean gestational ages at birth to be around 32 weeks.(9) The fact that TTTS infants are still born prematurely is mostly due to PPROM after laser surgery. Iatrogenic PPROM occurs in up to 30% of cases.(50, 51) Maternal complications of laser surgery are also not consistently reported, but appear to occur in about 5% of cases and include abdominal pain after leakage of amniotic fluid into the peritoneal cavity, chorioamnionitis, bleeding, pulmonary edema and placental abruption.(45)

Neonatal outcome

As the majority of TTTS survivors are still born prematurely, TTTS neonates are at risk for morbidity associated with prematurity, including respiratory disease, necrotizing enterocolitis, retinopathy of prematurity and cerebral injury.(52, 53) Additionally, specific TTTS-related neonatal complications include cardiovascular morbidity, renal failure and hematologic disorders.(53-55) The risk of congenital heart disease is increased 12-fold in TTTS survivors compared to singletons and is attributed to severe fetal hemodynamic instability, leading to altered cardiac development.(56) Although fetal cardiac functional and/or structural abnormalities improve after successful laser surgery, they can persist beyond birth and sometimes require intervention. Cardiovascular complications are primarily seen in the recipient twin and include hypertension, cardiomyopathy, right ventricular outflow tract obstruction (which affects about 4% of recipients at birth) and persistent pulmonary hypertension of the newborn.(56, 57) However, donors may be at increased risk for aortic coarctation, possibly due to reduced flow caused by hypovolemia.(58)

The most important and dreaded complication in TTTS survivors is cerebral injury, as it may have profound and lifelong impact on these infants.

Cerebral injury

Brain injury is a feared complication of MC pregnancies in general and of TTTS in particular. It is caused by the presence of vascular anastomoses and inter-twin transfusion. The risk is highest for single survivors after intrauterine demise of their co-twin, which can cause severe hypovolemia and anemia due to the acute transfer of blood from the surviving fetus into their dying or dead sibling through patent vascular connections.(59-61) Laser surgery performed before fetal demise is protective of cerebral injury, provided that the surgery is complete and no anastomoses were missed. The incidence of

cerebral injury in TTTS has dropped considerably since the introduction of laser therapy and reported ranges are now between 2 and 18%.(24, 25, 27, 62-66) In our own center, the incidence has decreased since the start of our laser surgery program in 2000 from 14% in the first five years, to 6% in the most recently studied cohort treated between 2011 and 2014.(48, 67) Various reasons have been suggested to explain the decrease in brain injury, including improvement in laser technique and a learning curve effect, both associated with a decrease in residual anastomoses. In a large cohort of 1023 TTTS pregnancies, postoperative TAPS and recurrence of TTTS after laser, both known to be caused by residual anastomoses, were associated with an increased risk of cerebral injury.(16, 42)

Several types of cerebral injury have been described in the literature, including (cystic) periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH), posthemorrhagic ventricular dilatation, cerebral atrophy and arterial ischemic stroke. Fetal and neonatal MRI studies have shed more light on brain abnormalities detected in TTTS in the last decade. Several studies have reported additional findings of MRI compared to ultrasonography alone, including polymicrogyria and other migrational disorders, sinovenous thrombosis, and more subtle and/or diffuse white matter injury.(42, 64, 68-71) Donors and recipients are equally affected by cerebral injury, although the occurrence of cerebral arterial stroke seems to be a specific risk for recipients. (42, 62, 72) The range of cerebral injury reported by different groups is quite wide. This is due to varying definitions of cerebral injury, differences in the timing and frequency of imaging, and the fact that some centers do not routinely perform cranial imaging in all TTTS survivors.

Prematurity is still more the rule than the exception in TTTS pregnancies, making survivors also prone to postnatally acquired cerebral damage related to (extreme) prematurity, especially IVH and PVL. Prematurity and low birth weight (with birth weight being strongly correlated with gestational age) have in fact been proven to be the most important risk factors for severe cerebral injuries in survivors of TTTS.(62, 66) Reduction of severe prematurity in TTTS could hypothetically be achieved by reduction of the risk of PPROM and intrauterine infection through further technical improvements in fetoscopic surgery (for example by developing smaller fetoscopic instruments). Besides postnatal brain injury caused by prematurity, antenatally acquired cerebral lesions are more common in the context of TTTS compared to dichorionic twins, presumably because of the severe hemodynamic disturbances during pregnancy.(62) When antenatal in origin, cerebral injury in donors is thought to be mainly caused by impaired cerebral perfusion as the result of hypovolemia and inter-twin shifts of blood, leading to hypoxic-ischemic insults.

Polycythemia and hyperviscosity with subsequent vascular sludging is the presumed mechanism for cerebral injury in recipients.

Given the remaining risk of cerebral injury for TTTS survivors, routine standardized antenatal and postnatal cerebral imaging protocols are strongly recommended to accurately evaluate origin, timing and type of damage. MRI may play a larger role in determining cerebral injury in the future. Increased awareness of the increased risk by neonatologists and pediatricians may improve neonatal and pediatric care for these children. The clinical relevance of neuroimaging findings should be determined using long-term neurodevelopmental outcome data of all TTTS survivors until at least school age.

Long-term outcome

Definition and incidence

The ultimate goal of fetal therapy should be survival without neurodevelopmental impairment (NDI). Especially now that survival rates and short-term outcome have greatly improved, long-term follow-up of survivors is essential to evaluate whether this goal is achieved.(46, 73) Severe NDI in most studies is defined as at least one of the following: cerebral palsy (CP), severe motor and/or cognitive developmental delay, bilateral blindness, or deafness requiring amplification with hearing aids. Determining NDI requires a follow-up regimen that includes a physical and neurological examination, as well as cognitive and motor developmental assessments. Psychomotor development is ideally evaluated using standardized tests, such as the Bayley Scales for Infant and Toddler Development. However, several studies rely on the parent interview-based Ages and Stages Questionnaire (ASQ) for the evaluation of NDI. The ASQ has been shown to be a good screening tool for identifying infants who are severely delayed at 24 months of age and require neurological follow-up or intervention, but it cannot give the detailed information provided by face-to-face developmental tests performed by trained professionals.(74)

When combining studies that have clearly reported rates of NDI and/or CP after laser surgery at ≥ 2 years of age, the incidence of severe NDI in TTTS after laser surgery is 9% (range between 3 and 18%) and CP is reported at an average of 5% (range 2-12%). The results of these studies are summarized in **table 1**. The incidences of severe NDI and CP have decreased over the last two decades. Different factors may explain this improvement, including the development of stringent fetal monitoring protocols for MC pregnancies, learning curve effect of the laser procedure, increased awareness and improved neonatal care strategies for TTTS survivors.(7, 75)

Table 1. Studies of neurodevelopmental impairment and cerebral palsy in TTTS treated with laser

Reference	Country	Age (y)	Patients	NDI %	CP %	Developmental test
Sutcliffe 2001 (76)	UK	2	66	9	9	GDS
Banek 2003 (77)	Germany	2-3	89	11	11	GDS, SOT
Graef 2006 (78)	Germany	2-4	167	8	6	GDS, SOT
Lenclen 2009 (25)	France	2	88	11	10	ASQ
Lopriore 2009 (79)	Netherlands	2	278	18	6	BSID-II
Salomon 2010 (23)	France	5	73	-	12	ASQ, WISC-IV
Gray 2011 (80)	Australia	2-4	113	12	4	GDS, BSID-II, Bayley-III
Graeve 2012 (81)	Germany	4-10	151	9	-	K-ABC, GNSP
Van Klink 2014 (46)*	Netherlands	2	155	6	3	Bayley-III
McIntosh 2014 (82)	Australia	1-3	50	4	2	WPPSI-III, questionnaire
Vanderbilt 2014 (83)	USA	2	100	4	3	BDI-2, ATNE
Tosello 2014 (47)	France	0-5	35	-	6	ASQ
Korsakissok 2018 (65)	France	2-7	58	9	5	ASQ
Schou 2019 (84)	Denmark	1-4	86	10	9	ASQ
Chmait 2019 (63)	USA	2	99	4	3	ATNE, BDI-2
Spruijt 2019 (48)**	Netherlands	2	258	3	2	Bayley-III
Overall				**9%**	**5%**	
				(151/1741)	**(93/1695)**	
Range				**3-18%**	**2-12%**	

GDS: Griffiths' Developmental Test Scales; SOT: Snijders-Oomen Non-Verbal Intelligence Test; ASQ: Ages and Stages Questionnaire; BSID: Bayley Scales of Infant Development; WISC: Wechsler Intelligence Scale for Children; K-ABC: Kaufman Assessment Battery for Children; GNSP: German National Screening Program; WPPSI: Wechsler Preschool and Primary Scale of Intelligence; BDI: Batelle Development Inventory; ATNE: Amiel-Tison Neurodevelopmental Examination

- : not assessed

* only numbers of the 2008-2010 cohort used here to prevent patient overlap with the 2009 Lopriore study

** only numbers of the 2011-2014 cohort used here to prevent patient overlap with the 2014 Van Klink study

Risk factors

Risk factors for long-term NDI identified in follow-up studies include advanced gestational age at laser surgery, Quintero stage, low gestational age at birth, low birth weight and severe cerebral injury.(48, 63, 79, 85, 86) The negative impact of advanced gestational age at laser and higher Quintero stage suggests that increasing disease severity may lead to increased long-term morbidity. Low gestational age and birth weight are well-recognized risk factors for developmental impairment in the general population as well: severe NDI is frequently found in children born preterm and is inversely associated with gestational age and birth weight.

Mild neurodevelopmental impairment

Long-term follow-up studies after TTTS have used widely varying methods with regard to inclusion criteria, timing of assessments, the definition of NDI and the used outcome measures and controls, making it difficult to compare results. Significant lost to follow-up rates are common amongst long-term follow-up studies and have the potential to cause bias, most likely causing an underestimation of developmental impairment.(87, 88) In the majority of studies children were assessed at the age of two years, adjusted for prematurity, when major developmental impairment that requires intervention can be distinguished. However, neurodevelopmental assessment during early childhood can only moderately predict longer-term developmental outcome, especially for cognitive ability and academic performance. Certain developmental problems such as learning difficulties, behavioral problems and autism spectrum disorder are not always detected until school age, when children become more socially and academically challenged. To understand the clinical relevance of milder forms of impairment diagnosed in early childhood, follow-up until at least school age is essential. Data on mild neurodevelopmental impairment are limited and inconsistent. However, because of the decreasing trend in the rate of severe NDI, attention is shifting towards more subtle problems, including mild CP and neurocognitive impairments. Minor impairments can have a significant impact on the care and educational requirements of children and are reported in up to 30% of TTTS survivors. (25, 47, 48, 77, 89, 90) In order to gain more information on the exact burden and severity of NDI after laser surgery for TTTS, we encourage international collaboration to obtain larger sample sizes and statistical power, using a standardized follow-up regimen including uniform and clearly defined criteria for long-term neuro-developmental impairment.(91)

Conclusion

The management of TTTS has evolved significantly in the 21st century. Placental injection studies have improved our understanding of underlying placental pathophysiology. Fetoscopic laser coagulation of placental anastomoses is the primary treatment option and many advancements have been made over the past decade. Laser surgery using the Solomon technique has been proven to decrease the risk of post-laser TAPS and recurrence of TTTS. The advancements in antenatal management of TTTS have led to improved survival, higher gestational ages at birth, a lower incidence of neonatal brain injury and a decrease in severe long-term neurodevelopmental impairment.

Expert Opinion

Although the outcome of TTTS has improved greatly over the past decades, opportunities for further progress certainly still exist. The ultimate goals in the field of TTTS include prompt diagnosis or even better, accurate prediction of development of the syndrome, followed by a preferably non-invasive treatment with minimal complications. This treatment should achieve quick normalization of hemodynamic imbalance, minimizing any damage to the fetuses, without increasing the risk of PPROM and prematurity. The final goal for every TTTS pregnancy is (near-) term birth of two healthy neonates with normal long-term cognitive and motor development.

There is still a lot of ground to cover before these goals can be achieved. High-intensity focused ultrasound (HIFU) is a non-invasive technique which can be used to ablate blood flow in placental vessels, and therefore holds promise for the future treatment of TTTS. Although some preliminary experience with HIFU has been reported in animals as well as human cases of twin reversed arterial perfusion sequence (TRAP), safety and efficacy in humans with TTTS remains to be established.(92, 93) In the meantime, technological innovations may be able to further improve visibility and accessibility of the vascular equator during laser procedures. The further development of instrumentation aimed at minimizing the risk of PPROM after laser surgery is another important focus of research in future years, hopefully helping to prolong pregnancies further beyond 32 weeks' gestation and thereby minimizing the risks associated with preterm birth. Also, as the severity of placental damage after laser surgery is associated with PPROM and earlier delivery, the further fine-tuning of laser technique using minimal energy but obtaining maximum effect can certainly contribute to even better perinatal outcome.

With concern to the neonatal outcome of TTTS survivors, fetal MRI studies have begun to shed more light on the timing and mechanisms of fetal brain injury in TTTS, but studies that link prenatal MRI findings to long-term neurodevelopment are still lacking. Knowledge on long-term development beyond the age of 2 years is also very limited, especially concerning potential risk factors for adverse outcome and mild neurodevelopmental impairment. Although follow-up programs are costly and hard to realize, TTTS remains a serious risk factor for NDI. In our opinion, the treatment of fetuses in utero comes with the responsibility of providing careful and long-term follow-up of survivors until at least school age, in order to ensure that they will receive the care they need, if they need it. Long-term follow-up studies are indispensable for the continuous evaluation of the outcome of fetal therapy in TTTS pregnancies, as well as for the facilitation of evidence-based counseling of future parents. The formation of a core outcome set by a group of international experts is an important step in the joining of forces to help TTTS fetuses survive without complications, by making it possible to compare and combine research results. As briefly discussed in our review, severe fetal hemodynamic changes in TTTS can interfere with cardiac development leading to both functional and structural cardiac abnormalities. Follow-up studies of these cardiac changes are still very limited, but are essential for determining the need for cardiac screening and long-term monitoring in TTTS survivors, as well as for the counseling of parents about the prognosis of their children.

Lastly, an important issue to address is the need for centralization of treatment for TTTS in high-output fetal therapy centers. Following the advancements of laser treatment, there is an increasing number of small centers starting up fetal therapy programs in different countries. However, centralization has proven to be vital for the quality of care in a highly technical procedure such as fetoscopic laser surgery.(94) The best possible care can only be realized in highly specialized centers with dedicated and experienced multidisciplinary teams, ideally comprising fetal therapists, neonatologists and psychologists and in collaboration with twin parents' organizations.

References

1. Lewi L, Van Schoubroeck D, Gratacos E, Witters I, Timmerman D, Deprest J. Monochorionic diamniotic twins: complications and management options. Current opinion in obstetrics & gynecology. 2003;15(2):177-94.
2. Dube J, Dodds L, Armson BA. Does chorionicity or zygosity predict adverse perinatal outcomes in twins? American journal of obstetrics and gynecology. 2002;186(3):579-83.
3. Pharoah PO. Risk of cerebral palsy in multiple pregnancies. Clinics in perinatology. 2006;33(2):301-13.
4. Glennon CL, Shemer SA, Palma-Dias R, Umstad MP. The History of Treatment of Twin-to-Twin Transfusion Syndrome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):168-74.
5. Weir PE, Ratten GJ, Beischer NA. Acute polyhydramnios--a complication of monozygous twin pregnancy. British journal of obstetrics and gynaecology. 1979;86(11):849-53.
6. Gratacos E, Ortiz JU, Martinez JM. A systematic approach to the differential diagnosis and management of the complications of monochorionic twin pregnancies. Fetal diagnosis and therapy. 2012;32(3):145-55.
7. Sueters M, Oepkes D. Diagnosis of twin-to-twin transfusion syndrome, selective fetal growth restriction, twin anaemia-polycythaemia sequence, and twin reversed arterial perfusion sequence. Best practice & research Clinical obstetrics & gynaecology. 2014;28(2):215-26.
8. Akkermans J, Peeters SH, Klumper FJ, Lopriore E, Middeldorp JM, Oepkes D. Twenty-Five Years of Fetoscopic Laser Coagulation in Twin-Twin Transfusion Syndrome: A Systematic Review. Fetal diagnosis and therapy. 2015;38(4):241-53.
9. Hecher K, Gardiner HM, Diemert A, Bartmann P. Long-term outcomes for monochorionic twins after laser therapy in twin-to-twin transfusion syndrome. The Lancet Child & adolescent health. 2018;2(7):525-35.
10. Galea P, Barigye O, Wee L, Jain V, Sullivan M, Fisk NM. The placenta contributes to activation of the renin angiotensin system in twin-twin transfusion syndrome. Placenta. 2008;29(8):734-42.
11. Djaafri F, Stirnemann J, Mediouni I, Colmant C, Ville Y. Twin-twin transfusion syndrome - What we have learned from clinical trials. Seminars in fetal & neonatal medicine. 2017;22(6):367-75.
12. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 1999;19(8 Pt 1):550-5.
13. Taylor MJ, Govender L, Jolly M, Wee L, Fisk NM. Validation of the Quintero staging system for twin-twin transfusion syndrome. Obstetrics and gynecology. 2002;100(6):1257-65.
14. Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ, et al. Accurate and simple evaluation of vascular anastomoses in monochorionic placenta using colored dye. Journal of visualized experiments : JoVE. 2011(55):e3208.
15. Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D, et al. Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta. 2013;34(7):589-93.
16. Lewi L, Deprest J, Hecher K. The vascular anastomoses in monochorionic twin pregnancies and their clinical consequences. American journal of obstetrics and gynecology. 2013;208(1):19-30.
17. Denbow ML, Cox P, Taylor M, Hammal DM, Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. American journal of obstetrics and gynecology. 2000;182(2):417-26.
18. Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP. Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta. 2007;28(1):47-51.
19. Lopriore E, Deprest J, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, et al. Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstetrics and gynecology. 2008;112(4):753-8.

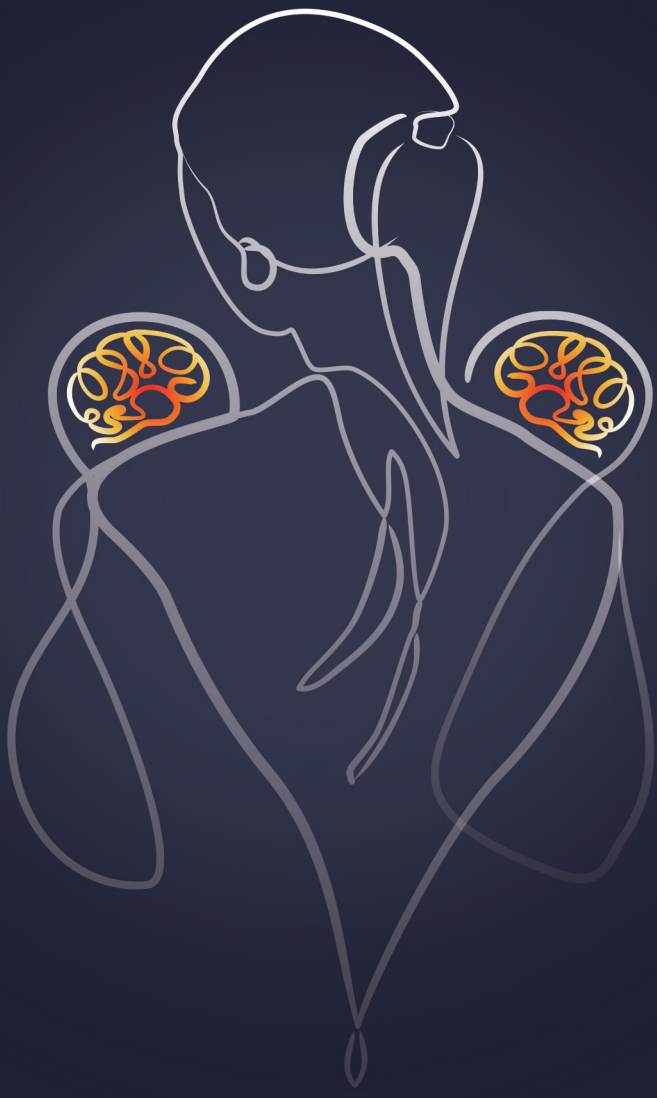
20. Slaghekke F, Kist WJ, Oepkes D, Middeldorp JM, Klumper FJ, Vandenbussche FP, et al. TAPS and TOPS: two distinct forms of feto-fetal transfusion in monochorionic twins. Zeitschrift fur Geburtshilfe und Neonatologie. 2009;213(6):248-54.
21. Tollenaar LSA, Lopriore E, Middeldorp JM, Haak MC, Klumper FJ, Oepkes D, et al. Improved prediction of twin anemia-polycythemia sequence by delta middle cerebral artery peak systolic velocity: new antenatal classification system. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2019;53(6):788-93.
22. Tollenaar LS, Slaghekke F, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D, et al. Twin Anemia Polycythemia Sequence: Current Views on Pathogenesis, Diagnostic Criteria, Perinatal Management, and Outcome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):222-33.
23. Salomon LJ, Ortqvist L, Aegerter P, Bussieres L, Staracci S, Stirnemann JJ, et al. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. American journal of obstetrics and gynecology. 2010;203(5):444.e1-7.
24. van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal diagnosis and therapy. 2013;33(2):81-9.
25. Lenclen R, Ciarlo G, Paupe A, Bussieres L, Ville Y. Neurodevelopmental outcome at 2 years in children born preterm treated by amnioreduction or fetoscopic laser surgery for twin-to-twin transfusion syndrome: comparison with dichorionic twins. American journal of obstetrics and gynecology. 2009;201(3):291.e1-5.
26. Li X, Morokuma S, Fukushima K, Otera Y, Yumoto Y, Tsukimori K, et al. Prognosis and long-term neurodevelopmental outcome in conservatively treated twin-to-twin transfusion syndrome. BMC pregnancy and childbirth. 2011;11:32.
27. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine. 2004;351(2):136-44.
28. Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. The Cochrane database of systematic reviews. 2014(1):Cd002073.
29. Hecher K, Plath H, Bregenzer T, Hansmann M, Hackeloer BJ. Endoscopic laser surgery versus serial amniocenteses in the treatment of severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 1999;180(3 Pt 1):717-24.
30. Rossi AC, D'Addario V. Survival outcomes of twin-twin transfusion syndrome stage I: a systematic review of literature. American journal of perinatology. 2013;30(1):5-10.
31. De Lia JE, Cruikshank DP, Keye WR, Jr. Fetoscopic neodymium:YAG laser occlusion of placental vessels in severe twin-twin transfusion syndrome. Obstetrics and gynecology. 1990;75(6):1046-53.
32. Quintero RA, Comas C, Bornick PW, Allen MH, Kruger M. Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2000;16(3):230-6.
33. Quintero RA, Morales WJ, Mendoza G, Allen M, Kalter CS, Giannina G, et al. Selective photocoagulation of placental vessels in twin-twin transfusion syndrome: evolution of a surgical technique. Obstetrical & gynecological survey. 1998;53(12 Suppl):S97-103.
34. Ville Y, Hecher K, Ogg D, Warren R, Nicolaides K. Successful outcome after Nd : YAG laser separation of chorioangiopagus-twins under sonoendoscopic control. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1992;2(6):429-31.
35. Ville Y, Hyett J, Hecher K, Nicolaides K. Preliminary experience with endoscopic laser surgery for severe twin-twin transfusion syndrome. The New England journal of medicine. 1995;332(4):224-7.

36. Yamamoto M, El Murr L, Robyr R, Leleu F, Takahashi Y, Ville Y. Incidence and impact of perioperative complications in 175 fetoscopy-guided laser coagulations of chorionic plate anastomoses in fetofetal transfusion syndrome before 26 weeks of gestation. American journal of obstetrics and gynecology. 2005;193(3 Pt 2):1110-6.
37. Khalil A, Cooper E, Townsend R, Thilaganathan B. Evolution of Stage 1 Twin-to-Twin Transfusion Syndrome (TTTS): Systematic Review and Meta-Analysis. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):207-16.
38. Quintero RA, Ishii K, Chmait RH, Bornick PW, Allen MH, Kontopoulos EV. Sequential selective laser photocoagulation of communicating vessels in twin-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2007;20(10):763-8.
39. Akkermans J, Peeters SH, Klumper FJ, Middeldorp JM, Lopriore E, Oepkes D. Is the Sequential Laser Technique for Twin-to-Twin Transfusion Syndrome Truly Superior to the Standard Selective Technique? A Meta-Analysis. Fetal diagnosis and therapy. 2015;37(4):251-8.
40. Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ, Vandenbussche FP. Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome. Placenta. 2007;28(2-3):204-8.
41. Slaghekke F, Lewi L, Middeldorp JM, Weingertner AS, Klumper FJ, Dekoninck P, et al. Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial. American journal of obstetrics and gynecology. 2014;211(3):285.e1-7.
42. Stirnemann J, Chalouhi G, Essaoui M, Bahi-Buisson N, Sonigo P, Millischer AE, et al. Fetal brain imaging following laser surgery in twin-to-twin surgery. BJOG : an international journal of obstetrics and gynaecology. 2018;125(9):1186-91.
43. Lewi L, Jani J, Cannie M, Robyr R, Ville Y, Hecher K, et al. Intertwin anastomoses in monochorionic placentas after fetoscopic laser coagulation for twin-to-twin transfusion syndrome: is there more than meets the eye? American journal of obstetrics and gynecology. 2006;194(3):790-5.
44. Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, et al. Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet (London, England). 2014;383(9935):2144-51.
45. Merz W, Tchatcheva K, Gembruch U, Kohl T. Maternal complications of fetoscopic laser photocoagulation (FLP) for treatment of twin-twin transfusion syndrome (TTTS). Journal of perinatal medicine. 2010;38(4):439-43.
46. van Klink JM, Koopman HM, van Zwet EW, Middeldorp JM, Walther FJ, Oepkes D, et al. Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology. 2014;210(6):540.e1-7.
47. Tosello B, Blanc J, Haumonte JB, D'Ercole C, Gire C. Short and medium-term outcomes of live-born twins after fetoscopic laser therapy for twin-twin transfusion syndrome. Journal of perinatal medicine. 2014;42(1):99-105.
48. Spruijt MS, Lopriore E, Tan R, Slaghekke F, Klumper F, Middeldorp JM, et al. Long-Term Neurodevelopmental Outcome in Twin-to-Twin Transfusion Syndrome: Is there still Room for Improvement? Journal of clinical medicine. 2019;8(8).
49. Chmait RH, Kontopoulos EV, Korst LM, Llanes A, Petisco I, Quintero RA. Stage-based outcomes of 682 consecutive cases of twin-twin transfusion syndrome treated with laser surgery: the USFetus experience. American journal of obstetrics and gynecology. 2011;204(5):393.e1-6.
50. Beck V, Lewi P, Gucciardo L, Devlieger R. Preterm prelabor rupture of membranes and fetal survival after minimally invasive fetal surgery: a systematic review of the literature. Fetal diagnosis and therapy. 2012;31(1):1-9.

51. Malshe A, Snowise S, Mann LK, Boring N, Johnson A, Bebbington MW, et al. Preterm delivery after fetoscopic laser surgery for twin-twin transfusion syndrome: etiology and risk factors. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017;49(5):612-6.
52. Halvorsen CP, Ek S, Dellgren A, Grunewald C, Kublickas M, Westgren M, et al. Survival and neonatal outcome after fetoscopic guided laser occlusion (FLOC) of twin-to-twin transfusion syndrome (TTTS) in Sweden. Journal of perinatal medicine. 2012;40(5):533-8.
53. Lopriore E, Oepkes D, Walther FJ. Neonatal morbidity in twin-twin transfusion syndrome. Early human development. 2011;87(9):595-9.
54. Verbeek L, Slaghekke F, Sueters M, Middeldorp JM, Klumper FJ, Haak MC, et al. Hematological disorders at birth in complicated monochorionic twins. Expert review of hematology. 2017;10(6):525-32.
55. Wohlmuth C, Boudreaux D, Moise KJ, Jr., Johnson A, Papanna R, Bebbington M, et al. Cardiac pathophysiology in twin-twin transfusion syndrome: new insights into its evolution. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2018;51(3):341-8.
56. Gijtenbeek M, Shirzada MR, Ten Harkel ADJ, Oepkes D, M CH. Congenital Heart Defects in Monochorionic Twins: A Systematic Review and Meta-Analysis. Journal of clinical medicine. 2019;8(6).
57. Manning N, Archer N. Cardiac Manifestations of Twin-to-Twin Transfusion Syndrome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):246-54.
58. van den Boom J, Battin M, Hornung T. Twin-twin transfusion syndrome, coarctation of the aorta and hypoplastic aortic arch: a case series report. Journal of paediatrics and child health. 2010;46(3):76-9.
59. Hillman SC, Morris RK, Kilby MD. Co-twin prognosis after single fetal death: a systematic review and meta-analysis. Obstetrics and gynecology. 2011;118(4):928-40.
60. van Klink JM, van Steenis A, Steggerda SJ, Genova L, Sueters M, Oepkes D, et al. Single fetal demise in monochorionic pregnancies: incidence and patterns of cerebral injury. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2015;45(3):294-300.
61. Adegbite AL, Castille S, Ward S, Bajoria R. Prevalence of cranial scan abnormalities in preterm twins in relation to chorionicity and discordant birth weight. European journal of obstetrics, gynecology, and reproductive biology. 2005;119(1):47-55.
62. Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2012;120(1):15-20.
63. Chmait RH, Chon AH, Schrager SM, Llanes A, Hamilton AH, Vanderbilt DL. Neonatal cerebral lesions predict 2-year neurodevelopmental impairment in children treated with laser surgery for twin-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2019;32(1):80-4.
64. Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2007;20(8):589-97.
65. Korsakissok M, Groussolles M, Dicky O, Alberge C, Casper C, Azogui-Assouline C. Mortality, morbidity and 2-years neurodevelopmental prognosis of twin to twin transfusion syndrome after fetoscopic laser therapy: a prospective, 58 patients cohort study. Journal of gynecology obstetrics and human reproduction. 2018;47(10):555-60.
66. Vanderbilt DL, Schrager SM, Llanes A, Chmait RH. Prevalence and risk factors of cerebral lesions in neonates after laser surgery for twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2012;207(4):320.e1-6.

67. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2006;194(5):1215-20.
68. Ascherl R, Sorge I, Thome U, Hirsch FW, Blaser A, Kiess W, et al. Severe gyration and migration disorder in fetofetal transfusion syndrome: two case reports and a review of the literature on the neurological outcome of children with lesions on neuroimaging. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2018;34(1):155-63.
69. Merhar SL, Kline-Fath BM, Meinzen-Derr J, Schibler KR, Leach JL. Fetal and postnatal brain MRI in premature infants with twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 2013;33(2):112-8.
70. Griffiths PD, Sharrack S, Chan KL, Bamfo J, Williams F, Kilby MD. Fetal brain injury in survivors of twin pregnancies complicated by demise of one twin as assessed by in utero MR imaging. Prenatal diagnosis. 2015;35(6):583-91.
71. Robinson A, Teoh M, Edwards A, Fahey M, Goergen S. Fetal brain injury in complicated monochorionic pregnancies: diagnostic yield of prenatal MRI following surveillance ultrasound and influence on prognostic counselling. Prenatal diagnosis. 2017;37(6):611-27.
72. Rossi AC, D'Addario V. Comparison of donor and recipient outcomes following laser therapy performed for twin-twin transfusion syndrome: a meta-analysis and review of literature. American journal of perinatology. 2009;26(1):27-32.
73. Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstetrics and gynecology. 2011;118(5):1145-50.
74. Gollenberg AL, Lynch CD, Jackson LW, McGuinness BM, Msall ME. Concurrent validity of the parent-completed Ages and Stages Questionnaires, 2nd Ed. with the Bayley Scales of Infant Development II in a low-risk sample. Child: care, health and development. 2010;36(4):485-90.
75. Morris RK, Selman TJ, Harbidge A, Martin WI, Kilby MD. Fetoscopic laser coagulation for severe twin-to-twin transfusion syndrome: factors influencing perinatal outcome, learning curve of the procedure and lessons for new centres. BJOG : an international journal of obstetrics and gynaecology. 2010;117(11):1350-7.
76. Sutcliffe AG, Sebire NJ, Pigott AJ, Taylor B, Edwards PR, Nicolaides KH. Outcome for children born after in utero laser ablation therapy for severe twin-to-twin transfusion syndrome. BJOG : an international journal of obstetrics and gynaecology. 2001;108(12):1246-50.
77. Banek CS, Hecher K, Hackeloer BJ, Bartmann P. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2003;188(4):876-80.
78. Graef C, Ellenrieder B, Hecher K, Hackeloer BJ, Huber A, Bartmann P. Long-term neurodevelopmental outcome of 167 children after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2006;194(2):303-8.
79. Lopriore E, Ortibus E, Acosta-Rojas R, Le Cessie S, Middeldorp JM, Oepkes D, et al. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2009;113(2 Pt 1):361-6.
80. Gray PH, Poulsen L, Gilshenan K, Soong B, Cincotta RB, Gardener G. Neurodevelopmental outcome and risk factors for disability for twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology. 2011;204(2):159 e1-6.
81. Graeve P, Banek C, Stegmann-Woessner G, Maschke C, Hecher K, Bartmann P. Neurodevelopmental outcome at 6 years of age after intrauterine laser therapy for twin-twin transfusion syndrome. Acta paediatrica (Oslo, Norway : 1992). 2012;101(12):1200-5.
82. McIntosh J, Meriki N, Joshi A, Biggs V, Welsh AW, Challis D, et al. Long term developmental outcomes of pre-school age children following laser surgery for twin-to-twin transfusion syndrome. Early human development. 2014;90(12):837-42.

83. Vanderbilt DL, Schrager SM, Llanes A, Hamilton A, Seri I, Chmait RH. Predictors of 2-year cognitive performance after laser surgery for twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2014;211(4):388 e1-7.
84. Schou KV, Lando AV, Ekelund CK, Jensen LN, Jorgensen C, Norgaard LN, et al. Long-Term Neurodevelopmental Outcome of Monochorionic Twins after Laser Therapy or Umbilical Cord Occlusion for Twin-Twin Transfusion Syndrome. Fetal diagnosis and therapy. 2019;46(1):20-7.
85. van Klink JM, Koopman HM, Rijken M, Middeldorp JM, Oepkes D, Lopriore E. Long-Term Neurodevelopmental Outcome in Survivors of Twin-to-Twin Transfusion Syndrome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):255-61.
86. Sananes N, Gabriele V, Weingertner AS, Ruano R, Sanz-Cortes M, Gaudineau A, et al. Evaluation of long-term neurodevelopment in twin-twin transfusion syndrome after laser therapy. Prenatal diagnosis. 2016;36(12):1139-45.
87. Aylward GP, Hatcher RP, Stripp B, Gustafson NF, Leavitt LA. Who goes and who stays: subject loss in a multicenter, longitudinal follow-up study. J Dev Behav Pediatr. 1985;6(1):3-8.
88. Wolke D, Sohne B, Ohrt B, Riegel K. Follow-up of preterm children: important to document dropouts. Lancet (London, England). 1995;345(8947):447.
89. Mullers SM, McAuliffe FM, Kent E, Carroll S, Mone F, Breslin N, et al. Outcome following selective fetoscopic laser ablation for twin to twin transfusion syndrome: an 8 year national collaborative experience. European journal of obstetrics, gynecology, and reproductive biology. 2015;191:125-9.
90. Campos D, Arias AV, Campos-Zanelli TM, Souza DS, Dos Santos Neto OG, Peralta CF, et al. Twin-twin transfusion syndrome: neurodevelopment of infants treated with laser surgery. Arquivos de neuro-psiquiatria. 2016;74(4):307-13.
91. Perry H, Duffy JMN, Umadia O, Khalil A. Outcome reporting across randomized trials and observational studies evaluating treatments for twin-twin transfusion syndrome: systematic review. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2018;52(5):577-85.
92. Shaw CJ, Civale J, Botting KJ, Niu Y, Ter Haar G, Rivens I, et al. Noninvasive high-intensity focused ultrasound treatment of twin-twin transfusion syndrome: A preliminary in vivo study. Science translational medicine. 2016;8(347):347ra95.
93. Seo K, Ichizuka K, Okai T, Dohi S, Nakamura M, Hasegawa J, et al. Treatment of twin-reversed arterial perfusion sequence using high-intensity focused ultrasound. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2019;54(1):128-34.
94. Diehl W, Diemert A, Grasso D, Sehner S, Wegscheider K, Hecher K. Fetoscopic laser coagulation in 1020 pregnancies with twin-twin transfusion syndrome demonstrates improvement in double-twin survival rate. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017;50(6):728-35.



Part THREE

Brain injury

Chapter 2

Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery

Marjolijn S. Spruijt
Sylke J. Steggerda
Mirjam E.A. Rath
Erik W. van Zwet
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Obstetrics & Gynecology 2012;120(1):15-20

Abstract

Objective

To estimate the incidence and risk factors for cerebral lesions in monochorionic twins with twin-twin transfusion syndrome treated with fetoscopic laser surgery compared with dichorionic twins.

Methods

We performed a case-control study on cerebral injury detected by postnatal cranial ultrasonography in monochorionic twin neonates with twin-twin transfusion syndrome treated with laser compared with a control group of dichorionic twin neonates matched for gestational age at birth. Severe cerebral lesions were defined as the presence of at least one of the following: intraventricular hemorrhage grade III, periventricular hemorrhagic infarction, periventricular leukomalacia grade II or greater, porencephalic cysts, arterial stroke, ventricular dilatation, or a combination of these.

Results

From 2004 until 2011, 267 twin neonates with twin-twin transfusion syndrome could be included and matched with 267 dichorionic twin neonates. Incidence of severe cerebral lesions in the twin-twin transfusion syndrome group and control group was 8.6% (23/267) and 6.7% (18/267), respectively ($p = 0.44$). Multivariable analysis revealed that only gestational age at birth was independently associated with increased risk for severe cerebral lesions (odds ratio [OR] 1.35 for each week, 95% confidence interval [CI] 1.14 to 1.59, $p < 0.01$). In 52.2% (12/23), the cerebral lesions in the twin-twin transfusion syndrome group were of antenatal origin compared with 16.7% (3/18) in the control group (OR 8.00, 95% CI 1.42 to 45.06, $p = 0.02$).

Conclusion

Incidence of severe cerebral lesions in twin–twin transfusion syndrome treated with laser is similar to a matched control group and is independently associated with prematurity. In contrast to dichorionic twins, cerebral injury in twins with twin–twin transfusion syndrome most often occurs antenatally.

Introduction

Twin-twin transfusion syndrome is a major complication of monochorionic twin pregnancies and is the result of intertwin blood transfusion through placental vascular anastomoses. Fetoscopic laser coagulation of the anastomoses is today considered to be the treatment of choice in twin–twin transfusion syndrome.(1)

Despite improved short-term and long-term outcome after laser surgery, twin-twin transfusion syndrome is still associated with an increased incidence of cerebral injury and neurodevelopmental impairment. Incidence of severe cerebral lesions on cranial ultrasonography ranges from 3% to 16% whereas the incidence of neurodevelopmental impairment ranges from 8% to 18%.(1-5)

The cause of cerebral injury in twin-twin transfusion syndrome is not fully understood and may result from antenatal injury resulting from hemodynamic imbalance, postnatal injury resulting from prematurity, or both. (2, 6-8) To date, most studies on cerebral injury in twin-twin transfusion syndrome are limited by small numbers of included patients and lack of a control group. Our objective was to estimate the incidence and risk factors of severe cerebral injury on postnatal cranial ultrasonography in a large prospective series of twins with twin-twin transfusion syndrome treated with laser surgery compared with a control group of dichorionic twins matched for gestational age at birth.

Patients and methods

All consecutive monochorionic twins with twin-twin transfusion syndrome treated with fetoscopic laser surgery at the Leiden University Medical Center and delivered at our center between January 2004 and December 2011 were included in this study. Neonates in the twin-twin transfusion syndrome group were matched according to gestational age at birth (± 1 week of gestation) with a control group of dichorionic twins born at our center during the same 8-year study period. The control dichorionic twins were identified from the hospital's database and were the next twin pregnancy delivered at our hospital with a matched gestational age.

The Leiden University Medical Center is a tertiary medical center and serves as the national referral center for intrauterine laser treatment in twin-twin transfusion syndrome pregnancies in The Netherlands.

Pregnancies complicated by intrauterine fetal demise of both twins, major congenital anomalies, triplets, or severe perinatal asphyxia were excluded from the study. Twin-twin transfusion syndrome was diagnosed using standard prenatal ultrasonographic criteria and staged according to the criteria of Quintero.(9)

Neonatal cranial ultrasonographic scans were performed when indicated according to our cranial ultrasonographic protocol. The clinical protocol at our neonatal intensive care unit requires a minimum of three scans during the first week of life (days 1, 3, and 7) for premature neonates with gestational ages less than 32 weeks or birth weights less than 1500 g and scans are repeated biweekly until discharge. Premature neonates with gestational ages between 32 weeks and (near-)term neonates admitted to our unit are routinely scanned at least once during the first week of life. If cerebral abnormalities are detected, scanning frequency is intensified around the date of detection and repeated at the time of the estimated date of confinement. Cranial ultrasonographic scans in our unit are performed with an Aloka 5000 scanner with a multifrequency (5–10 MHz) transducer. The cerebral anatomy was visualized in the standard coronal and sagittal planes. Experienced neonatologists performed all cranial ultrasonographic scans.

Intraventricular hemorrhages were classified according to Volpe, and periventricular leukomalacia was graded according to de Vries et al.(10, 11) Severe cerebral lesions on cranial ultrasound scans were defined as the presence of at least one of the following findings: intraventricular hemorrhage grade III, periventricular hemorrhagic infarction, periventricular leukomalacia grade II or greater, porencephalic cysts, arterial stroke, ventricular dilatation, or a combination of these. Diagnosis of arterial stroke was reached when a wedge-shaped area of echogenicity was detected on coronal and parasagittal views, in the region supplied by the middle or anterior or posterior cerebral artery with a linear demarcation line, followed by cystic evolution of this area of increased echogenicity after 2–4 weeks. Ventricular dilatation was present when the width of one or both lateral ventricles exceeded the 97th percentile.(12) Other severe cerebral abnormalities such as parenchymal hemorrhage, infarction, or both, which are 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 ultrasonographic scan on day 1. Periventricular white matter cysts detected within 2 weeks after birth were also considered to be of antenatal onset. Magnetic resonance imaging of the brain was performed in cases with suspected severe cerebral lesions.

The following data were recorded in the twin-twin transfusion syndrome group and control group: stage of twin-twin transfusion syndrome, treatment failure (defined as recurrent twin-twin transfusion syndrome or twin anemia-polycythemia sequence after laser surgery), gestational age at delivery, mode of delivery, birth weight, gender, and small for gestational age.(13)

The primary outcome measure was presence of severe cerebral lesions detected on cranial ultrasonographic scans. Outcome of the twin-twin transfusion syndrome group was compared with the matched control group of dichorionic twin neonates.

The following potential predictors for cerebral injury in the twin-twin transfusion syndrome group were studied in a univariable and multivariable logistic regression model: gestational age at birth, Quintero stage, donor (compared with recipient), treatment failure (defined as recurrent twin-twin transfusion syndrome or twin anemia-polycythemia sequence), and year of the laser procedure (assessed as a continuous variable to study the effect of the learning curve). Selection of the potential risk factors was based on previous studies.(14)

Based on previous studies (including our own data), we estimated that group sizes of at least 250 neonates were required to demonstrate a 10% difference (15% in the twin-twin transfusion syndrome group compared with 5% in the control group) in severe cerebral lesions with a significance of .05, a power of 90%, and an intracluster correlation coefficient ρ of 0.33 by two-tailed analysis. To take into account the dependence between siblings, we used the method of generalized estimating equations for all analyses.(15) The results of the logistic models were expressed as an odds ratio (OR) and 95% confidence intervals (CIs). A value of $p<.05$ was considered to indicate statistical significance. Analyses were performed using SPSS 17.0.

Results

During the 8-year study period (2004 - 2011), 313 twin-twin transfusion syndrome twin pregnancies were treated with laser surgery at our center. Overall perinatal survival was 77% (480/626). Of these 313 twin-twin transfusion syndrome pregnancies, 46% (144/313) were delivered at our center with at least one liveborn neonate (n=267 liveborn neonates). All 267 liveborn neonates were admitted to our neonatal intensive care unit and included in the study (twin-twin transfusion syndrome group). Patients' characteristics in the twin-twin transfusion syndrome group and the matched control group of dichorionic twins are presented in **table 1**. The neurologic

outcome in twin-twin transfusion syndrome survivors delivered at our center between June 2002 and September 2005 has been published before.(2)

Table 1. Baseline characteristics in neonates included in the twin-twin transfusion group and the control group

	TTTS Group (n = 267)	Control Group (n = 267)	*p value*
Gestational age at birth (wk)	32 (29-34)	32 (29-34)	0.71
Female	144 (53.9)	130 (48.7)	0.26
Vaginal delivery	135 (50.6)	191 (71.5)	< 0.01
Birth weight (g)	1634 ± 532 (683-3421)	1711 ± 540 (590-3785)	0.180
Small for gestational age	18 (6.7)	14 (5.2)	0.53
Quintero stage	3 (2-3)		
Recurrent TTTS after laser	2 (0.7)		
TAPS after laser	46 (17.2)		
Treatment failure*	48 (18.0)		

TAPS, twin anemia–polycythemia sequence.
Data are median (interquartile range), n (%), or mean ± standard deviation (range) unless otherwise specified.
* Treatment failure was defined as recurrent TTTS or TAPS after laser.

The incidence of severe cerebral lesions in the twin-twin transfusion syndrome group and control group was 8.6% (23/267) and 6.7% (18/267), respectively (p = 0.44). The majority of severe cerebral lesions in the twin-twin transfusion syndrome group were of antenatal origin. The incidence of antenatally acquired severe cerebral lesions in the twin-twin transfusion syndrome group and control group was 52.2% (12/23) and 16.7% (3/18), respectively (OR 8.00, 95% CI 1.42 to 45.06, p = 0.02). Details of the type of mild and severe cerebral lesions are presented in **table 2**.

In the twin-twin transfusion syndrome group, four neonates had a large parenchymal defect after an arterial stroke of the middle cerebral artery. All were ex-recipients and, in all cases, the stroke was localized in the left hemisphere (**figure 1**). None of the neonates in the control group had an arterial stroke.

Multiple logistic regression analysis was carried out to measure the independent associations between severe cerebral lesions and various possible risk factors in the twin-twin transfusion syndrome group (Quintero stage,

treatment failure [either recurrent twin-twin transfusion syndrome or twin anemia–polycythemia sequence], donor compared with recipient status, gestational age at birth, and treatment year). After multivariable analysis, lower gestational age at birth was the only factor independently associated with increased risk for severe cerebral lesions (OR 1.35 for each week less, 95% CI 1.14 to 1.59; p < 0.01) (**table 3**). The incidence of cerebral lesions decreased over the years, but the reduction did not reach statistical significance (OR 1.15, 95% CI .94 to 1.41, p = 0.16).

Table 2. Cerebral lesions detected by cranial ultrasound in the twin-twin transfusion group and the control group

	TTTS Group (n = 267)	Control Group (n = 267)	*p value*
Intraventricular hemorrhage grade III	10 (3.7)	10 (3.7)	0.95
Periventricular hemorrhagic infarction	9 (3.4)	7 (2.6)	0.63
PVL grade II	2 (0.7)	4 (1.5)	0.49
PVL grade III	5 (1.9)	3 (1.1)	0.81
Ventricular dilatation	8 (3.0)	7 (2.6)	0.77
Arterial stroke	4 (1.5)	0 (0.0)	0.12†
Other*	1 (0.4)	0 (0.0)	0.50†
Severe cerebral lesions	23 (8.6)	18 (6.7)	0.44
Antenatally acquired severe cerebral lesions	12/23 (52.2)	3/18 (16.7)	0.02

PVL, periventricular leukomalacia.
Data are n (%) or n/N (%) unless otherwise specified.
* Large venous hemorrhagic infarction of the right temporal lobe with parenchymal loss.
† Calculated with Fisher's exact test.

Table 3. Analysis of risk factors for severe cerebral injury in the twin-twin transfusion syndrome group

Characteristic	Cerebral Injury (n=23)	No Cerebral Injury (n=244)	Univariable OR (95% CI)	*p value*	Multivariable OR (95% CI)	*p value*
Gestational age at birth (wk)*	29.4 ± 3.1	31.9 ± 2.9	1.35 (1.15-1.59) for each week less	< 0.01	1.35 (1.14–1.59) for each week less	< 0.01
Stage 1	1 (4)	22 (9)	1			
Stage 2	8 (35)	82 (34)	2.17 (0.23–20.39)†	0.50	1.83 (0.21–16.07)	0.57
Stage 3	13 (57)	130 (53)	2.19 (0.24–19.61)†	0.48	1.74 (0.21–14.25)	0.60
Stage 4	1 (4)	10 (4)	2.45 (0.13–46.94)†	0.55	1.99 (0.10–37.87)	0.65
Treatment failure	6 (26)	42 (17)	1.69 (0.60–4.74)	0.32	1.34 (0.45–3.97)	0.60
Donor	12 (52)	122 (50)				
Recipient	11 (48)	122 (50)	0.93 (0.41–2.09)	0.86	0.92 (0.37–2.27)	0.86
Treatment year‡	2008 (2005-2010)	2009 (2007-2010)	1.19 (0.96–1.46)	0.12	1.15 (0.94–1.41)	0.16

OR, odds ratio; CI, confidence interval.

* Data are mean ± standard deviation or n (%) unless otherwise specified.

† Compared with the reference category = stage 1.

‡ Data are median (interquartile range).

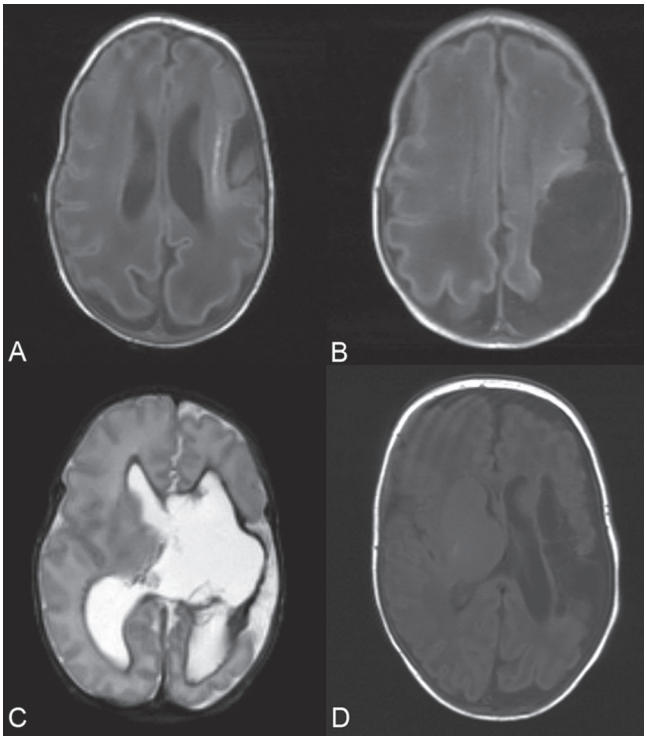


Figure 1. Four neonates from the twin-twin transfusion group with arterial ischemic stroke

A. Female neonate, ex-recipient, born at 29 weeks of gestation. T1-weighted magnetic resonance image (MRI) shows left-sided middle cerebral artery infarction with parenchymal destruction in the region of the left insula and operculum.
B. Female neonate, ex-recipient, born at 31 weeks of gestation. T1-weighted MRI shows left-sided middle cerebral artery infarction with extensive parenchymal destruction of the left hemisphere.
C. Male neonate, ex-recipient, born at 38 weeks of gestation. T2-weighted MRI shows a large parenchymal defect with a hemorrhagic component, contiguous with the lateral ventricle and involving both deeper and superficial structures of the left hemisphere. There was also severe parenchymal loss of both cerebellar hemispheres and vermis (not shown).
D. Female neonate, ex-recipient, born at 28 weeks of gestation. T1-weighted MRI shows left-sided middle cerebral artery infarction with parenchymal destruction of the temporal and parietal lobe and compensatory enlargement of the left lateral ventricle.

Discussion

In this study we compared the results of neonatal cranial ultrasonographic examination in 267 neonates with twin-twin transfusion syndrome treated with fetoscopic laser surgery with a matched control group of dichorionic twins and found the incidence of severe cerebral lesions to be similar in the two groups. These data suggest that twin-twin transfusion syndrome survivors after laser surgery are not at increased risk for cerebral injury compared with matched control participants.

The incidence of cerebral lesions reported in twin-twin transfusion syndrome survivors in our study was relatively low, 8.6%. In a previous study performed in the first cohort of twin-twin transfusion syndrome survivors born and examined at our center, the incidence of severe cerebral lesions in twin-twin transfusion syndrome survivors was 14.3%.(2) The methodology and definitions for severe cerebral lesions used in our studies remained the same during the last decade. One of the possible explanations for the lower incidence of cerebral injury detected in the present study could be related to the learning curve associated with improvement in laser surgery.(16) In this study, we detected a slight reduction in incidence of severe cerebral injury during the years. However, this difference did not reach statistical significance likely as a result of the relatively small numbers of patients.

Several large studies reported on the incidence of severe cerebral lesions in twin-twin transfusion syndrome treated with laser. In the study from Senat et al., the incidence of cerebral lesions seems to be lower (6%) compared with our study.(1) However, their definition of severe cerebral lesions was limited to the most severe form of leukomalacia (cystic periventricular leukomalacia grade 3 or greater) and intraventricular hemorrhage grade 3 or greater. In addition, other severe lesions such as arterial strokes or porencephalic cysts were not included in the definition, suggesting that the true incidence of severe cerebral lesions could be higher than reported. In addition, the denominator in the study from Senat et al. was the total number of fetuses instead of the total number of liveborn neonates in which a cranial ultrasonography was performed. The incidence of cystic periventricular leukomalacia (grade 3 or greater) in liveborn neonates was higher (8.6% [8 of 93]) compared with our findings (1.9% [5 of 267]).

In a recent study in 99 twin-twin transfusion syndrome survivors after laser surgery, Lenclen et al. reported a higher rate (16%) of cerebral lesions compared with our results.(3) However, they included only twin-twin transfusion syndrome survivors delivered before 34 weeks of gestation. Their studied cohort was therefore more premature and consequently more at risk for cerebral injury.

As shown in this study, lower gestational age at birth is the strongest predictor for cerebral damage in neonates. This is also in accordance with long-term outcome data in twin-twin transfusion syndrome showing that prematurity is the only independent predictor of adverse neurodevelopmental outcome.(14)

In another recent study in 143 twin-twin transfusion syndrome survivors from Cincotta et al., the authors reported an extremely low rate, only 2.8%, of severe cerebral abnormalities.(4) The discrepancies in reported rate may partly be the result of several methodological differences between the studies such as the use of different protocols for ultrasonographic scans, the criteria for cerebral injury, and the size of the various studies. Detection of cystic lesions is known to be less accurate when a restrictive scan regimen is used during the neonatal period. A restricted ultrasonographic regimen may lead to an underestimation of adverse outcome in twin-twin transfusion syndrome survivors because up to 33% of severe cerebral lesions may be missed.(17, 18)

Slightly more than half of severe cerebral lesions in the twin-twin transfusion syndrome group were present at birth, suggesting that the onset of brain injury in neonates with twin-twin transfusion syndrome is often of antenatal origin. The incidence of antenatally acquired cerebral lesions in the twin-twin transfusion syndrome group was eightfold higher than in the control group, which is accordance with our previous study.(2) The exact mechanism responsible for antenatal cerebral injury in twin-twin transfusion syndrome is not clear and may result from impaired cerebral perfusion resulting from hemodynamic imbalance and intertwin blood shifts. Impaired cerebral perfusion may then lead to hypoxic-ischemic insults and may occur before fetoscopic laser surgery. Previous studies have shown that severe cerebral injury in twin-twin transfusion syndrome may be detected before laser treatment.(19) Nevertheless, the timing of cerebral injury in twin-twin transfusion syndrome remains unclear and may occur before, during, or after fetoscopic laser surgery. This urgently requires further study, because more insight in the etiology of cerebral injury may lead to adaptation of treatment protocols, eg, earlier intervention in case of prelaser origin or altered technique if injury occurs during laser surgery.

Interestingly, we also detected rare cerebral lesions in the twin-twin transfusion syndrome group, such as perinatal arterial stroke. All arterial strokes occurred in ex-recipients and involved the left middle cerebral artery. Twin-twin transfusion syndrome has been shown to be the main risk factor for perinatal arterial strokes in preterm neonates.(20) The etiology of the focal ischemic stroke in the recipient is still obscure but could theoretically be related to sludging of polycythemic blood, hypoxia-ischemia, coagulation disorders, or all of these.(21)

The data on incidence of cerebral injury reported in this study should be interpreted with care because a selection bias may have been introduced as a result of the specific nature of our tertiary referral center. It is conceivable that the more complicated or more premature cases (particularly in the twin-twin transfusion syndrome group) may have been delivered at our center, whereas the less complicated or less premature cases were born elsewhere. However, by matching the twin-twin transfusion syndrome group with a control group of dichorionic twins, we eliminated the potential confounding bias resulting from prematurity.

In addition, although the predictive value of sequential cranial ultrasonography, magnetic resonance imaging, or both for detecting neurologic morbidity is increasing, the predictive accuracy of cerebral imaging remains controversial.(17) Neurodevelopmental impairment can only reliably be ascertained by adequate long-term evaluation up until childhood.

In conclusion, the risk of severe cerebral lesions in twin-twin transfusion syndrome treated with laser is similar to a matched control group of dichorionic twins. Cerebral injury in the twin-twin transfusion syndrome group is mainly of antenatal origin and is associated with premature delivery.

References

1. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine. 2004;351(2):136-44.
2. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2006; 194(5):1215-20.
3. Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, et al. Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. American journal of obstetrics and gynecology. 2007;196(5):450.e1-7.
4. Cincotta RB, Gray PH, Gardener G, Soong B, Chan FY. Selective fetoscopic laser ablation in 100 consecutive pregnancies with severe twin-twin transfusion syndrome. Aust N Z J Obstet Gynaecol. 2009;49(1):22-7.
5. van Klink JM, Koopman HM, Oepkes D, Walther FJ, Lopriore E. Long-term neurodevelopmental outcome in monochorionic twins after fetal therapy. Early human development. 2011;87(9):601-6.
6. Adegbite AL, Castille S, Ward S, Bajoria R. Prevalence of cranial scan abnormalities in preterm twins in relation to chorionicity and discordant birth weight. European journal of obstetrics, gynecology, and reproductive biology. 2005;119(1):47-55.
7. Denbow ML, Battin MR, Cowan F, Azzopardi D, Edwards AD, Fisk NM. Neonatal cranial ultrasonographic findings in preterm twins complicated by severe fetofetal transfusion syndrome. American journal of obstetrics and gynecology. 1998;178(3):479-83.
8. Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2007;20(8):589-97.
9. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 1999;19(8 Pt 1):550-5.
10. Volpe JJ. Intracranial hemorrhage: germinal matrix-intraventricular hemorrhage of the premature infant. In: Volpe JJ, editor. Neurology of the newborn. 4th ed. Philadelphia (PA): Saunders; 2001. p. 428-93.
11. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res. 1992;49(1):1-6.
12. Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. Arch Dis Child. 1981;56(12):900-4.
13. Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, et al. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal diagnosis and therapy. 2010;27(4):181-90.
14. Lopriore E, Ortibus E, Acosta-Rojas R, Le Cessie S, Middeldorp JM, Oepkes D, et al. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2009;113(2 Pt 1):361-6.
15. Liang KY ZS. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73:13-22.
16. Papanna R, Biau DJ, Mann LK, Johnson A, Moise KJ, Jr. Use of the Learning Curve-Cumulative Summation test for quantitative and individualized assessment of competency of a surgical procedure in obstetrics and gynecology: fetoscopic laser ablation as a model. American journal of obstetrics and gynecology. 2011;204(3):218.e1-9.
17. de Vries LS, van Haastert IC, Benders MJ, Groenendaal F. Myth: cerebral palsy cannot be predicted by neonatal brain imaging. Seminars in fetal & neonatal medicine. 2011;16(5):279-87.

18. De Vries LS, Van Haastert IL, Rademaker KJ, Koopman C, Groenendaal F. Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants. J Pediatr. 2004;144(6):815-20.
19. Banek CS, Hecher K, Hackeloer BJ, Bartmann P. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2003;188(4):876-80.
20. Benders MJ, Groenendaal F, Uiterwaal CS, de Vries LS. Perinatal arterial stroke in the preterm infant. Seminars in perinatology. 2008;32(5):344-9.
21. Miller V. Neonatal cerebral infarction. Semin Pediatr Neurol. 2000;7(4):278-88.

Chapter 3

Fetal and neonatal neuroimaging in twin-twin transfusion syndrome

Marjolijn S. Spruijt
Jeanine M.M. van Klink
Linda S. de Vries
Femke Slaghekke
Annemieke (J.M.) Middeldorp
Enrico Lopriore
Ratna N.G.B. Tan
Menno (J.P.) Toirkens
Sylke J. Steggerda

Ultrasound Obstet Gynecol. 2024 Jun;63(6):746-757

Abstract

Objectives

To describe the types of brain injury and subsequent neurodevelopmental outcome in fetuses and neonates from pregnancies with twin-twin transfusion syndrome (TTTS). Additionally, to determine risk factors for brain injury and to review the use of neuroimaging modalities in these cases.

Methods

This was a retrospective cohort study of consecutive TTTS pregnancies treated with laser surgery in a single fetal therapy center between January 2010 and January 2020. The primary outcome was the incidence of brain injury, classified into predefined groups. Secondary outcomes included adverse outcome (perinatal mortality or neurodevelopmental impairment (NDI)), risk factors for brain injury and the number of magnetic resonance imaging (MRI) scans.

Results

Cranial ultrasound was performed in all 466 TTTS pregnancies and 685/749 (91%) liveborn neonates. MRI was performed in 3% of pregnancies and 4% of neonates. Brain injury was diagnosed in 16/935 (2%) fetuses and 37/685 (5%) neonates and all predefined injury groups were represented. Four fetal and four neonatal cases of cerebellar hemorrhage were detected. Among those with brain injury, perinatal mortality occurred in 11/16 (69%) fetuses and 8/37 (22%) neonates. Follow-up was available for 29/34 (85%) long-term survivors with brain injury and the mean age at follow-up was 46 months. NDI was present in 9/29 (31%) survivors with brain injury. Adverse outcome occurred in 28/53 (53%) TTTS individuals with brain injury. The risk of brain injury was increased after recurrent TTTS/post-laser twin anemia polycythemia sequence (TAPS) (odds ratio (OR), 3.095, 95% CI 1.581 to 6.059, $p = 0.001$) and lower gestational age at birth (OR per 1-week decrease in gestational age, 1.381, 95% CI 1.238 to 1.541, $p < 0.001$).

Conclusions

Based on dedicated neurosonography and limited use of MRI, brain injury was diagnosed in 2% of fetuses and 5% of neonates with TTTS. Adverse outcome was seen in over half of cases with brain injury. Brain injury was related to recurrent TTTS/post-laser TAPS and a lower gestational age at birth.

Introduction

Twin-twin transfusion syndrome (TTTS) is a condition that results from intertwin transfusion through placental anastomoses in monochorionic twin pregnancy and puts both donor and recipient twins at risk for brain injury.(1, 2) Fetoscopic laser coagulation of placental anastomoses (FLC) is the first-line treatment for TTTS and reduces the risk of brain injury to 3- 16%.(3) Various pathophysiologic explanations for the occurrence of brain injury in TTTS have been suggested and include fetal hemodynamic instability, thromboembolism and anemia or polycythemia with vascular sludging.(4-7) Thromboembolism, due to TTTS itself or following fetal intervention, has been identified as an important pathway leading to focal brain injury, whereas symmetrical, diffuse injury is thought to result from hypoperfusion with or without fetal anemia.(6) In addition, preterm delivery is common after TTTS and exposes survivors to the risk of prematurity-related brain injury.(8)

While many previous studies have used cranial ultrasonography (cUS) to detect brain injury, recent work using prenatal and postnatal brain magnetic resonance imaging (MRI) have demonstrated the additional value of MRI over cUS alone.(9-17) The incidence of brain injury reported by these studies ranges widely and correlation of brain abnormalities on MRI with long-term outcome is lacking. The majority of recent studies report only on the outcomes of TTTS after treatment with laser surgery, as this is now the treatment of choice. However, it is important to realize that laser surgery is not always feasible and that TTTS fetuses not treated with FLC are at increased risk of adverse neonatal outcome, including brain injury.(3, 18)

Improving our understanding of the mechanisms that lead to brain injury in TTTS and their long-term consequences is important for counseling of future parents and may aid in the development of preventive measures based on pathophysiology. The aim of this study was to describe the types of fetal and neonatal brain injury and the long-term outcomes associated with these injuries in a large consecutive TTTS cohort. We also aimed to identify risk factors for brain injury and to review the use of neuroimaging modalities in TTTS cases in our center.

Methods

Study population

This was a retrospective cohort study of fetuses and neonates from consecutive twin and triplet pregnancies with TTTS treated with FLC at the Leiden University Medical Center (LUMC) between January 2010 and January 2020. The LUMC is the Dutch national referral center for fetal therapy. Triplets could be monochorionic or dichorionic, but only fetuses with TTTS were included in the study. After confirmation of TTTS using standard sonographic criteria, FLC was offered for Quintero stage 2 and higher.(19) Between 2011 and 2018, some patients with Quintero stage 1 TTTS were enrolled in a trial and were randomized to receive expectant management or FLC. For stage-1 patients outside the trial, therapy was individualized.(20) For this retrospective study, no formal ethical approval was required. The LUMC ethical review board waived the need for informed consent.

Neuroimaging

Standard prenatal ultrasonography included screening for fetal brain injury and was performed before laser surgery, 1 day and 1 week after laser surgery, and then continued at least once per fortnight. Fetal brain MRI was not part of standard care but was available at the discretion of the fetal therapy team. The fetal MRI protocol included T2-weighted sequences in the coronal, sagittal and transverse planes as well as susceptibility-weighted imaging (SWI), with the possibility of adding diffusion-weighted imaging (DWI). Postnatal cUS was recommended within 24 hours after birth for all TTTS neonates. Additional neonatal cUS scans were performed according to our previously described protocol.(21) Standard cUS images were made using the anterior and mastoid fontanelles.(22) Postnatal brain MRI images were retrieved from our neonatal intensive care unit (NICU) and that of four other Dutch centers. Indication for brain MRI was determined per individual case in our and three other centers, but MRI around term-equivalent age (TEA) was standard for all infants born at < 28 weeks in one center. MRI protocols differed between centers, but all included at least standard T1- and T2-weighted images as well as SWI.

Cerebral injury

Images from all fetal and neonatal MRI scans and all available cUS images were reviewed. Brain injury was categorized as either diffuse or focal injury and divided further into subgroups, as presented in **table 1**.

Table 1. Brain injury categories and groups

Category	Group	Injury
Diffuse	1	Cystic periventricular leukomalacia
	2	Multicystic or generalized encephalomalacia
	3	Migration or gyration disorders
	4	Ventriculomegaly and/or severe volume loss
Focal	5	Infarction
	6	Intraventricular hemorrhage (with or without post-hemorrhagic ventricular dilatation)
	7	Intraventricular hemorrhage with periventricular hemorrhagic infarction
	8	Cerebellar or cerebral parenchymal hemorrhage

Periventricular leukomalacia (PVL) was included in case of cystic white matter lesions (PVL ≥ grade 2).(23) Ventriculomegaly (VM) was defined as an atrial diameter ≥ 10 mm on fetal US as well as fetal and postnatal MRI, or a ventricular index above the 97th percentile according to Levene on neonatal cUS.(24) VM was termed post-hemorrhagic ventricular dilatation (PHVD) if preceded by intraventricular hemorrhage (IVH) ≥ grade 2, according to Volpe.(25-27) Infarction was diagnosed when cUS showed an area of increased echogenicity within an arterial territory, or MRI/DWI showed typical signal abnormalities in the acute stage, each with subsequent cyst formation. Periventricular hemorrhagic infarction (PVHI) was described according to Volpe.(27) Cerebellar hemorrhage (CBH) detected on postnatal cUS or MRI was subdivided as punctate, limited, or massive.(28) For all postnatal MRI scans performed at a postmenstrual age of between 36 and 46 weeks, the TEA scoring system developed by Kidokoro et al. was applied and included corrections for postmenstrual age for the measures of biparietal width, deep gray matter area and transcerebellar diameter.(25) The Kidokoro global brain abnormality score is the sum of several subscores and is classified as normal (0-3), mild (4-7), moderate (8-11) or severe (≥12). Severe volume loss (group 4) was diagnosed when the Kidokoro score was >3 based on reduced volume as represented by the subscores and without evidence of focal injury. Brain injury that was detected antenatally and confirmed postnatally was reported as fetal brain injury.

Follow-up

Standardized long-term follow-up was part of routine care and included visits at the ages of 2, 5 and 8 years. All follow-up visits consisted of a physical examination by a neonatologist and physiotherapist for the assessment of general health and neurological deficits, including cerebral palsy (CP). CP was classified according to the Gross Motor Function Classification System (GMFCS).(29) In addition, standardized tests were performed by trained professionals for the assessment of motor and cognitive development. At the 2-year visit, the Bayley Scales of Infant and Toddler Development were used. At the 5- and 8-year visits, cognitive functioning was assessed with the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and Wechsler Intelligence Scale for Children (WISC), respectively, whereas motor assessment was performed using the Movement Assessment Battery for Children (M-ABC). In specific cases, the Snijders-Oomen Nonverbal Intelligence Test (SON) was used for the assessment of cognitive functioning. For the purpose of this study, follow-up data from the latest known follow-up visit were retrieved for survivors with brain injury.

Outcome measures

The primary outcome was the incidence of fetal and neonatal brain injury, divided into predefined injury groups. Secondary outcomes were perinatal mortality, neurodevelopmental impairment (NDI), a composite called adverse outcome, risk factors associated with brain injury, Kidokoro scores for TEA MRI scans and the number of fetal and neonatal brain MRI scans. Adverse outcome was defined as perinatal mortality or NDI. Perinatal mortality was defined as fetal death or neonatal death within 28 days after birth. NDI was defined as CP, bilateral blindness or deafness, or cognitive or motor score on a standardized test more than 2 SD below the mean determined at the age of at least 2 years, corrected for prematurity. The following potential risk factors were studied: donor or recipient, TTTS stage, gestational age at FLC, recurrent TTTS after laser or post-laser twin anemia polycythemia sequence (TAPS)) and gestational age at birth.

Statistical analysis

Analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Data are reported as n (%), mean ± SD or median (interquartile range (IQR)), as appropriate. Statistical significance was defined as $p < 0.05$. Potential risk factors for brain injury were studied in a logistic regression analysis using Generalized Estimating Equations (GEE), since results within twin pairs are not independent. Results of the logistic regression are reported as odds ratios (OR) with 95% CI.

Results

We included 935 fetuses from 466 consecutive TTTS pregnancies managed at the LUMC during the study period. Characteristics of the included pregnancies are summarized in **table 2**. Mean ± SD gestational age at laser surgery was 20.0 ± 3.3 weeks. Mean ± SD gestational age at birth was 32.5 ± 3.4 weeks. There were 749/935 (80%) liveborn neonates and neonatal death occurred in 35/749 (5%); thus, the overall rate of perinatal survival was 76% (714/935 fetuses).

Table 2. Characteristics of 466 TTTS pregnancies (935 fetuses)

Twin pregnancies	456
Dichorionic triplet pregnancies*	7
Monochorionic triplet pregnancies*	3
Gestational age at laser surgery (weeks)	20.0 ± 3.3
Fetal death	109/935 (12)
Birth before viability	63/935 (7)
Selective fetal reduction/termination	14/935 (1)
Liveborn neonates	749/935 (80)
Neonatal death	35/749 (5)
Perinatal mortality†	221/935 (24)
Overall survival	714/935 (76)
Quintero stage	2 (1-4)
Stage 1	70/466 (15)
Stage 2	170/466 (36)
Stage 3	210/466 (45)
Stage 4	16/466 (3)
Gestational age at birth (weeks)	32.5 ± 3.4
Birth weight (g)	1736 ± 680
Delivery in LUMC	248/466 (53)

Data are given as n, mean ± SD, n/N (%) or median (range).
* For triplet pregnancy, only fetuses with TTTS were included
† Any cause of fetal death or neonatal death.
LUMC, Leiden University Medical Center.

Flowcharts summarizing the use of neuroimaging modalities in fetuses and neonates are presented in **figures 1a and 1b**, respectively. In all cases, both fetuses had normal intracranial ultrasound findings prior to laser surgery. For 64/749 (9%) liveborn neonates, of whom all were born in other hospitals, no postnatal neuroimaging results or reports could be identified. Consequently, neonatal neuroimaging results were available for 685/749 (91%) neonates. All 37 neonates with postnatally diagnosed brain injury had their first postnatal cUS scan within 24 hours after birth. This early scan showed brain injury in 12/37 (32%) neonates.

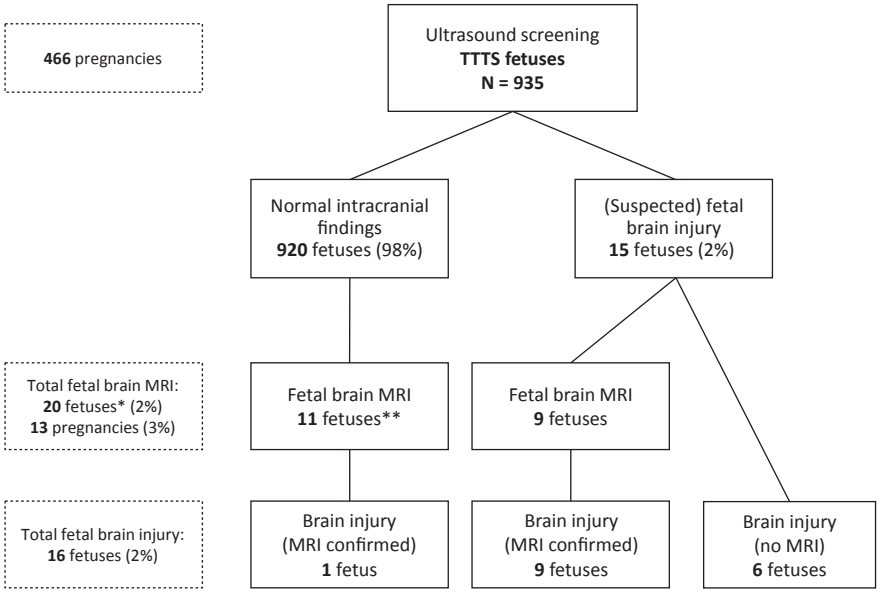


Figure 1a. Flowchart summarizing neuroimaging in fetuses from TTTS pregnancies.

† Reasons for fetal MRI: suspected skull anomaly (n=1; not confirmed on MRI); co-twin of fetus with indication for fetal MRI (n=6); incomplete laser surgery as reported by fetal surgeon (n=4).

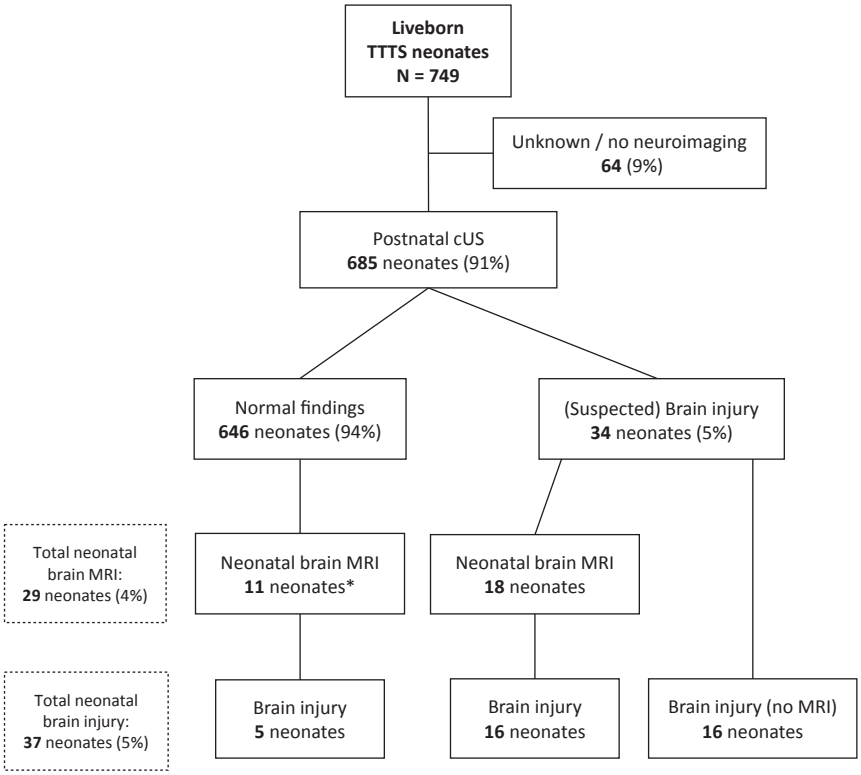


Figure 1b. Flowchart summarizing neuroimaging in neonates from TTTS pregnancies.

‡ Reasons for MRI at term-equivalent age: (extremely) preterm infant (n=10), incomplete laser surgery as reported by fetal surgeon (n=1). cUS, cranial ultrasound.

Primary outcome

Brain injury was diagnosed prenatally in 16/935 (2%) fetuses and postnatally in 37/685 (5%) neonates. The incidence of each predefined group of brain injury is presented in **table 3**. Injuries were diffuse (groups 1-4) in 9/16 (56%) fetal cases and 12/37 (32%) neonatal cases. The most common type of diffuse brain injury was VM or severe volume loss (group 4), occurring in 9/53 (17%) cases. Brain injury was focal (groups 5-8) in 7/16 (44%) fetal cases and 25/37 (68%) neonatal cases. The most common type of focal brain injury was IVH (groups 6 and 7), occurring in 18/53 (34%) cases.

Brain injury was confirmed after birth in 4/5 (80%) survivors diagnosed with fetal brain injury. In one case with a fetal diagnosis of grade 1 IVH, the postnatal cUS scan, performed 14 weeks after IVH was first seen, was normal. Details of the 16 fetuses in which cerebral injury was diagnosed prenatally are provided in **table 4**, arranged according to injury group. Details of the 37 neonates who were diagnosed with a brain injury postnatally are presented in **table 5**. Different brain injury groups visualized pre- and postnatally using different imaging modalities are depicted in **figure 2**.

Table 3. Incidence of predefined brain injury categories and groups

Brain injury group		Category	Fetal diagnosis (n=935)	Neonatal diagnosis (n=685)	Total
1	cPVL		3	3	6
2	Encephalomalacia		4	1	5
3	Migration/gyration disorder		0	1	1
4	VM / severe volume loss		2	7	9
		Diffuse	9	12	21 (40)
5	Infarction		1	3	4
6	IVH ± PHVD		2	8	10
7	IVH with PVHI		0	8	8
8	CBH / parenchymal hemorrhage		4	6	10
		Focal	7	25	32 (60)
		Total	16 (30)	37 (70)	53 (100)

Data are given as n or n (%)

Secondary outcomes

Perinatal mortality occurred in 11/16 (69%) fetuses and 8/37 (22%) neonates with brain injury, compared to 175/919 (19%) fetuses and 27/648 (4%) neonates without evidence of brain injury. An end-of-life decision (selective fetal reduction, termination of pregnancy or withdrawal of NICU treatment) was the cause of death in 9/11 (82%) cases of fetal brain injury and 8/8 (100%) cases of neonatal brain injury. Neurodevelopmental follow-up at the age of at least 2 years was available for 29/34 (85%) survivors with prior fetal or neonatal brain injury, and the mean age at follow-up was 46 months. NDI was present in 9/29 (31%) cases. None of the five children who survived after a fetal diagnosis of brain injury had NDI. These children had relatively mild forms

of brain injury: two had VM, two had grade 1 IVH and one had a unilateral CBH that did not appear to be massive on fetal or neonatal cUS. The details of their long-term follow-up are shown in **table 4**. All of the nine children with NDI had CP following postnatally diagnosed brain injury, with varying cognitive outcome (**table 5**). Overall, adverse outcome was present in 28/53 (53%) TTTS individuals with brain injury.

Results of the logistic regression analysis of potential risk factors for any brain injury are shown in **table 6**. Recurrent TTTS/post-laser TAPS (OR 3.095, 95% CI 1.581 to 6.059, $p = .001$) and lower gestational age at birth (OR per 1 week reduction in gestational age, 1.381, 95% CI 1.238 to 1.541, $p < .001$) were identified as significant risk factors for brain injury. A multivariate analysis could not be performed because gestational age at birth was assessed in a smaller group (only liveborn neonates). Mean ± SD gestational age at birth was 31.0 ± 3.5 weeks in liveborn neonates with recurrent TTTS/post-laser TAPS, compared to 32.7 ± 3.3 weeks in those without.

The Kidokoro score was assessed in 23 MRI scans obtained at a post-menstrual age of between 36 and 46 weeks. The overall median Kidokoro score was 5 (IQR, 2-9), indicating mild injury. Ten TEA MRI scans were made because of extreme prematurity after normal findings on repeated cUS examinations, and the median Kidokoro score in this subset was 3.5 (IQR, 1-5). In 11 infants who underwent MRI around TEA because of brain injury diagnosed on cUS, the median Kidokoro score was 9 (IQR, 5-11), indicating moderate injury.

Prenatal brain MRI was performed in 13/466 (3%) TTTS pregnancies. The indication for fetal MRI was brain abnormality on fetal neurosonography in nine fetuses (nine different pregnancies). The other fetal MRI scans were performed because of incomplete laser surgery (as reported by the fetal surgeon) in three pregnancies (four live fetuses) and a suspected fetal skull abnormality in one fetus. Fetal MRI confirmed sonographic abnormalities in all nine cases and identified new lesions in one fetus. Fetal MRI findings were followed by termination of pregnancy, selective reduction or palliative neonatal care in 5/13 (38%) cases.

Postnatal brain MRI was performed in 29/685 (4%) neonates. Suspected brain injury on cUS was the indication for MRI in 18/29 (62%) neonates. Brain injury was confirmed with MRI in 16/18 (89%) cases. In 10 infants born < 28 weeks' gestation without evidence of brain injury on prior cUS examinations, TEA MRI scans were made as a part of standard care, and five of these MRI scans identified new abnormalities. One revealed a massive unilateral CBH that had been missed on cUS, while the other four had VM and/or volume reduction that had been within the normal limits on earlier cUS examinations.

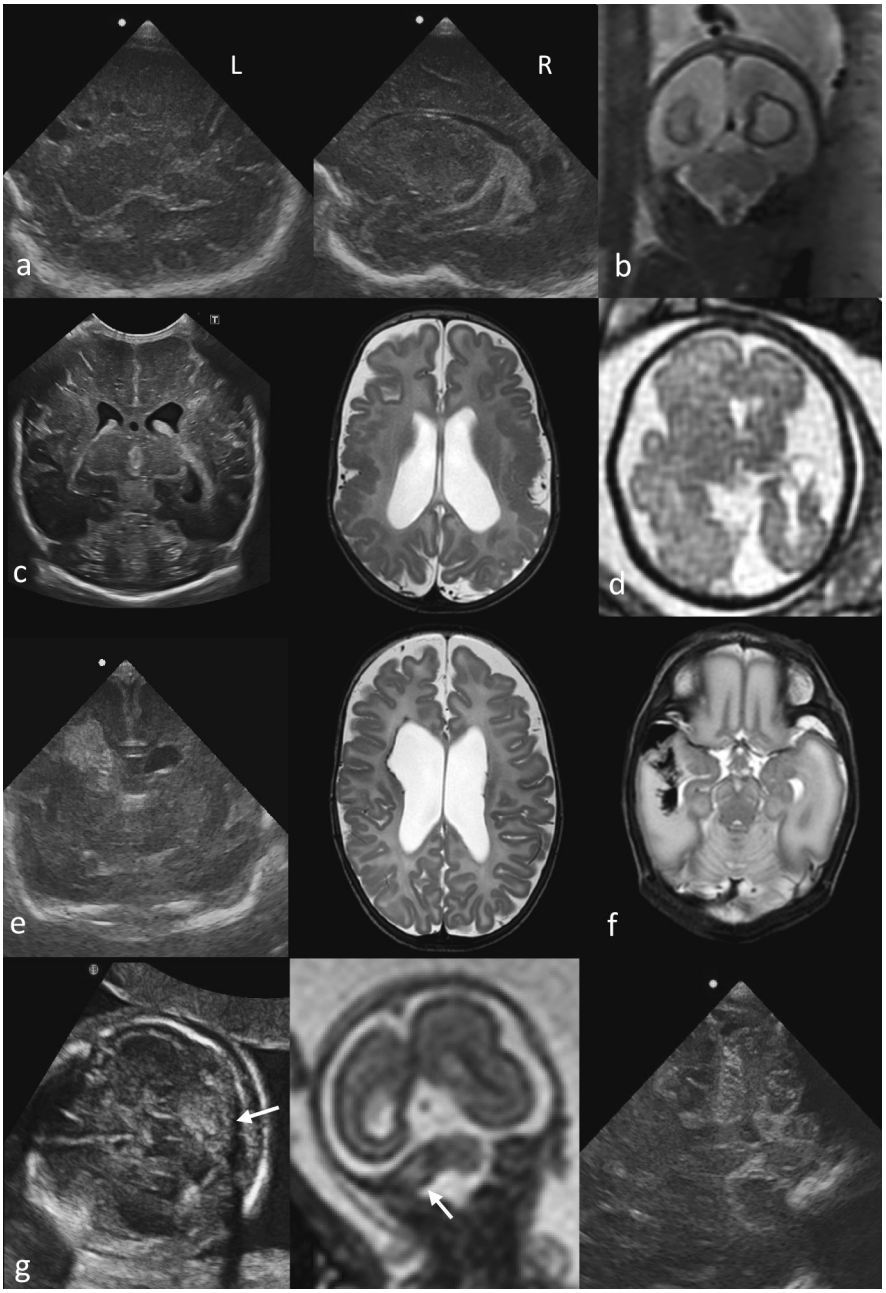


Figure 2. Different types of brain injury encountered in monochorionic twins with TTTS treated with fetoscopic laser coagulation.

a. Group 1 (periventricular leukomalacia): TTTS-recipient and subsequent donor in post-laser TAPS born at 32 weeks gestation. Postnatal cUS on day 1 with images in two parasagittal planes showing left frontal and right parieto-occipital cysts consistent with periventricular leukomalacia grade 3.
b. Group 2 (multicystic or generalized encephalomalacia): TTTS donor, fetal MRI at 26 weeks gestation, T2-weighted image in coronal plane showing near total cerebral atrophy.
c. Group 3 (migration or gyration disorders): TTTS donor, born at 31 weeks gestation. Postnatal cUS at 33 weeks (left) showing ventriculomegaly and bilateral suspected abnormal gyration around the insula. Transverse T2-weighted MR image at 45 weeks postmenstrual age (middle) showing perisylvian polymicrogyria, reduced white matter volume and ventriculomegaly.
d. Group 5 (infarction): TTTS recipient. Fetal T2-weighted MRI at 30 weeks' gestation in transverse plane showing large parenchymal defect in the territory of the left main cerebral artery.
e. Group 7 (intraventricular hemorrhage (IVH) with periventricular hemorrhagic infarction (PVHI)): TTTS donor born at 27 weeks gestation. Postnatal cUS on day 3 (left) showing right-sided IVH grade 3 with fan-shaped periventricular echogenicity, representing PVHI. T2-weighted transverse MRI at 45 weeks postmenstrual age (middle) showing right porencephalic cyst communicating with the lateral ventricle and bilateral posthemorrhagic ventricular dilatation.
f. Group 8 (cerebellar or cerebral parenchymal hemorrhage): TTTS recipient born at 30 weeks gestation. Postnatal transverse T2-weighted MRI at 31 weeks postmenstrual age, showing right temporal intraparenchymal hemorrhage.
g. Group 8 (cerebellar or cerebral parenchymal hemorrhage): TTTS donor. Fetal neurosonography (left) and fetal MRI (middle) around 23 weeks gestation, and postnatal cUS (right) at 33 weeks' gestation. Suspected right-sided fetal cerebellar hemorrhage (arrows), postnatal cUS showing the chronic stage with underdevelopment of right cerebellar hemisphere.

Withdrawal of NICU care occurred in one preterm infant after MRI had confirmed extensive cystic lesions seen on cUS. Care was withdrawn in six (extremely) preterm infants based on severe brain injury diagnosed with cUS alone. One infant underwent MRI because of incomplete laser surgery as reported by the fetal surgeon despite normal cUS findings, and the MRI was normal in this infant. None of the infants with a postnatal MRI report had previously undergone fetal MRI.

Table 4. Details of cases with prenatally diagnosed brain injury, according to injury group (n = 16)

Brain injury group	Donor/ recipient	Imaging	GA at FLC (weeks)	GA at Dx of brain injury (weeks)	Indication for MRI
1	Recipient	US	16	19	-
	Recipient	US	22	2	-
	Recipient	US	21	23	-
2	Donor	US, MRI	18	20	Abnormal fetal US
	Donor	US, MRI	23	25	Abnormal fetal US
	Donor	US, MRI	25	27	Incomplete FLC†, abnormal US
	Donor	US, MRI	16	24	Abnormal fetal US
4	Donor	US, MRI	18	20	Abnormal fetal US
	Donor	US, MRI	16	19	Abnormal fetal US
5	Recipient	US, MRI	23	28	Abnormal fetal US
6	Donor	US, MRI	23	27	Abnormal fetal US
	Donor	US, MRI	16	20	incomplete FLC†, sFD
8	Donor	US, MRI	20	21	Abnormal fetal US
	Donor	US	14	15	-
	Donor	US	19	23	-
	Recipient	US	18	23	-

*Results of Bayley test and Wechsler Intelligence Scale for Children (WISC) have mean of 100 and SD of 15: result of <70 is consistent with <-2SD. †As reported by fetal surgeon. GA: gestational age; MRI: magnetic resonance imaging; FLC: fetoscopic laser coagulation; cPVL: cystic periventricular leukomalacia; US: ultrasound; cUS: cranial ultrasound; PV: periventricular; sFD: single fetal demise; TOP: termination of pregnancy; VM: ventriculomegaly; WMI: white-matter injury; SFR: selective fetal reduction; Bayley-III:

Main findings at neuroimaging	Outcome/follow-up*
Bilateral frontal cystic PVL	sFD donor, TOP because of brain injury recipient
Progressive PV inhomogeneous echogenicity & VM	SFR
PV inhomogeneous echogenicity & VM, IVH	SFR
Cystic degeneration of almost complete right hemisphere, left VM	Died after palliative neonatal care (GA at birth 32 wks)
Progressive PV cystic lesions, followed by near total atrophy, brain stem & cerebellum spared	Died after palliative neonatal care (GA at birth26 wks)
Symmetrical, extensive frontoparietal cystic degeneration	Died after palliative neonatal care (GA at birth 30 wks)
Bilateral WMI with severe atrophy, VM	SFR of recipient at 19 weeks for recurrent TTTS, TOP donor after fetal brain MRI
Bilateral VM 13 mm	Birth at 36 wks, stable VM on neonatal cUS; Bayley-III at 2 years: cog 105, mot 92
Bilateral VM 11 mm	Birth at 36 weeks; stable VM on neonatal cUS; hydrocephalus, VP shunt at 2 years; BSID-II at 2 years: MDI 115, PDI unknown
Large parenchymal defect in left MCA territory	SFR
Bilateral grade 1 IVH	Birth at 29 weeks; PVL gr 1, no IVH on neonatal cUS; WISC at 8 years: TIQ 86, M-ABC p5
Unilateral grade 1 IVH	Birth at 34 weeks; normal postnatal cUS; Bayley-III at 2 years: cog 115, mot 89
Right-sided CBH	Birth at 33 weeks; underdeveloped right cerebellar hemisphere on neonatal cUS; Bayley-III at 2 years: cog 125, mot 100, normal v/h
CBH, symmetrical VM	sFD of recipient after FLC; TOP of donor for brain injury
CBH and IVH	Spontaneous sFD at 25 weeks
CBH	Spontaneous birth before viability

Bayley Scales of Infant and Toddler Development, third edition; cog: cognitive composite score of Bayley-III, mot: motor composite score of Bayley-III; VP: ventriculoperitoneal; BSID-II: Bayley Scales of Infant and Toddler Development, second edition; MDI: mental development index, PDI: psychomotor development index; IVH: intraventricular hemorrhage; TIQ: total intelligence quotient; M-ABC: Movement Assessment Battery for Children; CBH: cerebellar hemorrhage; v/h: vision/hearing; p5, 5th percentile.

Table 5. Details of cases with postnatally diagnosed brain injury, according to injury group (n=37)

Brain injury group	Donor/ recipient	Imaging	GA at FLC (weeks)	GA at birth (weeks)	BW (g)	GA at MRI (weeks)	Indication for MRI
1	Donor	cUS	20	29+4	1288	—	—
	Recipient	cUS	24	29+2	1400	—	—
	Recipient/ donor†	cUS	23	32+4	1635	—	—
2	Recipient/ donor†	cUS, MRI	21	27+4	980	28	Abnormal cUS
3	Donor	cUS, MRI	16	31+6	726	45	Abnormal cUS
4	Donor	cUS, MRI	18	30+5	625	33	Abnormal cUS
	Donor	cUS, MRI	21	26+6	865	40	Extreme prematurity
	Donor	cUS, MRI	25	26+6	890	46	Extreme prematurity
	Recipient	cUS, MRI	25	26+6	795	46	Extreme prematurity
	Donor	cUS, MRI	16	31+2	528	36	Abnormal cUS
	Donor	cUS, MRI	19	26+6	889	45	Extreme prematurity
	Donor	cUS, MRI	21	26+4	820	40	Abnormal cUS
5	Recipient	cUS, MRI	18	31+2	2084	38	Abnormal cUS
	Recipient	cUS, MRI	20	33+6	1740	34	Abnormal cUS
	Recipient	cUS, MRI	26	32+4	1263	33	Abnormal cUS
6	Recipient	cUS, MRI	20	25 + 4	625	43	Abnormal cUS
	Recipient	cUS, MRI	21	26+6	1075	40	Abnormal cUS
	Donor	cUS	17	25+4	800	—	—
	Donor	cUS	23	26+0	1070	—	—
	Recipient	cUS	17	25+4	880	—	—
	Donor	cUS	27	27+6	1190	—	—
	Recipient	cUS	16	28+2	1170	—	—
	Donor	cUS	17	26+2	840	—	—

Main findings at neuroimaging	Kidokoro global score	Outcome/follow-up*
Unilateral PVL Grade 2	—	8 years: USCP, GMFCS 1, WISC: TIQ 100
Unilateral PVL Grade 2, IVH Grade 1	—	2 years: USCP, GMFCS unknown, BSID-II: MDI 82
Bilateral PVL Grade 3	—	2 years: BSCP, GMFCS 1, BSID-II: MDI 100
Multiple cysts in basal ganglia and thalamus, extensive PWML, IVH, CBH	N/A	Died after withdrawal of care
Bilateral perisylvian PMG, markedly reduced WM volume with VM	15	2 years: BSCP, GMFCS 2 with general developmental delay, gastrostomy
Generalized cerebral atrophy	N/A	2 years: Bayley-III: cog 77, mot 75
Bilateral VM, reduced WM volume	5	6 years: WPPSI: TIQ 102, normal v/h
Bilateral VM, germinolytic cysts	5	8 years: WISC: TIQ 97, M-ABC p50
Bilateral VM, germinolytic cysts	6	8 years: WISC: TIQ 115, M-ABC p50
VM, severely reduced WM volume, two punctate CBH	11	1 year: motor delay and tubefeeding
Reduced WM volume	4	8 years: WISC: TIQ 97, M-ABC p75
Bilateral VM, unilateral IVH Grade 1	5	2 years: Bayley-III: cog 105, mot 87
Focal infarction thalamus just outside PLIC	3	2 years: Bayley-III: mot 76, cog incomplete
Cystic degeneration in left MCA territory	N/A	2 years: USCP GMFCS II, BSID-II: MDI 95
Bilateral caudate stroke, small IVH	N/A	Lost to follow-up
Unilateral IVH Grade 2, VM	5	2 years: Bayley-III: cog 77, mot 98
Bilateral IVH Grade 2, PHVD	6	6 years: WPPSI: TIQ 105, normal v/h
Bilateral IVH Grade 3, PHVD	—	Died after withdrawal of care
Bilateral IVH Grade 3	—	Died after withdrawal of care
Bilateral IVH Grade 3, PHVD	—	Discharged alive, lost to follow-up
Bilateral IVH Grade 2–3	—	2 years: Bayley-III: cog 105 mot 98
IVH Grade 3, PHVD	—	2 years: no CP, BSID-II: MDI 87
Unilateral IVH Grade 2, bilateral VM	—	Lost to follow-up

Table 5. Continued

Brain injury group	Donor/ recipient	Imaging	GA at FLC (weeks)	GA at birth (weeks)	BW (g)	GA at MRI (weeks)	Indication for MRI
7	Recipient	cUS	23	26+0	1053	—	—
	Donor	cUS	18	30+1	1693	—	—
	Donor	cUS, MRI	20	25+4	730	42	Abnormal cUS
	Donor	cUS, MRI	19	27+3	984	45	Abnormal cUS
	Recipient	cUS, MRI	19	27+3	890	45	Abnormal cUS
	Donor	cUS	21	25+3	910	—	—
	Recipient	cUS, MRI	17	29+2	1450	6 months	Abnormal cUS
	Recipient	cUS	21	26+0	701	—	—
8	Donor	cUS, MRI	16	24+3	630	42	Extreme prematurity
	Recipient	cUS, MRI	20	33+4	2020	38	Abnormal cUS
	Recipient	cUS, MRI	20	30+1	1589	31, 46	Abnormal cUS
	Recipient	cUS	18	25+6	705	—	—
	Recipient	cUS	26	27+2	987	—	—
	Recipient	cUS	21	26+4	880	—	—

*Results of Bayley, Wechsler Intelligence Scale for Children (WISC), Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and Snijders-Oomen Nonverbal Intelligence Test (SON) have mean of 100 and SD of 15: result of <70 is consistent with <-2SD. †Recipient in TTTS, donor in post-laser twin anemia polycythemia sequence and received intrauterine transfusion. GA: gestational age; FLC: fetoscopic laser coagulation; BW: birth weight; cUS: cranial ultrasound; CP: cerebral palsy; BSCP: bilateral spastic cerebral palsy; USCP: unilateral spastic cerebral palsy; GMFCS: Gross Motor Function Classification System; TIQ: total intelligence quotient;

Main findings at neuroimaging	Kidokoro global score	Outcome/follow-up*
Bilateral massive IVH and PVHI	—	Died after withdrawal of care
Bilateral IVH and PVHI, extensive cystic evolution in WM	—	Died after withdrawal of care
Bilateral IVH and PVHI, PHVD, developing porencephalic cyst	9	5 years: VP shunt, USCP, GMFCS 1, mild cognitive delay
Bilateral IVH, unilateral PVHI, PHVD, developing porencephalic cyst	10	5 years: WPPSI: TIQ 103. M-ABC p91, no asymmetry
Bilateral IVH, unilateral PVHI, PHVD, developing porencephalic cyst	10	5 years: USCP, GMFCS 1, WPPSI: TIQ 105
Bilateral IVH, unilateral PVHI developing porencephalic cyst	—	6 years: SON: TIQ 66, USCP, GMFCS I, ASS
Bilateral IVH, PHVD, unilateral PVHI, porencephalic cyst, interrupted PLIC	N/A	2 years: USCP, GMFCS I, BSID-II: MDI 100
Bilateral IVH, unilateral PVHI, contralateral cPVL	—	Died after withdrawal of care
Massive unilateral CBH, small IVH	7	2 years: Bayley-III cog 96, mot 95, normal v/h
Multiple CBH of limited size, small IVH, diffusely high WM signal	8	Discharged home in good condition, lost to follow-up
Right temporal hemorrhagic venous infarction, punctate CBH	12	5 years: WPPSI: TIQ 104 8 years: M-ABC p91
Massive bilateral CBH	—	Died after withdrawal of care
Hemorrhage right thalamus, small IVH	—	2 years: Bayley-III cog 101, mot 104
Bilateral large parenchymal hemorrhage	—	Died after withdrawal of care

BSID-II: Bayley Scales of Infant Development, second edition; MDI: Mental Developmental Index; N/A: not applicable; PMWL: punctate white-matter lesion; CBH: cerebellar hemorrhage; WM: white matter; Bayley-III: Bayley Scales of Infant and Toddler Development, third edition; cog: cognitive composite score of Bayley-III, mot: motor composite score of Bayley-III; M-ABC: Movement Assessment Battery for Children; PLIC: posterior limb of internal capsule; MCA: middle cerebral artery; VP: ventriculoperitoneal; ASS: autism spectrum disorder; v/h: vision/hearing; p50, 50th percentile.

Table 6. Potential risk factors for any brain injury

Risk factor	Brain injury (n=53)	No Brain injury (n=818)	Univariate OR (95%-CI)	p value
Donor	27/53 (51)	413/818 (51)	1.029 (0.627 - 1.689)	0.909
TTTS Stage	2 (2-3)	3 (2-3)	1.030 (0.681 - 1.557)	0.890
GA at laser*	20.4 ± 3.3	19.9 ± 3.3	0.956 (0.882 - 1.036)	0.273
Recurrent TTTS/ post-laser TAPS	14/53 (26)	85/818 (10)	3.095 (1.581 - 6.059)	0.001
GA at birth*†	29.1 ± 3.3	32.8 ± 3.2	1.381 (1.238 - 1.541)	<0.001

Data are given as n (%), median (interquartile range) or mean ± SD, unless specified otherwise.
*Per 1 week reduction in gestational age (GA).
†Analyzed only in liveborn neonates with known neuroimaging results (n=685).
OR, odds ratio; TAPS, twin anemia polycythemia sequence.

Discussion

Brain injury was detected in 2% of fetuses and 5% of neonates in this large consecutive TTTS cohort treated with FLC. Antenatally, diffuse types of brain injury were slightly more common, whereas postnatally over two thirds of cases had focal injury. Adverse outcome was present in the majority of cases with brain injury. Recurrent TTTS/post-laser TAPS and lower gestational age at birth were identified as risk factors for brain injury. Fetal and neonatal brain MRI were performed in 3% of pregnancies and 4% of survivors, respectively.

Previous fetal MRI studies in pregnancies with TTTS have reported an incidence of brain injury between 2 and 33%.(9, 14, 15, 17, 30, 31) In a recent systematic review that included MRI and ultrasound results, the incidence of fetal brain injury was 2%.(32) Two small studies performing neonatal brain MRI reported abnormalities in as many as 40% and 68% of TTTS infants.(16, 17) The relatively low incidence of brain injury in our study compared with studies that used MRI exclusively likely reflects the limitations of ultrasound, which may show only overt brain lesions. Since none of the previous MRI studies have reported on neurodevelopmental outcome, the true relevance of abnormalities seen only on MRI remains unknown. In the current study, NDI was present in 31% of TTTS survivors with brain injury, but follow-up data were not retrieved for children without brain injury. However, in a previous study on neurodevelopmental outcome at 2 years of age in TTTS survivors treated between 2011 and 2014, NDI was seen in 3% of all TTTS survivors and

brain injury was found to be predictive of NDI.(33) Even though the presence of brain injury increases the risk of NDI, the positive and negative predictive values of prenatal neuroimaging remain limited. In fact, none of the five survivors after fetal brain injury in this study met our criteria for NDI, although all had relatively mild forms of brain injury that may be associated with mild neurodevelopmental sequelae that manifest at a later age.

Long-term neurodevelopmental outcome depends on many different factors. Prematurity remains common after TTTS and is a well-known risk factor for brain injury as well as NDI. Our results confirm the increased risk of postnatal brain injury at a lower gestational age. However, based on our previous findings that early (<24 hours) postnatal cUS abnormalities are more common in TTTS compared with non-TTTS neonates, we assume that a portion of postnatally diagnosed brain injuries actually originated prenatally as the result of TTTS or fetal treatment.(21, 34) Moreover, in at least three cases with "postnatal" brain injury and subsequent CP (one with evidence of arterial infarction, one with cystic PVL and one with polymicrogyria), findings on early postnatal neuroimaging were consistent with injuries that originated during fetal life.

Although the types of brain injury described in this study are mostly comparable to the literature, some discrepancies stand out. A remarkable finding is the relatively frequent occurrence of CBH. One of the fetal CBH cases in this study was described previously in a small case series from our center of three fetuses that had undergone fetal therapy.(35) Besides that report, only Merhar et al. described one case of fetal CBH in TTTS.(17) Aside from fetal therapy, known risk factors for antenatal CBH are various causes of maternal hemodynamic disturbance, as well as fetal factors including coagulopathies and fetal anemia. In the newborn, CBH has been mostly attributed to preterm birth, impaired autoregulation of cerebral blood flow and other risk factors that compromise the cerebral circulation.(36, 37) Interestingly, two studies have reported an association between CBH and multiple gestation. (38, 39) In recent years, there has been an increased focus on the preterm cerebellum. Advances in neuroimaging, including cUS with mastoid fontanelle views, have increased the recognition of CBH.(40) Our data show that special attention should be paid to the posterior fossa when screening for brain injury in TTTS fetuses and neonates. CBH may have a significant impact on neuro-development and, therefore, should not be overlooked.(41, 42)

The brain injury groups in this study were adapted from Conte et al., who investigated fetal brain injury in survivors of monochorionic twin pregnancy complicated by single intrauterine death and proposed that focal brain injury was more common in TTTS because of thromboembolic phenomena.(6)

We found diffuse types of injury in 56% of prenatal cases, suggesting that hypoperfusion is at least an equally important mechanism leading to fetal brain injury in TTTS.

Recurrent TTTS/post-laser TAPS and lower gestational age were identified as risk factors for brain injury. We assume that persistence of TTTS poses a direct risk of fetal brain injury due to prolonged hemodynamic instability, whereas post-laser TAPS increases the risk by means of polycythemia or anemia. These results highlight the importance of ultrasonographic follow-up after FLC. Due to small numbers, we could not perform a reliable analysis of potential risk factors for prenatal and postnatal brain injury separately.

Strengths of our study include the detailed description of fetal and neonatal brain injuries found in TTTS cases, the reports of long-term follow-up and the large size of this cohort. Limitations include the retrospective design, the small number of MRI scans and the lack of a standardized protocol that indicates when to perform MRI. Because the majority of TTTS infants were born preterm, our data on postnatal brain injury should be interpreted with care, as prematurity is an important confounding factor.

Future research into brain injury in TTTS should include serial imaging, including fetal ultrasound and MRI and postnatal cUS and (TEA) MRI, with long-term follow-up. MRI studies should preferably use state-of-the-art techniques, including SWI, DWI and quantitative measures of brain volume and maturation, to enable the description of more subtle brain injuries and disturbed growth, supporting the counseling of future parents.(14, 30, 31, 43)

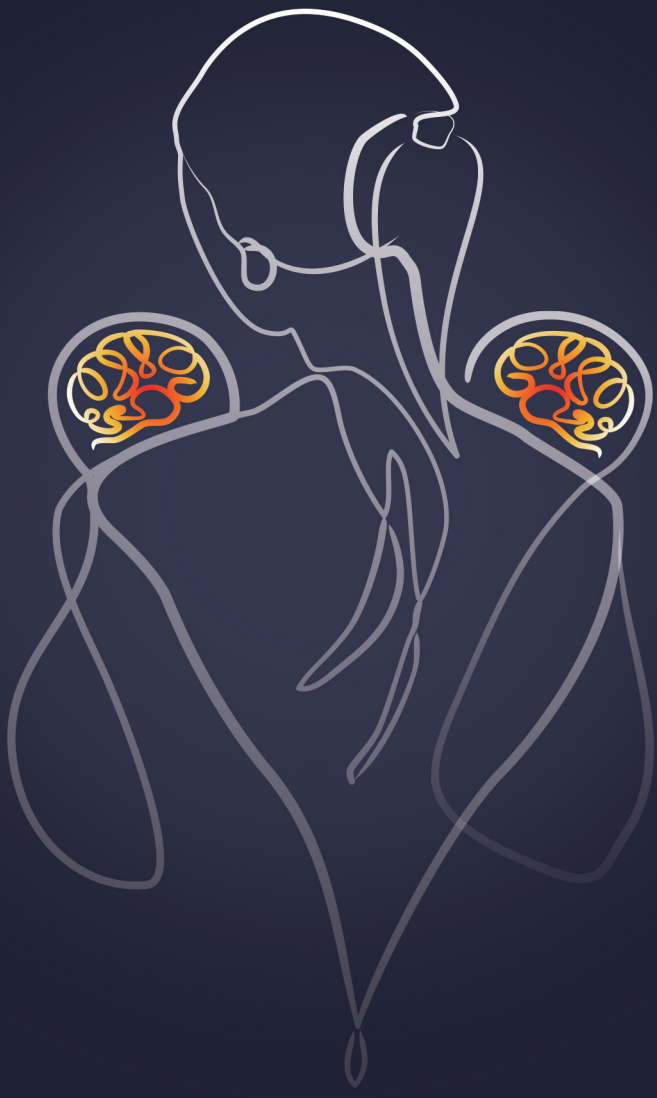
In conclusion, a wide range of brain injuries reflecting different patho-physiological mechanisms were encountered in TTTS fetuses and neonates treated with laser surgery. The incidence of brain injury was likely under-estimated due to the limited use of MRI. Adverse outcome was common after brain injury. Recurrent TTTS/post-laser TAPS and a lower gestational age at birth increased the risk of brain injury. The relatively frequent finding of CBH means that attention should be paid to the posterior fossa by clinicians taking care of women and children with TTTS.

References

1. Rossi AC, D'Addario V. Comparison of donor and recipient outcomes following laser therapy performed for twin-twin transfusion syndrome: a meta-analysis and review of literature. Am J Perinatol 2009; 26: 27-32.
2. Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Doné E, Boes AS, Hecher K, Gratacós E, Lewi P, Deprest J. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008; 199: 514.e511-518.
3. van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal Diagn Ther 2013; 33: 81-89.
4. Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. J Matern Fetal Neonatal Med 2007; 20: 589-597.
5. Larroche JC, Droulle P, Delezoide AL, Narcy F, Nessmann C. Brain damage in monozygous twins. Biol Neonate 1990; 57: 261-278.
6. Conte G, Righini A, Griffiths PD, Rustico M, Lanna M, Mackie FL, Pinelli L, Prefumo F, Persico N, Igra MS, Parazzini C, Doneda C, Fichera A, Ambrosi C, Kilby M, Severino M, Triulzi F, Rossi A, Skipper N. Brain-injured Survivors of Monochorionic Twin Pregnancies Complicated by Single Intrauterine Death: MR Findings in a Multicenter Study. Radiology 2018; 288: 582-590.
7. Denbow ML, Battin MR, Cowan F, Azzopardi D, Edwards AD, Fisk NM. Neonatal cranial ultrasonographic findings in preterm twins complicated by severe fetofetal transfusion syndrome. Am J Obstet Gynecol 1998; 178: 479-483.
8. Akkermans J, Peeters SH, Klumper FJ, Lopriore E, Middeldorp JM, Oepkes D. Twenty-Five Years of Fetoscopic Laser Coagulation in Twin-Twin Transfusion Syndrome: A Systematic Review. Fetal Diagn Ther 2015; 38: 241-253.
9. Aertsen M, Van Tieghem De Ten Berghe C, Deneckere S, Couck I, De Catte L, Lewi L. The prevalence of brain lesions after in utero surgery for twin-to-twin transfusion syndrome on third trimester MRI: a retrospective cohort study. Eur Radiol 2021; 31: 4097-4103.
10. Griffiths PD, Sharrack S, Chan KL, Bamfo J, Williams F, Kilby MD. Fetal brain injury in survivors of twin pregnancies complicated by demise of one twin as assessed by in utero MR imaging. Prenat Diagn 2015; 35: 583-591.
11. Jarvis D, Mooney C, Cohen J, Papaioannou D, Bradburn M, Sutton A, Griffiths PD. A systematic review and meta-analysis to determine the contribution of mr imaging to the diagnosis of foetal brain abnormalities In Utero. Eur Radiol 2017; 27: 2367-2380.
12. Kline-Fath BM, Calvo-Garcia MA, O'Hara SM, Crombleholme TM, Racadio JM. Twin-twin transfusion syndrome: cerebral ischemia is not the only fetal MR imaging finding. Pediatr Radiol 2007;37: 47-56.
13. Robinson A, Teoh M, Edwards A, Fahey M, Goergen S. Fetal brain injury in complicated monochorionic pregnancies: diagnostic yield of prenatal MRI following surveillance ultrasound and influence on prognostic counselling. Prenat Diagn 2017; 37: 611-627.
14. Hochberg A, Silber R, Avnet H, Rosen H, Katorza E, Hoffmann C, Mazkereth R, Lipitz S, Weisz B, Yinon Y. Fetal and neonatal brain lesions following laser ablation for twin-to-twin-transfusion syndrome as detected by pre- and post-natal brain imaging. Prenatal diagnosis 2021; 41: 1531-1540.
15. Stirnemann J, Chalouhi G, Essaoui M, Bahi-Buisson N, Sonigo P, Millischer AE, Lapillonne A, Guigue V, Salomon LJ, Ville Y. Fetal brain imaging following laser surgery in twin-to-twin surgery. BJOG 2018; 125: 1186-1191.
16. Boyle M, Lyons A, Ryan S, Malone F, Poran A. Postnatal MRI Brain in Infants Treated for Twin-Twin Transfusion Syndrome. Ir Med J 2015; 108: 240-243.

17. Merhar SL, Kline-Fath BM, Meinzen-Derr J, Schibler KR, Leach JL. Fetal and postnatal brain MRI in premature infants with twin-twin transfusion syndrome. J Perinatol 2013; 33: 112-118
18. Lopriore EA, Slaghekke F, Verweij EJ, Haak MC, Middeldorp AJM, Lopriore E. Neonatal Outcome in Twin-to-Twin Transfusion Syndrome Not Treated with Fetoscopic Laser Surgery. Twin Res Hum Genet 2022; 25: 45-49.
19. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. J Perinatol 1999; 19: 550-555.
20. Stirnemann J, Slaghekke F, Khalek N, Winer N, Johnson A, Lewi L, Massoud M, Bussieres L, Aegerter P, Hecher K, Senat MV, Ville Y. Intrauterine fetoscopic laser surgery versus expectant management in stage 1 twin-to-twin transfusion syndrome: an international randomized trial. Am J Obstet Gynecol 2021; 224: 528 e521-528 e512.
21. Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, Lopriore E. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstet Gynecol 2012; 120: 15-20.
22. Meijler GS, S.J. Neonatal Cranial Ultrasound (Third Edition edn). Springer Nature Switzerland AG: Switzerland, 2019.
23. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res 1992; 49: 1-6.
24. Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with realtime ultrasound. Arch Dis Child 1981; 56: 900-904.
25. Kidokoro H, Neil JJ, Inder TE. New MR imaging assessment tool to define brain abnormalities in very preterm infants at term. AJNR Am J Neuroradiol 2013; 34: 2208-2214.
26. Norton ME, Fox NS, Monteagudo A, Kuller JA, Craigo S. Fetal Ventriculomegaly. Am J Obstet Gynecol 2020; 223: B30-b33.
27. Volpe JJ. Volpe's neurology of the newborn (Sixth Edition edn). Elsevier: Philadelphia, PA, 2018.
28. Boswinkel V, Steggerda SJ, Fumagalli M, Parodi A, Ramenghi LA, Groenendaal F, Dudink J, Benders MN, Knol R, de Vries LS, van Wezel-Meijler G. The CHOPIn Study: a Multicenter Study on Cerebellar Hemorrhage and Outcome in Preterm Infants. Cerebellum 2019; 18: 989-998.
29. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol 1997;39: 214-223.
30. Kocaoglu M, Kline-Fath BM, Calvo-Garcia MA, Zhang B, Nagaraj UD. Magnetic resonance imaging of the fetal brain in monochorionic diamniotic twin gestation: correlation of cerebral injury with ultrasound staging and survival outcomes. Pediatr Radiol 2020; 50: 1131-1138.
31. Tarui T, Khwaja OS, Estroff JA, Robinson JN, Gregas MC, Grant PE. Altered fetal cerebral and cerebellar development in twin-twin transfusion syndrome. AJNR Am J Neuroradiol 2012; 33: 1121- 1126.
32. Sileo FG, Curado J, D'Antonio F, Benlioglu C, Khalil A. Incidence and outcome of prenatal brain abnormalities in twin-to-twin transfusion syndrome: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2022. DOI: 10.1002/uog.24895.
33. Spruijt MS, Lopriore E, Tan R, Slaghekke F, Klumper F, Middeldorp JM, Haak MC, Oepkes D, Rijken M, van Klink JMM. Long-Term Neurodevelopmental Outcome in Twin-to-Twin Transfusion Syndrome: Is there still Room for Improvement? J Clin Med 2019; 8.
34. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006; 194: 1215-1220.
35. Aziz NA, Peeters-Scholte CM, de Bruine FT, Klumper FJ, Adama van Scheltema PN, Lopriore E, Steggerda SJ. Fetal cerebellar hemorrhage: three cases with postnatal follow-up. Ultrasound Obstet Gynecol 2016; 47: 785-786.
36. Hayashi M, Poretti A, Gorra M, Farzin A, Graham EM, Huisman TA, Northington FJ. Prenatal cerebellar hemorrhage: fetal and postnatal neuroimaging findings and postnatal outcome. Pediatr Neurol 2015; 52: 529-534.

37. Limperopoulos C, Benson CB, Bassan H, Disalvo DN, Kinnamon DD, Moore M, Ringer SA, Volpe JJ, du Plessis AJ. Cerebellar hemorrhage in the preterm infant: ultrasonographic findings and risk factors. Pediatrics 2005; 116: 717-724.
38. Vesoulis ZA, Herco M, Mathur AM. Divergent risk factors for cerebellar and intraventricular hemorrhage. J Perinatol 2018; 38: 278-284.
39. Sehgal A, El-Naggar W, Glanc P, Asztalos E. Risk factors and ultrasonographic profile of posterior fossa hemorrhages in preterm infants. J Paediatr Child Health 2009; 45: 215-218.
40. Steggerda SJ, van Wezel-Meijler G. Cranial ultrasonography of the immature cerebellum: Role and limitations. Semin Fetal Neonatal Med 2016; 21: 295-304.
41. Limperopoulos C, Bassan H, Gauvreau K, Robertson RL, Jr., Sullivan NR, Benson CB, Avery L, Stewart J, Soul JS, Ringer SA, Volpe JJ, duPlessis AJ. Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? Pediatrics 2007; 120: 584-593.
42. Bodensteiner JB, Johnsen SD. Cerebellar injury in the extremely premature infant: newly recognized but relatively common outcome. J Child Neurol 2005; 20: 139-142.
43. Weisz B, Hoffmann C, Ben-Baruch S, Yinon Y, Gindes L, Katorza E, Shrim A, Bar Yosef O, Schiff E, Lipitz S. Early detection by diffusion-weighted sequence magnetic resonance imaging of severe brain lesions after fetoscopic laser coagulation for twin-twin transfusion syndrome. Ultrasound Obstet Gynecol 2014; 44: 44-49.



Part FOUR

Neurodevelopmental outcome

Chapter 4

Long-term neurodevelopmental outcome in twin-twin transfusion syndrome: Is there still room for improvement?

Marjolijn S. Spruijt
Enrico Lopriore
Ratna N.G.B. Tan
Femke Slaghekke
Frans J.C.M. Klumpe
Annemieke (J.M.) Middeldorp
Monique C. Haak
Dick Oepkes
Monique Rijken
Jeanine M.M. van Klink

Journal of Clinical Medicine 2019:8:1226

Abstract

Despite many developments in its management, twin-to-twin transfusion syndrome (TTTS) remains an important risk factor for long-term neurodevelopmental impairment (NDI). Our objective was to compare the incidence of severe NDI in a recent cohort of TTTS survivors, treated with laser surgery from 2011 to 2014, with a previous cohort treated from 2008 to 2010. Neurological, cognitive, and motor development were assessed at two years of age. We determined risk factors associated with Bayley-III scores. Severe NDI occurred in 7/241 (3%) survivors in the new cohort compared to 10/169 (6%) in the previous cohort ($p = 0.189$). Disease-free survival (survival without severe impairment) did not significantly differ. Low birth weight and being small for gestational age (SGA) were independently associated with lower cognitive scores (both $p < 0.01$). Severe cerebral injury was related to decreased motor scores ($B = -14.10$; 95% CI -25.04 to -3.16; $p = 0.012$). Children with severe NDI were born ≥32 weeks' gestation in 53% of cases and had no evidence of cerebral injury on cranial ultrasound in 59% of cases. Our results suggest that improvement in outcome of TTTS has reached a plateau. Low birth weight, SGA, and cerebral injury are risk factors for poor neurodevelopmental outcome. Neither gestational age above 32 weeks nor the absence of cerebral injury preclude severe NDI.

Introduction

Monochorionic twin pregnancies complicated by twin-to-twin transfusion syndrome (TTTS) carry a high risk of fetal and neonatal complications, including death and long-term neurodevelopmental impairment (NDI).(1, 2) Fetoscopic laser coagulation of the causative placental vascular anastomoses has proven to be superior to serial amnioreduction in the treatment of TTTS.(3-7) Laser surgery has been the standard treatment for TTTS in the Leiden University Medical Center (LUMC) since 2000. Because of the invasive nature of the treatment for this high-risk disease, we have since then advocated that long-term follow-up of surviving infants should be an integral part of any fetal therapy program.

In a previous study, we showed a marked improvement in overall survival in TTTS treated at our center, from 70% in a cohort of pregnancies treated in the first six years of our laser surgery program (2000–2005), to 80% in a cohort treated between 2008 and 2010. Improved survival was associated with a decrease in severe NDI from 18% to 6%.(8, 9) This may be explained by the growing experience of our fetal therapy team, leading to improved monitoring and management, as well as a learning-curve effect of the highly technical fetoscopic laser procedure.(10) Other authors have also suggested that there is a decreasing trend in the incidence of severe NDI in more recent studies.(11)

The purpose of the current study was to evaluate if neurodevelopmental outcome has continued to improve over time. We assessed neurodevelopment in TTTS survivors treated with laser surgery at our center between 2011 and 2014 and compared these results with a previous cohort treated between 2008 and 2010. In addition, we aimed to identify predictors for adverse neurodevelopmental outcome after laser treatment for TTTS using follow-up data from all children treated between 2008 and 2014.

Experimental section

The LUMC is the national referral center for TTTS in the Netherlands. For this study, we included all consecutive TTTS pregnancies treated with laser surgery in the LUMC between 1 January 2011 and 31 December 2014 (new cohort) and compared the neurodevelopmental outcome of surviving children with an earlier cohort, treated between 1 January 2008 and 31 December 2010 (previous cohort). The 2-year outcome of a large part of this 2008 to 2010 cohort has been published previously.(9) TTTS diagnosis and staging were determined with ultrasound criteria according to Quintero.(12, 13) During the

two study periods, the selective laser technique and the Solomon technique, which was studied in the Solomon trial that ran from March 2008 until July 2012, were used for the treatment of TTTS.(14) After conclusion of the trial, the Solomon technique became the standard technique for all laser procedures.

Spontaneous monoamniotic pregnancies, children with major congenital anomalies, and children with other causes of severe developmental delay unrelated to TTTS were excluded from this study. For each TTTS pregnancy, we recorded the following data from the medical records: gestational age (GA) at laser surgery, TTTS stage, fetal demise, delivery <24 weeks gestation, selective cord coagulation after laser therapy, occurrence of twin anemia polycythemia sequence (TAPS) after laser, and recurrence of TTTS after laser. Fetal demise included post-laser demise and death due to birth before 24 weeks gestation, the legal limit for neonatal resuscitation in the Netherlands. For each neonate, the following data were recorded: former donor or recipient status, gender, GA at birth, birth weight, whether or not small for gestational age (SGA, defined as birth weight below the 10th percentile), presence of severe cerebral injury, and neonatal death. TAPS was diagnosed according to antenatal and/or postnatal criteria.(15) Severe cerebral injury was defined as the presence of at least one of the following findings on cerebral ultrasound: intraventricular hemorrhage (IVH) ≥ grade III, periventricular leukomalacia (PVL) ≥ grade II, ventricular dilatation ≥97th percentile, and porencephalic cyst, arterial infarction, venous hemorrhagic infarction, or other severe cerebral lesions associated with adverse neurological outcome.(16) Neonatal cranial ultrasonography was performed by experienced neonatologists in accordance with our clinical protocol, as described in detail in our earlier studies.(17) Our protocol states that TTTS is an indication for at least one cranial ultrasound in the first week of life, independent of GA at birth. Neonatal death was defined as the death of a neonate born at or after 24 weeks 0 days of gestation, within 28 days after birth.

All TTTS survivors were invited for follow-up assessment at two years of age, corrected for prematurity. This visit consisted of a neurological and physical examination by a pediatrician and physiotherapist. When cerebral palsy (CP) was diagnosed, it was defined according to the European CP Network and classified using the Gross Motor Function Classification System (GMFCS).(18) Additionally, cognitive and motor development were evaluated by a child psychologist using the Bayley Scales of Infant and Toddler Development-third edition (Bayley-III).(19) The results of the Bayley-III are expressed as cognitive and motor composite scores. Each of these scores has a normal distribution with a mean of 100 and standard deviation of 15. Until the Dutch version (Bayley-III-NL) became available in 2015, children were

tested with the United States (US) version. The Bayley-III-NL is the translated and slightly adapted version of the original Bayley-III. For this study, the raw US test results were re-evaluated according to Dutch norms, which resulted in slightly different motor and cognitive composite scores. These 'Dutch' composite scores were used for all analyses in this study.

We determined our primary outcome, called severe neurodevelopmental impairment (NDI), as any of the following: CP GMFCS grade >1, cognitive or motor composite score <70, bilateral blindness or bilateral deafness requiring amplification. The primary outcome was determined in children when at least the visit with the physician and physiotherapist and the cognitive subtest of the Bayley-III were completed.

For this study, we determined the rate of disease-free survival, defined as the number of children who were free of NDI at two years adjusted age divided by the total number of included fetuses for whom the outcome was known, that is, either fetal/neonatal death or the presence or absence of severe NDI. Severe NDI and disease-free survival were compared between the two cohorts. For the recent cohort, we also evaluated the incidence of mild NDI, defined as either CP GMFCS grade 1, or Bayley-III cognitive or motor composite score ≥70 and <85.

The long-term outcome data from both cohorts together were analyzed to determine which risk factors influence cognitive and motor scores of the Bayley-III.

No formal ethical approval was required for this anonymized retrospective study. The institutional review board of the LUMC reviewed the study protocol and declared a statement of no objection.

Statistical analysis

Results are reported as percentages, mean and SD or median and range, as appropriate. A p value below 0.05 was considered to be statistically significant. Analyses were performed using generalized estimating equations (GEE), to account for the fact that results are not independent within twin pairs. The relationship between potential risk factors with Bayley-III scores was investigated with a linear regression analysis using the GEE approach. The following risk factors were studied: former donor or recipient status, TTTS stage, GA at laser, incomplete laser (either TAPS or recurrent TTTS after laser), fetal demise of the co-twin, GA at birth, birth weight, SGA, and severe cerebral injury. Significant factors in the univariate analysis were included in a multivariate model to estimate the independent effects. Results of the GEE risk factor analysis are expressed as regression coefficient B with 95% confidence interval (95% CI). Our data were analyzed using IBM SPSS Statistics, Version 23.0; Chicago, IL, USA.

Results

Perinatal characteristics of included TTTS pregnancies

Between January 2011 and December 2014, all consecutive 204 TTTS pregnancies treated with laser at the LUMC were included in this follow-up study. We compared the outcome of this new cohort with 116 TTTS pregnancies from the previous cohort treated between 2008 and 2010. This adds up to a total of 320 TTTS pregnancies, or 640 fetuses, treated with laser surgery in seven years' time. A flow chart showing the total study population divided into the two cohorts is depicted in **figure 1**.

There were no significant differences in any of the perinatal characteristics between the two cohorts. Fetoscopic laser surgery took place at a mean GA of 19.6 ± 3.3 weeks in our most recent cohort. Median Quintero stage at time of treatment was 3 in both cohorts. Post-laser TAPS and recurrent TTTS were seen in 11% and 10% of pregnancies in the two cohorts, respectively. The incidences of fetal demise in the new and previous cohort were 19% and 15%, respectively (p = 0.163). For our most recent cohort, we determined the type of fetal demise. In total, fetal demise occurred in 79/408 (19%) of cases, of which 39 single and 40 double fetal demises (i.e., 20 twin pairs). In 52/79 (66%) cases, fetal demise occurred in utero after laser therapy. In 12 pregnancies, or 24/79 fetuses (30%), spontaneous delivery took place before 24 weeks of gestation, which is the legal limit for active neonatal resuscitation in the Netherlands. Mean GA at delivery in these pregnancies was 20.8 weeks (range 17–23 weeks). The remaining 3/79 (4%) died after selective cord coagulation because of complications detected after laser surgery (one with severe cerebral injury, one with severe growth restriction, and one with TAPS after incomplete laser). Mean GA at live birth was 32.4 weeks and mean birth weight 1742 grams in the new cohort, both similar to the previous cohort. The incidences of severe cerebral injury were 6% in the new cohort and 7% in the previous one. The rate of neonatal death also remained stable over time and occurred in 6% (new cohort) versus 5% (previous cohort). Overall, 309/408 (76%) of treated TTTS fetuses survived to at least 28 days after birth, which is not significantly different from the previous cohort (81%, p = 0.159).

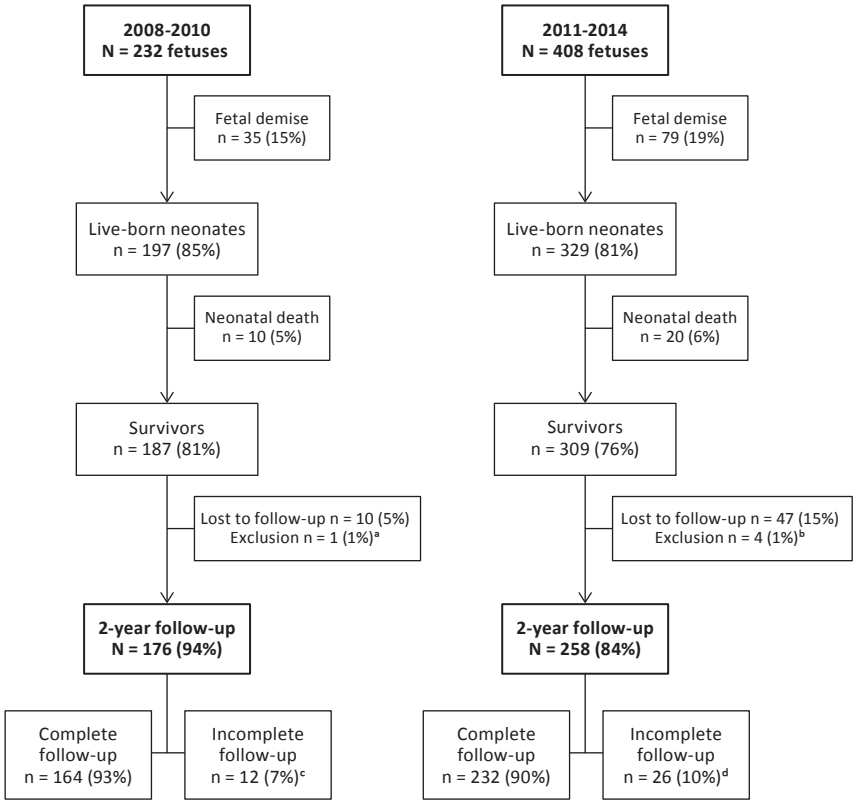


Figure 1. Flow chart of study population.

[a] infantile Tay-Sachs disease; [b] n = 2: neurofibromatosis type 1, n = 2: familial severe hearing loss; [c] all 176 children were assessed by a pediatrician and physiotherapist; n = 5: only cognitive subtest of Bayley-III completed, no motor score; n = 7: neither cognitive nor motor subtests of the Bayley-III completed; [d] all 258 children were assessed by a pediatrician and physiotherapist; n = 8: only cognitive subtest of Bayley-III completed, no motor score; n = 2: only motor subtests of Bayley-III completed, no cognitive score; n = 16: neither cognitive nor motor subtests of the Bayley-III completed.

Table 1. Baseline characteristics of long-term TTTS survivors included for follow-up

	Previous cohort: 2008-2010 n = 176	New cohort: 2011-2014 n = 258	p value
Donor	90 (51)	130 (50)	0.685
Gestational age at laser (weeks)	20.3 ± 3.4	20.0 ± 3.4	0.663
TTTS stage - median (range)	3 (1-4)	3 (1-4)	0.515
I	24 (14)	35 (14)	
II	48 (27)	89 (34)	
III	102 (58)	127 (49)	
IV	2 (1)	7 (3)	
Post-laser TAPS or recurrent TTTS	24/176 (14)	31/258 (12)	0.788
Fetal demise of co-twin	18 (10)	29 (11)	0.771
Gestational age at birth (weeks)	32.4 ± 3.4	32.9 ± 3.1	0.516
>37 weeks	16 (9)	21 (8)	
33-36 weeks	74 (42)	116 (45)	
26-32 weeks	86 (49)	112 (43)	
24-25 weeks	0	9 (3)	
Birth weight (grams)	1771 ± 596	1826 ± 610	0.717
Small for gestational age[a]	16 (9)	22 (9)	0.835
Severe cerebral injury[b]	9 (5)	11 (4)	0.681
Female	95 (54)	127 (49)	0.353

Data are presented as n (%), mean ± standard deviation or median (range).

[a] Birth weight <p10 for gestational age according to the Netherlands Perinatal Registry (PRN) 2007.

[b] Severe cerebral injury: at least one of the following findings on cerebral ultrasound: intraventricular hemorrhage (IVH) ≥ grade III, periventricular leukomalacia (PVL) ≥ grade II, ventricular dilatation ≥97th percentile, porencephalic cyst, arterial infarction, venous hemorrhagic infarction, or other severe cerebral lesions associated with adverse neurological outcome.

Long-term follow-up

In our most recent cohort, 47/309 (15%) survivors were lost to follow-up. Four survivors were excluded from analysis of long-term outcome because of other causes of neurodevelopmental delay. One twin pair was diagnosed with neurofibromatosis type 1. Another twin pair had severe, familial, bilateral hearing loss, and a genetic cause was suspected. Follow-up data were available for the remaining 258/309 (84%) survivors. **Table 1** shows the baseline characteristics of all TTTS survivors included for follow-up. No differences between the cohorts were found.

The perinatal characteristics of the included versus the lost-to-follow-up group in our most recent cohort were compared. Mean GA at birth in the follow-up group was higher compared to the lost-to-follow-up group (32.9 weeks vs. 31.7 weeks, p = 0.039). For all other characteristics, the groups were similar.

Neurodevelopmental outcome

The results of the follow-up assessments at the corrected age of two years are presented in **table 2**. The incidences of severe NDI, CP, and disease-free survival were not significantly different in the new compared to the previous cohort. There was no difference in mean Bayley-III cognitive scores between the new and previous cohort, whereas mean motor composite score in the new cohort was significantly lower compared to the previous cohort.

In the new cohort, follow-up was complete in 232/258 (90%) children. Out of 258 children, 8 completed only the cognitive subtest and 2/258 children only the motor subtests of the Bayley-III. The presence or absence of severe NDI could be determined in 241 children: 240 children who completed at least the cognitive Bayley-III, and 1 child without a Bayley-III test who was bilaterally deaf. In the previous cohort, follow-up was complete in 164/176 (93%) children, and in 5/176 children, only the cognitive subtest of the Bayley-III was completed. The presence or absence of severe NDI could be determined in these 169 children.

For all children in the previous cohort and 142/242 children in the new cohort, the original Bayley-III was used, and the raw scores were re-evaluated according to the Dutch norms for this study. For the other 100/242 children in the new cohort, the Bayley-III-NL was used.

We did not observe blindness in any of our follow-up patients. Bilateral deafness was present in one former recipient (0.4%) in our most recent cohort. Mild NDI was assessed in the new cohort and was present in 53/232 (23%) children with a complete Bayley-III. A cognitive score between 70 and 84 was found in 28/240 (12%). A motor score between 70 and 84 was found in 47/234 (20%).

Table 2. Neurodevelopmental impairment in survivors included for follow-up

	Previous cohort: 2008-2010 n = 176	New cohort: 2011-2014 n = 258	p value
Severe NDI[a]	10/169 (6)	7/241 (3)	0.189
Disease-free survival[b]	162/215 (75)	234/340 (68)	0.263
Cerebral Palsy Cerebral Palsy grade I Cerebral Palsy grade II-V	5/176 (3) 1 (1) 4 (2)	4/258 (2) 3 (1) 1 (0.4)	0.356
Cognitive composite score	101 ± 14	99 ± 13	0.220
Cognitive composite score < -2SD	5/169 (3)	1/240 (0.4)	0.097
Motor composite score	102 ± 15	97 ± 14	0.003
Motor composite score < -2SD	5/164 (3)	6/234 (3)	0.723

N, number; SD, standard deviation. Data are expressed as n/N (%) or mean ± SD

[a] Severe NDI included any of the following: Cerebral Palsy GMFCS > I, cognitive development <-2SD, motor development <-2SD, bilateral deafness or blindness.

[b] Disease-free survival: the number of children without NDI at follow-up divided by the total number of included fetuses for whom the outcome was known (outcome either fetal/neonatal death or the presence or absence of severe NDI)

Of the total of 17 children with severe NDI, 9 (53%) were born at gestational ages ≥ 32 0/7 weeks, and 10 (59%) had no evidence of severe cerebral injury on cranial ultrasound performed during the neonatal period. Six children (35%) fell into both categories (i.e., they were >32 weeks without evidence of severe cerebral damage).

Risk factors

Results of the univariate linear regression analysis of potential risk factors for all survivors from the two cohorts taken together are shown in **table 3**. It shows a significant association between birth weight and cognitive scores: for each 100 grams increase in birth weight, cognitive scores increased with 0.41 point (B 0.41; 95% CI 0.18 - 0.64, p = 0.000). We found a trend towards an association between higher GA at birth and higher cognitive scores (B 0.49; 95% CI -0.01 to 0.99, p = 0.056). There was a strong positive correlation between GA at birth and birth weight (r = 0.86; p = 0.01). In addition, children who were SGA had significantly lower cognitive scores than children with birth weights above the 10th percentile for GA (B -5.67; 95% CI -9.35 to -1.99, p = 0.003). In a multivariate analysis, both birth weight (B 0.34; 95% CI 0.11–0.57, p = 0.004)

Table 3. Association of potential risk factors with cognitive and motor composite scores

Risk factor	Cognitive composite score *Univariate*		Motor composite score *Univariate*	
	B (95% CI)	p value	B (95% CI)	p value
Donor	-1.10 (-2.46 – 0.27)	0.115	1.29 (-0.67 – 3.25)	0.196
TTTS stage	0.10 (-2.108 – 2.30)	0.928	0.51 (-1.64 – 2.66)	0.642
GA at laser	-0.27 (-0.77 – 0.23)	0.291	-0.13 (-0.66 – 0.40)	0.634
Incomplete laser	-2.96 (-8.20 – 2.28)	0.268	-4.65 (-9.69 – 0.39)	0.071
Fetal demise co-twin	3.17 (-1.02 – 7.36)	0.138	1.89 (-2.71 – 6.48)	0.421
GA at birth	0.49 (-0.01 – 0.99)	0.056	-0.10 (-0.64 – 0.43)	0.707
Birth weight[a]	0.41 (0.18 – 0.64)	0.000	-0.79 (-0.32 – 0.16)	0.521
Growth restriction[b]	-5.67 (-9.35 - -1.99)	0.003	-0.47 (-4.52 – 3.58)	0.822
Severe cerebral injury	-2.11 (-8.96 – 4.73)	0.545	-14.10 (-25.04 - -3.16)	0.012

B, regression coefficient; CI, confidence interval; GA, gestational age

[a] Birth weight in grams/100

[b] Birth weight < p10 for gestational age according to the Netherlands Perinatal Registry (PRN) 2007

and SGA (B -4.28; 95% CI -7.91 to -0.65, p = 0.021) remained independently associated with cognitive scores (not shown in table). For motor scores, univariate analysis revealed a significant negative association with severe cerebral injury, leading to motor scores that were 14.10 points lower in children with severe cerebral injury compared to children without severe cerebral injury (B -14.10; 95% CI -25.04 to -3.16, p = 0.012).

Discussion

This is the largest study describing neurodevelopmental outcome of TTTS survivors published to date, studying over 600 fetuses, of which 434 children were available for follow-up at two years of age. Overall survival was 76% in the cohort treated between 2011 and 2014, which was similar to 81% survival in the previous cohort.

In our most recent cohort, the incidences of severe NDI and CP were 3% and 2%, respectively. Earlier follow-up studies of laser-treated TTTS twins, including our own, have reported incidences of severe NDI between 6% and 18% and of CP between 3% and 11%, with a trend toward lower incidences of NDI in more recent studies.(9, 11, 20) We found no significant differences in severe NDI or disease-free survival between the two consecutive cohorts in the current study. The low numbers of children with severe NDI and CP presumably contribute to the lack of significance. However, the fact that disease-free survival has not increased further seems to indicate that the considerable improvement in outcome for TTTS that was achieved in the first ten years of our laser surgery program has now reached a plateau. In a devastating disease such as TTTS, bringing disease-free survival up to the level of the general population is probably not feasible. In addition to the remaining risk of severe NDI due to the severe nature of the disease, important limiting factors for further improvement of disease-free survival are intrauterine fetal demise and spontaneous premature delivery after laser surgery. Together, fetal demise and birth before the threshold of viability occurred in 19% of fetuses in our latest cohort. Finding ways to reduce these risks may contribute to further improvement of disease-free survival after laser treatment for TTTS.

In this study, we found a significantly lower mean motor score in our new cohort compared to the previous cohort. A possible explanation for the difference in motor score is that different child psychologists have tested the children since 2000. Although the inter-observer agreement for the Bayley-III is substantial with a kappa coefficient of 0.77, an individual tendency to score

a certain way may have an effect on the average results.(19) Another possible explanation for this difference is that about half of the children in the new cohort were tested with the Bayley-III-NL, whereas the rest of the children were tested with the original US version of the Bayley-III. In the Bayley-III-NL, the reversal rule of the gross motor subtest was adapted and became stricter. (21) This adaptation may have contributed to lower motor scores in the children tested with the Bayley-III-NL, which is in favor of the previous cohort. The decrease in motor score that was found in this study may also reflect a true, unexplained effect.

In the current study, we chose to also report mild NDI for several reasons. First, the incidence of severe NDI has become lower over the years, shifting attention to children with mild impairments, which can still have a major impact on the care and educational requirements of children. Secondly, several studies have shown that test scores of the Bayley-III are higher when compared to the previous version (Bayley-II), resulting in the risk of under-estimation of NDI with the use of the Bayley-III.(22) Our study shows that the incidence of mild NDI in the new cohort was considerable, as 23% of children fell into this category. Data on mild -sometimes called minor or moderate- NDI are limited, but it was present in 11% of children in a study that used the Griffiths Scales of Child Development 2nd edition, and in respectively 7% and 29% of children in two studies that used the Ages and Stages Questionnaire (ASQ).(23-25) Due to different testing methods and definitions of mild NDI, a direct comparison cannot be made.

Cerebral injury in TTTS is thought to occur at different stages and may be caused by fetal hemodynamic imbalance, anemia or polycythemia before laser surgery. Sudden changes in hemodynamics during the procedure may also cause ischemic or hemorrhagic injury to the fetal brain.

The association between birth weight and growth restriction with cognitive performance found in our study has been described in several other studies. (26, 27) However, in contrast with other studies, the association between GA at birth and neurodevelopment in this large group of 434 children was not significant ($p = 0.056$). In fact, more than half of the children with severe NDI were born at gestational ages of 32 weeks or more. The overall improvement in neonatal intensive care treatment at younger gestational ages, in combination with the low absolute number of TTTS survivors with severe NDI, may cause the association between lower gestational age and NDI in TTTS to weaken. Motor scores were significantly lower for children with severe cerebral injury. However, of the children with severe NDI, 59% had no evidence of severe cerebral injury. Fetal and postnatal magnetic resonance imaging (MRI) is not part of our routine care for preterm infants with or

without TTTS. MRI is only performed when ultrasound scans show brain abnormalities. Some recent studies have shown the added value of MRI over ultrasound in the detection of cerebral injury in the context of TTTS.(28, 29) However, the evidence is insufficient to confirm a predictive value of fetal MRI for long-term NDI, as this was never studied in large groups. Our findings emphasize the importance of long-term follow-up for all TTTS survivors, not just the ones born before 32 weeks of gestation or those with severe cerebral injury on ultrasound, as is the current practice in some fetal therapy centers around the world.(30)

Strengths of this study are the very large group of included TTTS twins, as well as the use of a standardized psychometric instrument. Finally, every known TTTS pregnancy in the Netherlands during the study periods was treated in the LUMC. Consequently, there is no selection in terms of ethnicity, socioeconomic status or other factors that may be influenced by geographical differences. A limitation of this study is that survivors were assessed at two years of age, and testing at this young age only partially predicts NDI at a later age.(31) Therefore, it is important that follow-up for these children is continued until -at least- school age. Another limitation is that we experienced a slightly higher loss to follow-up rate than we did in our previous studies, with 15% of children lost to follow-up in the new cohort.

Conclusions

After a considerable decrease in NDI and an improvement in disease-free survival in the first decade of our laser program, this improvement now seems to have reached a plateau. With the incidence of severe NDI now being low at 3%, further improvement of disease-free survival may especially be gained by attempting to lower the incidence of immature and premature birth, because of the concomitant risks of low birth weight and cerebral injury, which affect long-term neurodevelopmental outcome, and death. Our data show the importance of assuring long-term follow-up for all TTTS survivors at least until school age, regardless of their gestational age at birth or the presence of severe cerebral injury on ultrasound scans.

References

1. De Lia JE, Kuhlmann RS, Cruikshank DP, O'Bee LR. Current topic: placental surgery: a new frontier. Placenta. 1993;14(5):477-85.
2. Minakami H, Honma Y, Matsubara S, Uchida A, Shiraishi H, Sato I. Effects of placental chorionicity on outcome in twin pregnancies. A cohort study. The Journal of reproductive medicine. 1999;44(7):595-600.
3. Hecher K, Plath H, Bregenzer T, Hansmann M, Hackeloer BJ. Endoscopic laser surgery versus serial amniocenteses in the treatment of severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 1999;180(3 Pt 1):717-24.
4. Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. The Cochrane database of systematic reviews. 2014(1):Cd002073.
5. Rossi AC, D'Addario V. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome: a metaanalysis and review of literature. American journal of obstetrics and gynecology. 2008;198(2):147-52.
6. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine. 2004;351(2):136-44.
7. van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal diagnosis and therapy. 2013;33(2):81-9.
8. Lopriore E, Ortibus E, Acosta-Rojas R, Le Cessie S, Middeldorp JM, Oepkes D, et al. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2009;113(2 Pt 1):361-6.
9. van Klink JM, Koopman HM, van Zwet EW, Middeldorp JM, Walther FJ, Oepkes D, et al. Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology. 2014;210(6):540.e1-7.
10. Papanna R, Biau DJ, Mann LK, Johnson A, Moise KJ, Jr. Use of the Learning Curve-Cumulative Summation test for quantitative and individualized assessment of competency of a surgical procedure in obstetrics and gynecology: fetoscopic laser ablation as a model. American journal of obstetrics and gynecology. 2011;204(3):218.e1-9.
11. Hecher K, Gardiner HM, Diemert A, Bartmann P. Long-term outcomes for monochorionic twins after laser therapy in twin-to-twin transfusion syndrome. The Lancet Child & adolescent health. 2018;2(7):525-35.
12. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 1999;19(8 Pt 1):550-5.
13. Wittmann BK, Baldwin VJ, Nichol B. Antenatal diagnosis of twin transfusion syndrome by ultrasound. Obstetrics and gynecology. 1981;58(1):123-7.
14. Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, et al. Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet (London, England). 2014;383(9935):2144-51.
15. Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, et al. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal diagnosis and therapy. 2010;27(4):181-90.
16. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2006;194(5):1215-20.

17. Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2012; 120(1):15-20.
18. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Developmental medicine and child neurology. 1997;39(4):214-23.
19. A.L. van Baar LJPS, M. Verhoeven, D.J. Hessen. Bayley-III-NL Technische Handleiding. Amsterdam: Pearson Assessment and Information BV.; 2014.
20. Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstetrics and gynecology. 2011;118(5):1145-50.
21. Steenis LJ, Verhoeven M, Hessen DJ, van Baar AL. Performance of Dutch children on the Bayley III: a comparison study of US and Dutch norms. PLoS One. 2015;10(8):e0132871.
22. Sharp M, DeMauro SB. Counterbalanced Comparison of the BSID-II and Bayley-III at Eighteen to Twenty-two Months Corrected Age. Journal of developmental and behavioral pediatrics : JDBP. 2017;38(5):322-9.
23. Banek CS, Hecher K, Hackeloer BJ, Bartmann P. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2003;188(4):876-80.
24. Korsakissok M, Groussolles M, Dicky O, Alberge C, Casper C, Azogui-Assouline C. Mortality, morbidity and 2-years neurodevelopmental prognosis of twin to twin transfusion syndrome after fetoscopic laser therapy: a prospective, 58 patients cohort study. Journal of gynecology obstetrics and human reproduction. 2018;47(10):555-60.
25. Lenclen R, Ciarlo G, Paupe A, Bussieres L, Ville Y. Neurodevelopmental outcome at 2 years in children born preterm treated by amnioreduction or fetoscopic laser surgery for twin-to-twin transfusion syndrome: comparison with dichorionic twins. American journal of obstetrics and gynecology. 2009;201(3):291.e1-5.
26. Korzeniewski SJ, Allred EN, Joseph RM, Heeren T, Kuban KCK, O'Shea TM, et al. Neurodevelopment at Age 10 Years of Children Born <28 Weeks With Fetal Growth Restriction. Pediatrics. 2017;140(5).
27. Miller SL, Huppi PS, Mallard C. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. The Journal of physiology. 2016;594(4):807-23.
28. Weisz B, Hoffmann C, Ben-Baruch S, Yinon Y, Gindes L, Katorza E, et al. Early detection by diffusion-weighted sequence magnetic resonance imaging of severe brain lesions after fetoscopic laser coagulation for twin-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2014;44(1):44-9.
29. Robinson A, Teoh M, Edwards A, Fahey M, Goergen S. Fetal brain injury in complicated monochorionic pregnancies: diagnostic yield of prenatal MRI following surveillance ultrasound and influence on prognostic counselling. Prenatal diagnosis. 2017;37(6):611-27.
30. Chmait RH, Chon AH, Schrager SM, Llanes A, Hamilton AH, Vanderbilt DL. Neonatal cerebral lesions predict 2-year neurodevelopmental impairment in children treated with laser surgery for twin-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2019;32(1):80-4.
31. Graeve P, Banek C, Stegmann-Woessner G, Maschke C, Hecher K, Bartmann P. Neurodevelopmental outcome at 6 years of age after intrauterine laser therapy for twin-twin transfusion syndrome. Acta paediatrica (Oslo, Norway : 1992). 2012;101(12):1200-5.

Chapter 5

Behavioral outcome in twin-twin transfusion syndrome survivors treated with laser surgery

Fieke Brandsma
Marjolijn S. Spruijt
Monique Rijken
Ratna N.G.B. Tan
Dick Oepkes
Enrico Lopriore
Jeanine M.M. van Klink

Archives of Disease in Childhood - Fetal and Neonatal Edition
2020;105(3):304-309

Abstract

Objective
Evaluate the incidence of and risk factors for behavioral problems in twin-twin transfusion syndrome (TTTS) survivors treated with fetoscopic laser coagulation.

Design
Observational cohort study.

Setting
National referral center for fetal therapy, Leiden University Medical Center, the Netherlands.

Patients
Behavioral outcome was assessed in 417 TTTS survivors, at the age of 2 years.

Interventions
Parents completed the Child Behavior Checklist for their twins. Antenatal, neonatal and follow-up data including Bayley-III and a neurological exam were recorded from the medical database.

Main outcome measures
The incidence of risk factors for behavioral problems.

Results
332 twin pregnancies (664 fetuses) were treated with fetoscopic laser for TTTS between 2008 and 2015. For 517 children eligible for follow-up, 417 (81%) Child Behavior Checklist questionnaires were completed. The study group was born at a mean gestational age of 32.8 weeks $\pm$ 3.2. Total behavioral problems within the borderline to clinical range were reported in 8% (95% CI 5.9 to 11.2) of survivors, compared with 10% in the general Dutch population ($p = 0.12$). No difference between donors and recipients was detected ($p = 0.84$). Internalizing and externalizing problems were reported in 9.4% (95% CI 6.9 to 12.6) and 11.5% (95% CI 8.8 to 15.0), respectively. Severe neurodevelopmental impairment was more frequent in the children with behavioral problems. High maternal educational level was associated with lower behavioral problem scores.

Conclusion
Parents of twins treated with fetoscopic laser therapy for TTTS do not report more behavioral problems compared with general population norms. More behavioral problems are reported in children with severe neurodevelopmental impairment.

Introduction

Twin-to-twin-transfusion-syndrome (TTTS) is a severe complication of monochorionic (MC) twin gestations. TTTS develops in approximately 10% of MC twin pregnancies and is the result of an unbalanced net transfusion of blood between one twin, the donor, and the other twin, the recipient, via placental vascular anastomoses. The donor twin becomes hypovolemic, resulting in oliguria and oligohydramnios. The recipient becomes hypervolemic, resulting in polyuria and polyhydramnios.(1) Once TTTS is diagnosed by ultrasound, fetoscopic laser coagulation of the placental vascular anastomoses is the treatment of choice.

Increased survival rate and improved short-term outcome of both donor and recipient has led to a shift in focus towards the long-term neurodevelopmental outcome of TTTS survivors. Long-term follow-up studies report cerebral palsy in 3 to 12% (overall 6%) of survivors and neurodevelopmental impairment (NDI) in 4 to 17% (overall 10%).(2) However, even in children without obvious neurodevelopmental impairment, subtle problems may occur including behavioral and social-emotional problems such as attention problems and rule-breaking behavior. These 'subtle' problems can have a significant impact on care and educational requirements of children. For example, hyperactive/inattentive behavior may result in fewer opportunities to learn in the class room, thereby reducing opportunities to develop age-appropriate academic skills. Up until now, these outcome measures are lacking in the follow-up of TTTS survivors treated with fetoscopic laser surgery. The aim of this study was to assess the behavioral outcome of TTTS survivors treated with laser surgery and to evaluate potential risk factors for long-term problems.

Methods

Participants

All TTTS survivors treated with fetoscopic laser coagulation at the Leiden University Medical Center (LUMC) between March 2008 and March 2015 were eligible for this study. The LUMC is a tertiary medical center and the national referral center for laser treatment in TTTS pregnancies in the Netherlands. TTTS was diagnosed using standard prenatal ultrasound criteria and staged I to V according to Quintero.(3, 4) All parents gave written informed consent for their children.

The following antenatal and neonatal data were recorded: gestational age at laser surgery, Quintero stage, fetal demise, incomplete laser surgery (post-laser twin anemia polycythemia sequence (TAPS) or recurrence of TTTS), gestational age at birth, birth weight, severe neonatal morbidity, cerebral injury and neonatal death (death within 28 days after birth). The presence of TAPS was diagnosed according to previously published antenatal and postnatal criteria.(5)

Severe neonatal morbidity was defined as: respiratory distress syndrome needing surfactant and mechanical ventilation, severe chronic lung disease defined as the need for ≥30% oxygen and/or positive pressure ventilation or nasal continuous positive airway pressure at 36 weeks postmenstrual age or at discharge (whichever comes first), patent ductus arteriosus needing medical therapy or surgical closure, necrotizing enterocolitis ≥ Bell stage 2, retinopathy of prematurity ≥ stage 3, ischemic limb injury, amniotic band syndrome and/or severe cerebral injury. Severe cerebral injury includes: intra-ventricular hemorrhage ≥ grade 3, cystic periventricular leukomalacia ≥ grade 2, ventricular dilatation ≥ 97th percentile, porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome. (6-9) Neuroimaging was performed using either fetal or neonatal ultrasound. In case of suspected cerebral injury, MRI was performed. Mild NDI was defined as the presence of at least one of the following: Cerebral Palsy (Gross Motor Functioning Classification System (GMFCS) grade I), cognitive and/or motor test score between 70 (≥ -2 SD) and 84 (< -1 SD) using the Dutch version of the Bayley scales of Infant and Toddler Development (Bayley-III), vision or hearing loss requiring aids.(10, 11) Severe NDI was defined as the presence of at least one of the following: Cerebral Palsy (GMFCS grade ≥ II), Bayley III cognitive and/or motor test score of less than 70 (< -2 SD), bilateral blindness or bilateral deafness requiring hearing aids.(11)

Maternal educational level was recorded and divided into three levels. A score of 1 was given when the mother's education was low (primary school), a score of 2 for an intermediate educational level (secondary school and intermediate vocational school), and a score of 3 for higher levels of education (higher vocational school and university).

Procedure

At 2 years of age (corrected for prematurity), all TTTS survivors treated with fetoscopic laser surgery were invited for a follow-up visit at our outpatient clinic. According to our follow-up protocol a visit includes a physical and neurological examination and an assessment of cognitive and motor development using Bayley-III.(12)

Measures

At follow-up, parents completed a behavioral questionnaire, the Child Behavior Checklist (CBCL/1½-5).(13) The checklist obtains parents' ratings of 99 problem items. Parents are instructed to rate their child's behavior as it occurs now or within the previous 2 months on a 3-point scale (*not true*, *somewhat or sometimes true* and *very true or often true*). Similar problem items are grouped into syndrome scale scores and their scores are summed up to produce a raw score for that syndrome: Emotionally Reactive (e.g. upset by new people or situations), Anxious/Depressed (e.g. too fearful or anxious), Somatic Complaints (e.g. stomachaches without medical cause), Withdrawn (e.g. avoids eye contact), Sleep Problems (e.g. Resists going to bed), Attention Problems (e.g. cannot concentrate) and Aggressive Behavior (e.g. angry moods).

Two broad band scales combine the syndrome scales: Internalizing Problems sums the Emotionally Reactive, Anxious/Depressed, Somatic Complaints and Withdrawn scores. Externalizing problems combines Attention Problems and Aggressive behavior. The Total problems score is the sum of the scores of all the problem items.

The CBCL/1½-5 also produces five DSM-oriented scales consisting of problem items matching the diagnostic criteria for DSM disorders: Depressive Problems, Anxiety Problems, Autism Spectrum Problems, Attention Deficit/Hyperactivity Problems and Oppositional Defiant Problems.

A Dutch normative sample was used to create standard T scores. These scores compare the raw score to what would be 'normal' compared with responses for preschoolers of the same age and gender. The T scores of the normative sample are scaled with a mean of 50 and a standard deviation (SD) of 10. Higher scores indicate greater severity of problems. For each syndrome, T scores can be interpreted as falling in the normal ($T \leq 64$, $\leq 92^{nd}$ percentile), borderline ($T = 65\text{-}69$; $93^{rd}\text{-}97^{th}$ percentile), or clinical range ($T \geq 70$; $\geq 98^{th}$ percentile). For the broadband scales (Internalizing, Externalizing, Total Problems) the cut points are $T = 60\text{-}63$ ($84^{th}\text{-}90^{th}$ percentiles) for the borderline- and $T \geq 64$ ($\geq 91^{st}$ percentile) for the clinical range. Emotional and behavioral problems are reported in approximately 10% of 2- and 3-year-olds in the Dutch population.(14, 15)

The primary aim was to evaluate the incidence of behavioral problems within the borderline to clinical range. We compared the incidence of behavioral problems between donors and recipients. Secondary outcome was to determine potential risk factors associated with behavioral problem scores including Quintero stage, gestational age at laser surgery, post-laser TAPS or recurrent TTTS, gestational age at birth, birth weight, severe neonatal morbidity (including severe cerebral injury) and maternal level of education.

Statistical analysis

Results are presented as median (IQR and/or minimum-maximum), mean ± SD or n (%). For comparisons between donors and recipients, a paired t-test was used. A binomial test was used to compare the incidence of behavioral problems in our study group to the general Dutch population (10%). Potential risk factors contributing to behavioral problems were tested using an univariable linear regression model. Only variables that showed significant association in the univariate analysis were included in a multivariate analysis. Results are expressed as regression coefficients (B) with 95% CI. A p value of less than 0.05 was considered significant. All analyses were conducted using the Generalized Estimated Equation (GEE) to account for the fact that observations between MC twins are not independent. All statistical analyses were executed with SPSS version 23 (IBM).

Results

Between March 2008 and March 2015, a total of 332 TTTS pregnancies were treated with fetoscopic laser therapy at our center. **Figure 1** shows the derivation of the study population. There were 75 (11%) cases of fetal demise and 33 (5%) neonatal deaths. Three children were excluded from follow-up analyses due to Tay Sachs disease (n=1, the co-twin was a fetal demise) and Neurofibromatosis Type 1 (n=2). In total, 517 children were eligible for follow-up and 417 (81%) Child Behavior Checklists were completed by parents. Ninety- seven children were lost to follow-up due to loss of contact address (n=75), refusal (n=6) or language problems (n=16). The study group had a higher birth weight compared with the lost-to follow-up group (B 2.04, 95% CI 0.51 to 3.56; p = 0.01) and a significant larger proportion of children in the study group were born at-term (B 0.08, 95% CI 0.23 to 0.14; p = 0.01). Baseline characteristics of both groups are presented in **table 1**.

Table 2a shows the incidence of behavioral problems in the 417 children included for behavioral follow-up at a corrected median age of 26 months (IQR 25-29 months). Total behavioral problem scores were within the borderline to clinical range in 34/417 (8.2%, 95% CI 5.9 to 11.2) children.

Compared with 10% in the general Dutch population, parents did not report more behavioral problems for their twins (p = 0.12). Internalizing problems and externalizing problems were reported in 39/417 (9.4%, 95% CI 6.9 to 12.6) and 48/417 (11.5%, 95% CI 8.8 to 15.0) children respectively. We found no significant differences between donors and recipients for total behavioral problem score (t (176) = -0.21, p = 0.84), internalizing (t (176) = -0.17, p = 0.86) or externalizing problems (t (176) = 1.09, p = 0.28). Baseline characteristics did not differ between the children with and without behavioral problems (**table 2b**).

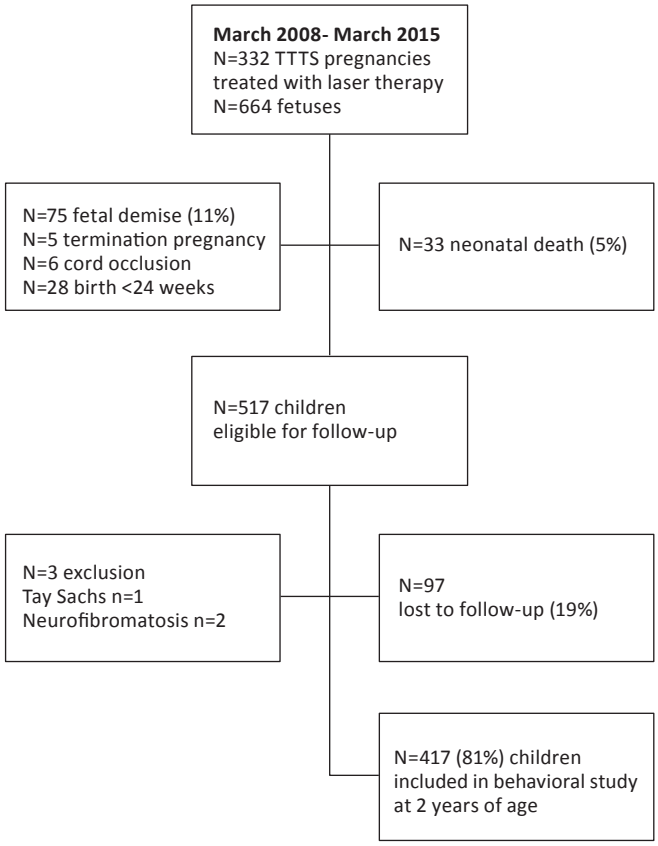


Figure 1. Flow chart showing the derivation of the study population.

Of the 417 children included in our behavioral study, 408 (98%) children had a complete neurodevelopmental assessment according to our follow-up protocol. Severe NDI was detected in 18/408 (4.4%) children. Mean cognitive development score was 99.94 ± 13.5 (55-139). Mean motor development score was 99.22 ± 14.7 (49-138). The incidence of severe NDI including severe cognitive and motor impairment was however more frequent in the children with behavioral problems compared with the children without behavioral problems (**table 3**).

Univariate analysis of potential risk factors associated with total behavioral problem scores was performed (**table 4**). Compared with mothers with a low educational level, mothers with a high educational level reported less behavioral problems (B -5.36, 95% CI -9.56 to -1.15, p = 0.01).

Table 1. Baseline characteristics of the study group and the 97 children lost-to follow-up

Characteristics	Study group N=417	Lost-to follow-up group N=97	p value
TTTS Quintero stage	3 (1-4)	3 (1-4)	0.65
Stage I, n(%)	53 (12.7)	11 (11.3)	
Stage II, n(%)	129 (22.1)	32 (33.0)	
Stage III, n(%)	226 (54.2)	53 (54.6)	
Stage IV, n(%)	9 (2.2)	1 (1)	
Donor, n(%)	210 (50.4)	48 (49.5)	0.46
Gestational age at laser, weeks	20.02 ± 3.44	19.45 ± 2.89	0.11
Recurrent TTTS or TAPS	54 (13)	9 (9)	0.72
Double survivor*, n (%)	354 (85)	86 (89)	0.30
Gestational age at birth, weeks	32.77 ± 3.23	32.0 ± 3.23	0.10
Term 37-40 weeks, n (%)	40 (9.6)	2 (2.1)	0.01
Late preterm 33-36 weeks	182 (43.6)	37 (38.1)	0.65
Very preterm 26-32 weeks	188 (45.1)	55 (56.7)	0.18
Extremely preterm 24-25 weeks	7 (1.7)	3 (3.1)	0.55
Birth weight, grams	1825 ± 597.75	1623 ± 536.26	0.01
Severe neonatal morbidity, n (%)	92/415 (22)	24/91 (26)	0.40
Severe cerebral injury, n (%)	19/415 (5)	3/90 (3.3)	0.71
Female, n (%)	206 (49.4)	46 (47.4)	0.59
Maternal education			
High, n(%)	184 (44)	NA	-
Intermediate, n(%)	184 (44)		
Low, n(%)	49 (12)		

Data are presented as median (minimum-maximum), n (%) or mean ± SD
*Double survivor, survival of both twins beyond the first 28 days of life.
NA, not assessed; TAPS, twin anemia-polycythemia sequence

Table 2a. Behavioral outcome in the 417 TTTS survivors included for follow-up

Child Behavior Checklist	T score ± SD*	Clinical n (%)	Borderline n (%)	Borderline-Clinical n (%)
Behavioral problems, total score	45.27 ± 10.27	25 (6)	9 (2.2)	34 (8.2)
Internalizing behavior	44.70 ± 10.44	22 (5.3)	17 (4.1)	39 (9.4)
Emotionally Reactive	53.49 ± 6.06	10 (2.4)	25 (6)	35 (8.4)
Anxious/Depressed	51.68 ± 4.21	3 (0.7)	8 (1.9)	11 (2.7)
Somatic Complaints	52.85 ± 4.94	11 (2.7)	13 (3.1)	24 (5.8)
Withdrawn behavior	52.64 ± 4.82	6 (1.4)	11 (2.7)	17 (4.1)
Externalizing behavior	46.84 ± 10.32	23 (5.5)	25 (6.0)	48 (11.5)
Attention Problems	53.13 ± 5.22	8 (1.9)	19 (4.6)	27 (6.5)
Aggressive behavior	53.06 ± 5.33	10 (2.4)	13 (3.1)	23 (5.5)
Sleep Problems	52.75 ± 5.67	14 (3.4)	3 (0.7)	17 (4.1)
Stress Problems	53.09 ± 4.82	10 (2.4)	7 (1.7)	17 (4.1)
DSM-V scales				
Depressive Problems	53.02 ± 4.88	10 (2.4)	7 (1.7)	17 (4.1)
Anxiety	52.47 ± 5.27	12 (2.9)	7 (1.7)	19 (4.6)
Autism Spectrum	53.91 ± 6.46	19 (4.6)	25 (6.0)	44 (10.6)
Attention Deficit/Hyperactivity	52.12 ± 3.89	3 (0.7)	7 (1.7)	10 (2.4)
Oppositional Defiant	53.60 ± 5.83	20 (4.8)	11 (2.7)	31 (7.5)

Data are presented as mean ± SD or n (%)
*T scores of the CBCL normative sample have a mean of 50 ± SD 10. For each syndrome score: T = 65-69 for the borderline range and T ≥ 70 for the clinical range. For Internalizing, Externalizing and Total Problems: T = 60-63 for the borderline range and T ≥ 64 for the clinical range.
CBCL, Child Behavior Checklist; DSM-V, Diagnostic and Statistical Manual of Mental Disorders 5th edition

Table 2b. Baseline characteristics of the TTTS survivors with borderline to clinical behavioral problem scores and scores within the normal range

Characteristics	n = 34 children Borderline - clinical range	n = 383 children Normal range	p value
TTTS Quintero stage	3 (1-4)	3 (1-4)	0.89
Stage I, n(%)	2 (5.9)	362 (11.3)	0.39
Stage II, n(%)	15 (44.1)	32 (33.0)	0.34
Stage III, n(%)	15 (44.1)	53 (54.6)	0.54
Stage IV, n(%)	2 (5.9)	1 (1)	0.20
Donor, n(%)	210 (50.4)	48 (49.5)	0.27
Gestational age at laser, weeks	20.38 ± 4.28	19.45 ± 2.89	0.82
Recurrent TTTS or TAPS	54 (13)	9 (9)	0.32
Double survivor, n (%)	354 (85)	86 (89)	0.99
Gestational age at birth, weeks	32.73 ± 3.54	32.0 ± 3.23	0.89
Term 37-40 weeks, n (%)	40 (9.6)	2 (2.1)	0.92
Late preterm 33-36 weeks	182 (43.6)	37 (38.1)	0.69
Very preterm 26-32 weeks	188 (45.1)	55 (56.7)	0.81
Extremely preterm 24-25 weeks	7 (1.7)	3 (3.1)	0.77
Birth weight, grams	1825 ± 597.75	1623 ± 536.26	0.68
Severe neonatal morbidity, n (%)	92/415 (22)	24/91 (26)	0.86
Severe cerebral injury, n (%)	19/415 (5)	3/90 (3.3)	0.57
Female, n (%)	206 (49.4)	46 (47.4)	0.88
Maternal education			0.39
High, n(%)	184 (44)	163/354 (42.6)	0.29
Intermediate, n(%)	184 (44)	155/354 (40.5)	0.40
Low, n(%)	49 (12)	36/354 (9.4)	0.83

TAPS, Twin Anemia Polycythemia Sequence

Table 3. Outcome of the 34 TTTS survivors with behavioral problems in the borderline to clinical range compared with the 383 TTTS survivors with behavioral scores in the normal range

Characteristics	n = 34 children Borderline - clinical range	n = 383 children Normal range	p value
Mild-moderate neurodevelopmental impairment	9/33 (27.3)	59/375 (15.7)	0.15
Severe neurodevelopmental impairment	6/33 (18.2)	12/375 (3.2)	0.00
Cerebral Palsy	1/34 (2.9)	8/383 (2.1)	0.77
CP grade I	-	4/383 (1.0)	
CP grade ≥ II	1/34 (2.9)	4/383 (1.0)	
Cognitive development score	91.6 ± 17.84 (55-129)	100.7 ± 12.77 (55-139)	0.02
Cognitive development <85 and >70 (-1 SD), n (%)	7/33 (21.2)	28/371 (7.5)	0.05
Cognitive development < 70 (-2 SD), n (%)	3/33 (9.1)	2/371 (0.5)	0.01
Motor development score	93.03 ± 16.82 (64-135)	99.7 ± 14.36 (49-138)	0.02
Motor development <-1 SD, n (%)	7/33 (20.6)	45/364 (12.4)	0.08
Motor development <-2 SD, n (%)	4/33 (12.1)	7/364 (1.9)	0.00
Deafness	0/34 (0)	2/383 (0.5)	-

Data are presented as mean ± SD (minimum-maximum) or n/N (%)

Table 4. Analysis of potential risk factors associated with total behavior problem scores

Characteristics	Univariate analysis B (95% CI)	SE	p value
TTTS Quintero			
Stage I	-5.71 (-13.79 – 2.36)	4.12	0.17
Stage II	-3.66 (-11.40 – 4.08)	3.95	0.35
Stage III	-5.07 (-12.61 – 2.46)	3.84	0.19
Stage IV	-		
Gestational age at laser therapy, weeks	0.17 (-0.20 – 0.54)	0.19	0.37
Recurrent TTTS or TAPS	0.36 (-3.06 – 3.79)	1.75	0.84
Fetal demise co-twin	-0.18 (-3.47 – 3.11)	1.68	0.92
Gestational age at birth, weeks	-0.11 (-0.51 – 0.29)	0.20	0.59
Birth weight, grams	-0.05 (-0.23 – 0.12)	0.09	0.55
Severe neonatal morbidity	0.14 (-1.83 – 2.11)	1.00	0.89
Severe cerebral injury	0.49 (-3.07 – 4.04)	1.81	0.79
Maternal education			
High	-5.36 (-9.56 – -1.15)	2.15	0.01
Intermediate	-3.80 (-8.10 – 0.48)	2.18	0.08
Low	-		

Values are regression coefficient B (95% CI), SE and p value.
TAPS, twin anemia-polycythemia sequence

Discussion

This is the first study evaluating the behavioral outcome in over 400 TTTS survivors treated with fetoscopic laser surgery. Despite the improving rate of survival to birth, the neurodevelopmental outcome for TTTS survivors has not been reported consistently, let alone behavior and socio-emotional development.(16, 17) At 2 years of age behavioral problems were reported in 8.2% (95% CI 5.9% to 11.2%) of TTTS survivors. This proportion is comparable to cohorts of 2-year-old children from the general population, with approximately 10% in the general Dutch population.(14, 15, 18) Dickinson and colleagues reported clinical behavior problems in 12% of TTTS survivors treated with serial amnioreduction.(19) In our cohort, donor twins did not differ from recipient twins in long-term behavioral outcome. An important finding of our study is that severe impairment was more frequent in children with

behavioral problems. The association between cognitive impairment and behavioral problems has been reported previously, particularly among preterm born children (below 32 weeks gestational age). For TTTS survivors, often born between 32 to 33 weeks' gestation, this association has not been reported before. This finding suggests that caregivers and health care professionals need to be aware of comorbid behavioral problems in children with severe impairments including cognitive and motor delay. Behavior problems among children with developmental delay are already evident at 2 years of age and seem to increase as children move toward school age.(20) Early identification, evaluation and referral to specialist care is necessary to support parents and to improve outcomes for these children. In addition, TTTS diagnosis and treatment, often followed by complicated neonatal courses due to prematurity and/or other complications constitute traumatic events with an important risk of posttraumatic stress, anxiety and a possible alteration of the prenatal attachment.(21) Insecure attachment to parents is strongly related to externalizing problem behavior in children.(22) Prenatal and postnatal psychological support is therefore important for both mothers and fathers.

In our study group, a relatively large proportion of mothers, 44%, reported a high level of education compared to 30% in the general Dutch population.(23) The mothers with a high educational level reported less behavioral problems in their twins compared to mothers with a low educational level. The strong link between maternal education and children's outcomes is one of the most well-established findings in developmental psychology.(24, 25)

Unfortunately, 19% of the children were lost to follow-up. Comparison of the antenatal and neonatal characteristics showed a significantly lower birth weight and a lower proportion of term born children in the lost-to follow-up group. Preterm born children with low birth weight are at higher risk of developing behavioral problems than term born children with normal birth weight.(26, 27) If these children had been included for follow-up the incidence of behavioral problems may have been higher.

An important limitation is the absence of a control group of uncomplicated MC twins matched for gestational age to assess the effect of TTTS and treatment on outcome. In addition, although we have included over 400 children for behavioral assessment at 2 years of age, assessment at this young age only partially predicts outcome at a later age. At this young age it is possible to discover major developmental abnormalities that require and benefit from early intervention. However, developmental outcomes assessed during early childhood are only moderate predictors of long-term neurodevelopment, particularly for scores on behavioral functioning and academic performance.

Some developmental problems, including learning difficulties or autism spectrum disorder, cannot be detected until later on, once the children start becoming more socially and academically challenged at school age. Follow-up of children treated with laser for TTTS is recommended until at least school age.

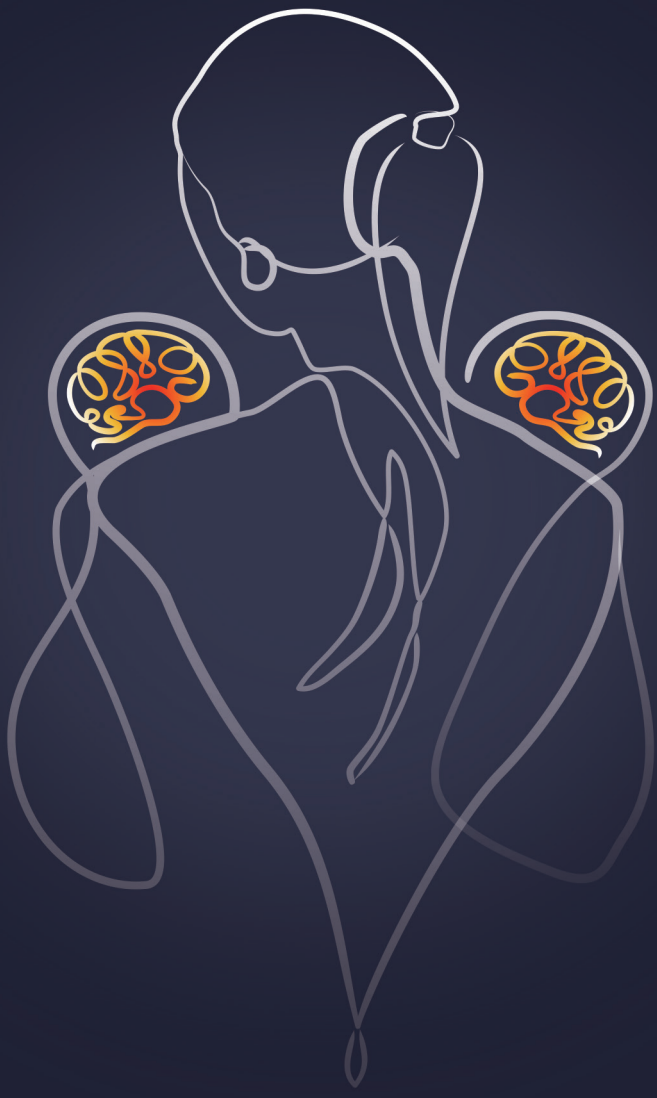
Conclusion

In conclusion, parents of twins treated with fetoscopic laser therapy for TTTS do not report more behavioral problems at 2 years of age compared to general populations. Behavioral problems were more frequent in twins with severe developmental delay. This study should be repeated at school age when the academic and social environment becomes more complex and challenging for children.

References

1. Maschke C, Diemert A, Hecher K, Bartmann P. Long-term outcome after intrauterine laser treatment for twin-twin transfusion syndrome. Prenatal diagnosis. 2011;31(7):647-53.
2. van Klink JM, Koopman HM, Rijken M, Middeldorp JM, Oepkes D, Lopriore E. Long-Term Neurodevelopmental Outcome in Survivors of Twin-to-Twin Transfusion Syndrome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):255-61.
3. Wittmann BK, Robinson HP, Aitchison T, Fleming JE. The value of diagnostic ultrasound as a screening test for intrauterine growth retardation: comparison of nine parameters. Am J Obstet Gynecol. 1979;134(1):30-5.
4. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. J Perinatol. 1999;19:550-5.
5. Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, et al. Twin anemia-polycythemia sequence: Diagnostic criteria, classification, perinatal management and outcome. Fetal Diagnosis and Therapy. 2010;27(4):181-90.
6. Volpe JJ. Intraventricular hemorrhage and brain injury in the premature infant. Neuropathology and pathogenesis. Clinics in perinatology. 1989;16(2):361-86.
7. de Vries LS, Eken P, Dubowitz LM. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res. 1992;49(1):1-6.
8. Levene MI. Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound. Arch Dis Child. 1981;56(12):900-4.
9. Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Vandenbussche FP, Walther FJ. Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses. J Pediatr. 2005;147(5):597-602.
10. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Developmental medicine and child neurology. 1997;39(4):214-23.
11. van Klink JM, Slaghekke F, Balestriero MA, Scelsa B, Introvini P, Rustico M, et al. Neurodevelopmental outcome at 2 years in twin-twin transfusion syndrome survivors randomized for the Solomon trial. Am J Obstet Gynecol. 2015;214(1):113.e1-7.
12. Albers CA, Grieve AJ. Test Review: Bayley, N.(2006). Bayley Scales of Infant and Toddler Development– Third Edition. San Antonio, TX: Harcourt Assessment. Journal of Psychoeducational Assessment. 2007;25(2):180-90.
13. Achenbach TM, Rescorla LA. Manual for the ASEBA preschool forms and profiles: University of Vermont, Research Center for Children, Youth and Families, Burlington, VT; 2000 2000.
14. Tick NT, van der Ende J, Koot HM, Verhulst FC. 14-year changes in emotional and behavioral problems of very young Dutch children. J Am Acad Child Adolesc Psychiatry. 2007;46(10):1333-40.
15. Achenbach TM, Becker A, Döpfner M, Heiervang E, Roessner V, Steinhausen H, et al. Multicultural assessment of child and adolescent psychopathology with ASEBA and SDQ instruments: research findings, applications, and future directions. Child Psychology and Psychiatry. 2008;49:251-75.
16. Perry H, Duffy JMN, Reed K, Baschat A, Deprest J, Hecher K, et al. A core outcome set for the evaluation of treatments for twin-twin transfusion syndrome. Ultrasound Obstet Gynecol. 2018.
17. Khalil A, Perry H, Duffy J, Reed K, Baschat A, Deprest J, et al. Twin-Twin Transfusion Syndrome: study protocol for developing, disseminating, and implementing a core outcome set. Trials. 2017;18(1):325.
18. Briggs-Gowan MJ, Carter AS, Skuban EM, Horwitz SM. Prevalence of social-emotional and behavioral problems in a community sample of 1- and 2-year-old children. J Am Acad Child Adolesc Psychiatry. 2001;40(7):811-9.
19. Dickinson JE, Duncombe GJ, Evans SF, French NP, Hagan R. The long term neurologic outcome of children from pregnancies complicated by twin-to-twin transfusion syndrome. BJOG. 2005;112(1):63-8.

20. Cheng ER, Palta M, Kotelchuck M, Poehlmann J, Witt WP. Cognitive delay and behavior problems prior to school age. Pediatrics. 2014;134(3):e749-57.
21. Beauquier-Maccotta B, Chalouhi GE, Picquet AL, Carrier A, Bussieres L, Golse B, et al. Impact of Monochorionicity and Twin to Twin Transfusion Syndrome on Prenatal Attachment, Post Traumatic Stress Disorder, Anxiety and Depressive Symptoms. PLoS One. 2016;11(1):e0145649.
22. Fearon RP, Bakermans-Kranenburg MJ, van Ijzendoorn MH, Lapsley AM, Roisman GI. The significance of insecure attachment and disorganization in the development of children's externalizing behavior: a meta-analytic study. Child Dev. 2010;81(2):435-56.
23. CBS. Bevolking; hoogstbehaald onderwijsniveau en onderwijsrichting https://opendata.cbs.nl/statline/#/CBS/nl/dataset/82816ned/table?dl=8083: Centraal Bureau voor de Statistiek; 2018 [Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/82816ned/table?dl=8083.
24. Reardon SF. The widening academic achievement gap between the rich and the poor: New evidence and possible explanations. In: Murnane R, Duncan G, editors. Whither opportunity: Rising inequality and the uncertain life chances of low-income children. New York: Russell Sage Foundation Press; 2011. p. 91-116.
25. Harding JF. Increases in maternal education and low-income children's cognitive and behavioral outcomes. Dev Psychol. 2015;51(5):583-99.
26. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. JAMA. 2002;288(6):728-37.
27. Schappin R, Wijnroks L, Uniken Venema M, Jongmans M. Exploring predictors of change in behavioral problems over a 1-year period in preterm born preschoolers. Infant Behav Dev. 2018;50:98-106.



Part FIVE

Intentional demise

Chapter 6

Incidence and causes of intentional fetal or neonatal demise in twin-twin transfusion syndrome

Marjolijn S. Spruijt
Ellen Tameeris
Danny P. Zhao
Annemieke (J.M.) Middeldorp
Monique C. Haak
Dick Oepkes
Enrico Lopriore

Fetal Diagnosis and Therapy 2018;43(1):19-25

Abstract

Introduction

The aim of this study is to evaluate the incidence and causes of intentional fetal and neonatal demise in twin-twin transfusion syndrome (TTTS).

Material and methods

All TTTS pregnancies managed at our center between 2000 and 2014 were included. We evaluated incidence and causes of intentional fetal/neonatal demise, defined as termination of pregnancy, selective fetal reduction, or withdrawal of neonatal intensive care.

Results

Intentional fetal/neonatal demise occurred in 9.8% (110/1122) of fetuses and was due to termination of pregnancy (2.2%), selective fetal reduction (4.2%), or withdrawal of neonatal intensive care (3.4%). Reasons for termination of pregnancy included complications of laser treatment (72.0%), severe fetal anomaly (20.0%), and unwanted pregnancy (8.0%). Reasons for selective fetal reduction were technical difficulties to perform laser surgery (51.1%), fetal complications (38.3%), and parental preference for fetal reduction rather than laser treatment (10.6%). Reasons for withdrawal of neonatal intensive care treatment were severe cerebral injury (47.4%), severe pulmonary complications (15.8%), birth asphyxia (5.3%), multiple complications of TTTS and/or prematurity combined (21.1%), or other (10.5%).

Conclusions

Intentional fetal or neonatal demise in TTTS occurs frequently and is often due to complications after laser surgery and/or severe (cerebral) injury in affected fetuses or neonates.

Introduction

Twin-twin transfusion syndrome (TTTS) results from imbalanced inter-twin blood flow through placental vascular anastomoses and is associated with an increased risk of fetal and neonatal mortality.(1, 2) Treatment with fetoscopic laser surgery strongly reduces the risks of morbidity and mortality.(3, 4) Although short- and long-term outcome in survivors has improved over time owing to improvements in fetal and neonatal care, TTTS is still associated with an increased incidence of cerebral injury and neurodevelopmental impairment.(5, 6)

The incidence of fetal or neonatal mortality as well as short- or long-term morbidity in TTTS after laser surgery varies greatly between reported cohorts.(4, 7-12) These differences may be due to multiple reasons including methodological differences between the studies, variations in definitions of severe morbidity, type of laser treatment, and level of experience in fetal therapy centers. An additional reason could be related to varying rates of termination of pregnancy, selective fetal reduction and withdrawal of neonatal care in cases with unfavorable prognosis and/or severe cerebral injury.(4, 8, 13) If severe fetal injury is detected during pregnancy, termination of pregnancy or selective feticide may be considered within the constriction of the law. If severe cerebral injury is detected close to or after delivery, withholding life-sustaining neonatal treatment or withdrawal of intensive care treatment may be taken into consideration. A higher rate of (intentional or unintentional) fetal or neonatal demise in cases with unfavorable prognosis will lead to a higher rate of mortality and indirectly to a lower rate of neurodevelopmental impairment in survivors due to selection. This important factor is usually not reported or discussed when reporting the long-term outcome in TTTS series. Decisions on intentional fetal or neonatal demise are influenced by cultural, legal and social factors, and incidence rates are bound to be different between treatment centers in different countries.

The aim of this study is to describe the incidence and causes of termination of pregnancy, selective fetal reduction and withdrawal of neonatal intensive care treatment in TTTS pregnancies treated at our center during the past 15 years.

Material and methods

We conducted a cohort study of all TTTS pregnancies treated at the Leiden University Medical Center (LUMC) since the beginning of laser therapy at our center in 2000. The LUMC is the single Dutch tertiary referral center for TTTS.

All monochorionic twin pregnancies with TTTS referred to and treated at the LUMC between 2000 and 2014 were included in this study. Most TTTS pregnancies were treated with fetoscopic laser surgery. We also included TTTS pregnancies in which selective fetal reduction or termination of pregnancy was chosen as the primary management option. TTTS was diagnosed using standard prenatal ultrasound criteria and staged according to the criteria of Quintero.(10, 11, 14) Triplets or higher order multiple gestations were excluded from the study.

The following data were recorded for every individual fetus of the included pregnancies: year of TTTS treatment, Quintero stage, laser technique, donor or recipient, gender, fetal demise, mode of delivery, neonatal survival (up to 1 month after birth) and the presence of severe cerebral injury. Severe cerebral lesions were defined in the same way as in our previous study, summarized as the presence of at least one of the following: intraventricular hemorrhage grade III, periventricular hemorrhagic infarction, periventricular leukomalacia grade II or greater, porencephalic cysts, arterial stroke, ventricular dilatation, or a combination of these.(15)

We recorded all cases with intentional demise during the fetal or neonatal period and reasons for these management choices. Intentional fetal demise included termination of pregnancy or selective feticide. Termination of pregnancy was defined as termination of both twins or of the surviving co-twin after spontaneous demise of the first. Selective fetal reduction was defined as termination of one fetus by means of bipolar umbilical cord coagulation, radio-frequency ablation or the administration of intracardiac potassium chloride after laser therapy. Intentional neonatal demise was defined as the withholding of neonatal intensive care treatment (treatment is never started) or the withdrawal of life-sustaining treatment in severely ill neonates because of a suspected poor prognosis, resulting in death.

The primary outcome in this study was a composite outcome termed intentional demise. As a secondary outcome measure we analysed differences in the incidence of intentional demise over time, comparing three 5-year subcohorts: 2000-2004, 2005-2009 and 2010-2014.

Finally, we analysed the reasons for intentional fetal demise or neonatal demise and divided these into categories.

Importantly, a new national guideline concerning the care for extremely premature infants was implemented in the Netherlands in 2010, advising active neonatal intervention for very preterm neonates born at gestational ages at or above 24 0/7 weeks.(16) The previous national guideline advised active neonatal treatment only after 25 0/7 weeks, unless there were compelling arguments to decide otherwise. This change in policy occurred therefore during the period of our study.

The primary composite outcome, intentional demise, was reported as a percentage of the total number of TTTS-affected fetuses and as a percentage of the total number of TTTS pregnancies. The secondary outcome was analysed by dividing the study cohort into three 5-year subcohorts. Analysis of the incidence of intentional demise over the studied years was calculated by χ^2 test for trend. The final secondary outcome, reasons for intentional fetal and neonatal demise, was reported per category, i.e., termination of pregnancy, selective fetal reduction, and withdrawal of neonatal treatment, as a percentage of the total number of fetuses, pregnancies, and/or neonates per category. Statistical analysis was performed using SPSS Statistics version 23.0 (SPSS, Inc., Chicago, IL, USA).

No ethical approval was required for this anonymized retrospective study. The institutional review board of the LUMC reviewed the study protocol and declared a statement of no objection.

Results

A total of 561 twin pregnancies (1122 fetuses) with TTTS treated at our center with laser therapy or selective fetal reduction between 2000 and 2014 were included in this study. The baseline characteristics of these patients are shown in **table 1**. In 29 cases, the outcome of the pregnancy or neonate(s) was unknown. In 147 cases, unintentional fetal demise occurred due to death of one or both twins after laser therapy or death of the co-twin after selective fetal reduction of the other fetus. In 50 cases, death occurred after spontaneous immature birth before gestational ages of 24 0/7 weeks (see flowchart in **figure 1**).

Termination of pregnancy was performed after a mean gestational age of 20.0 weeks (range 16.0 - 23.4 weeks). The mean gestational age at which selective fetal reduction was performed was 19.9 weeks (range 13.9 – 33.0 weeks).

Selective fetal reduction was performed in the recipient in 68.1% (32/47) of cases and the donor in the remaining 31.9% of cases. Neonatal intensive care was withdrawn in the donor in 60.5% (23/38) and in the recipient in the remaining 39.5% of neonates.

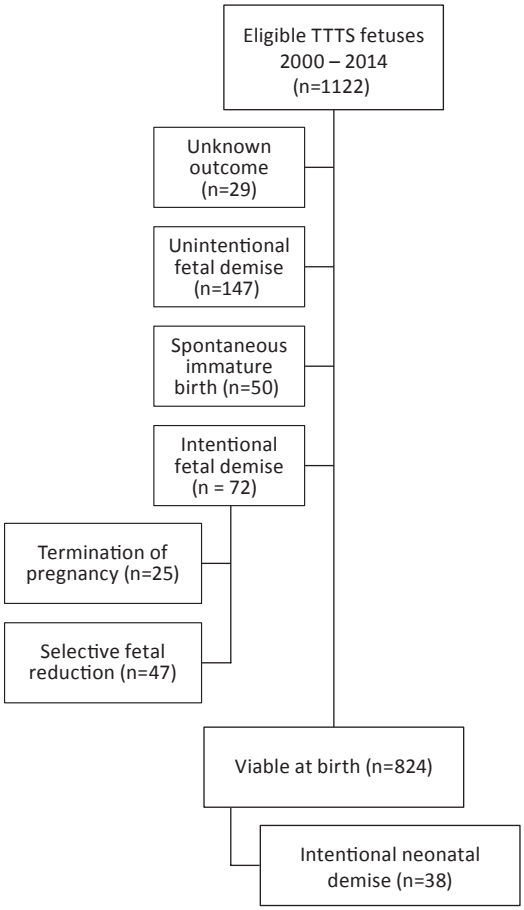


Figure 1. Flow chart of study population.

In 14 of 47 (29.8%) pregnancies in which selective feticide was performed, prior laser therapy had taken place, but turned out to be incomplete (recurrent or persistent TTTS, reversal of TTTS, or twin anemia polycythemia sequence (TAPS) occurred following laser therapy), after which re-intervention was necessary (not shown in table).

The technique used for selective fetal reduction was bipolar cord coagulation in 44 of 47 cases. Fetal intracardiac potassium chloride injection was used after laser surgery in two cases, and radio-frequency ablation was used in one fetus.

Table 1. Patient characteristics of the TTTS cohort

Total number of pregnancies	561
Total number of fetuses	1122
Gender	
Female	542 (48.3)
Male	552 (49.2)
Unknown	28 (2.5)
Delivery mode	
Caesarean section	347 (30.9)
Vaginal	725 (64.6)
Unknown	50 (4.5)
Gestational age at birth*	33.0 ± 3.5 (24-42)
Birth weight*	1852 ± 682 (502 - 4320)
Quintero stage (number of pregnancies)	
1	62 (11.1)
2	190 (33.9)
3	274 (48.8)
4	26 (4.6)
Unknown	9 (1.6)
Technique used at intervention (number of pregnancies)	
Laser therapy (n=528 pregnancies)	
Sequential selective laser	311 (58.9)
Solomon technique	217 (41.1)
Selective fetal reduction (n=47 pregnancies)	
Bipolar cord coagulation	44 (93.6)
Radiofrequency ablation	1 (2.1)
Intracardiac potassium (after laser)	2 (4.3)

Data are n (%) or mean ± standard deviation (range)
Denominator is total no. of fetuses unless otherwise specified
*Presented only for liveborns > 24 0/7 weeks gestation (n= 848)

Primary outcome

The primary composite outcome, intentional fetal or neonatal demise, occurred in 9.8% (110/1122) of fetuses (**table 2**). When calculated per total number of pregnancies, intentional demise occurred in 16.8% (94/561) of pregnancies.

Table 2. Primary outcome: intentional fetal and neonatal demise

	Fetuses (n =1122)	Pregnancies (n =561)
Intentional fetal demise	72 (6.4)	62 (11.1)
Selective fetal reduction	47 (4.2)	47 (8.4)
Termination of pregnancy	25 (2.2)	15 (2.7)
Intentional neonatal demise	38 (3.4)	32 (5.7)
Total intentional fetal/neonatal demise	110 (9.8)	94 (16.8)

Data are presented as n (%).

Intentional fetal demise

Intentional fetal demise occurred in 6.4% of fetuses (72/1122) and included selective fetal reduction in 4.2% (47/1122) and termination of pregnancy in 2.2% (25/1122) of fetuses. In five pregnancies, termination of pregnancy was performed after one fetus had already died unintentionally in utero.

Intentional neonatal demise

The incidence of intentional neonatal demise was 3.4% (38/1122) of all fetuses, or 4.6% (38/824) of TTTS neonates who were alive at birth. In six pregnancies, withdrawal of neonatal care occurred in both co-twins, consequently the number of unique pregnancies affected by intentional neonatal demise is reduced by six compared to the number of fetuses (or really, neonates) affected. One pregnancy was affected by selective fetal reduction of one co-twin and withdrawal of neonatal care in the other, therefore the sum of the number of pregnancies affected by fetal and neonatal demise is reduced by one.

Secondary outcomes

Intentional demise over time

The incidence of intentional fetal and neonatal demise did not change significantly over time. The incidence was 8.8% (19 of 216 fetuses) in the first subcohort (2000-2004), 9.8% (41 of 420) in the second (2005-2009) and 10.3% (50 of 486) in the third subcohort (p = 0.83).

Reasons for intentional fetal and neonatal demise

An overview of reasons for intentional demise is given in **table 3**.

Table 3. Secondary outcome: reasons for intentional demise

Termination of pregnancy	**25 fetuses**	**15 pregnancies**
Complications after laser therapy	18 (72.0)	11 (73.3)
Early PPROM	12 (48.0)	7 (46.7)
Intra uterine infection	5 (20.0)	3 (20.0)
Reversal of TTS	1 (4.0)	1 (6.7)
Chromosomal or other severe anomaly of fetus(es)	5 (20.0)	3 (20.0)
Unwanted pregnancy and multiple complications after laser	2 (8.0)	1 (6.7)
Selective fetal reduction	**47 fetuses**	
Complete laser therapy not possible (anatomical/ positional)	19 (40.4)	
Complete laser therapy not possible (intra-amniotic bleeding)	5 (10.6)	
Complications in one fetus	18 (38.3)	
Severe cerebral injury	6 (12.7)	
Hydrops fetalis	6 (12.7)	
Multiple injury due to amniotic bands	1 (2.1)	
Congenital anomaly	5 (10.6)	
Choice of parents, no complications besides TTTS	5 (10.6)	
Withdrawal of neonatal intensive care treatment	**38 neonates**	
Severe cerebral injury	18 (47.4)	
Prenatally acquired	13 (34.2)	
Postnatally acquired	5 (13.2)	
Severe pulmonary complications	6 (15.8)	
Multiple complications (of TTS and prematurity, not cerebral)	8 (21.1)	
Severe asphyxia	2 (5.3)	
Hydrops fetalis	1 (2.6)	
Deemed previable at birth, no active treatment	3 (7.9)	

Data are presented as n (%)
PPROM, preterm prelabor rupture of membranes

Reasons for termination of pregnancy

Severe complications after laser therapy were the most common reason to perform termination of pregnancy, as these were established in 72.0% (18/25) of involved fetuses. Complications included preterm prelabor rupture of membranes (PPROM) in 48.0% (12/25). In all of these cases, PPROM was associated with severe oligohydramnios or anhydramnios, in some cases combined with imminent immature delivery. Evident intrauterine infection was the reason for termination of pregnancy in 20.0% (5/25) of cases and these included two cases (4 fetuses) of severe maternal sepsis. Termination of pregnancy was performed after reversal of TTTS after laser surgery without the possibility of a successful second intervention in one pregnancy (4.0% of fetuses). In three pregnancies (5 living fetuses), termination of pregnancy was performed due to congenital or syndromal fetal abnormalities (not known at the time of laser treatment), i.e. trisomy 21 (1 pregnancy), Jeune syndrome, a rare skeletal dysplasia (1 pregnancy), and in one case bilateral clubfeet combined with cardiac hypertrophy, the latter most likely due to TTTS in a recipient (detected after unintentional fetal demise of the donor shortly after laser therapy). There was one unwanted pregnancy, which was terminated after the development of complications after laser therapy (severe growth retardation of one fetus, followed by TAPS after laser therapy).

Reasons for selective fetal reduction

Selective feticide was performed in 47 TTTS pregnancies. In 51.1% (24/47), selective fetal reduction was chosen as the mode of therapy during the procedure (primarily planned as laser surgery) because complete laser surgery was impossible to achieve due to either anatomical or positional reasons (19 cases), or because of intra-amniotic bleeding occurring during the procedure, obscuring fetoscopic visualization (5 cases). Anatomical or positional causes included unfavorable (anterior) placental position, a stuck donor twin overlying the site of the vascular equator and in one case, severe maternal obesity. A total of 528 pregnancies in our cohort were treated with laser therapy. These 24 cases treated with selective fetal reduction due to the impossibility to perform laser, represent 4.3% (24 / (528 + 24) of pregnancies in which laser therapy was the intended treatment.

The reason for selective feticide was the occurrence of complications in one of the fetuses in 38.3% (18/47) of cases, including severe cerebral injury in 6 cases, hydrops fetalis in 6 cases, injury due to amniotic band syndrome in 1 fetus and discordant congenital anomaly in 5 fetuses. The congenital anomalies seen in these five fetuses were double outlet right ventricle, severe lower urinary tract obstruction, bilateral multicystic kidney disease, gastroschisis and anencephaly.

In 10.6% of cases (5/47), parents decided to perform selective fetal reduction as a primary treatment option to optimize the chance of having at least one healthy infant. These five pregnancies were staged as Quintero stages 1, 2 and 3 and there were no additional complications besides TTTS nor any significant growth discordance in these fetuses.

Reasons for withdrawal of neonatal care

Of the 38 cases in which intentional neonatal demise took place, the reason for cessation of neonatal intensive care was severe cerebral injury in 47.4% (18/38). In 13 of these 18 neonates, cerebral injury was acquired antenatally. One of these, a donor born at 26 0/7 weeks of gestation, had a grade 3 intraventricular hemorrhage at birth, and in addition a hypoplastic right heart with severe pulmonary valve stenosis. The combination of severe prematurity, severe cerebral injury and a complex congenital heart defect led to the decision to withdraw life-sustaining treatment.

Severe pulmonary complications were the reason to withdraw treatment in 15.8% (6/38) of neonates. These included 3 cases of pulmonary hypoplasia after PPROM following fetal therapy, and 3 cases of severe pulmonary disease associated with prematurity. In eight neonates (21.1%), the decision to stop life-sustaining treatment was made because of a combination of multiple complications of TTTS and/or prematurity (not including severe cerebral injury).

Severe birth asphyxia was reason to abstain from further therapy in 2 cases. Both these neonates were former recipients after laser therapy, born after 34 and 38 weeks of gestation, respectively, and residual anastomoses were found in neither case.

One ex-donor was born with massive fetal hydrops at 31 2/7 weeks and resuscitation at birth was withheld. Three babies were born alive but did not receive intensive care treatment because they were deemed too immature due to national protocols concerning the management of extremely premature infants at that time. These included a pair of twins born after a gestational age of 24 2/7 weeks in 2005 and a baby born at 25 0/7 weeks in 2010. The co-twin of this last baby did receive treatment because he was vital at birth (Apgar scores of 6, 7 and 9), but treatment was withdrawn on the fifteenth day because of multiple complications including a candida sepsis.

Discussion

This study shows that intentional demise in TTTS occurs in 9.8% of fetuses or neonates and 16.7% of pregnancies. To our knowledge, there are no earlier reports on the incidences and causes of intentional demise in TTTS. These results are important as they may partly explain differences in mortality and morbidity rates between reported TTTS cohorts.

It is reasonable to assume that centers with a higher rate of intentional demise (mainly when due to unfavorable prognosis or cerebral injury) will have a higher rate of mortality but a lower rate of adverse neonatal and long-term neurodevelopmental outcome, because a selection takes place of fetuses with a better prognosis. Conversely, centers with a lower rate of intentional demise, possibly due to cultural or legal reasons, will most probably have a lower mortality rate, but a higher rate of adverse neonatal and long-term (neurological) complications in surviving neonates. Awareness of the impact of intentional demise of fetuses and neonates affected by TTTS is of crucial importance to gain insight into which factors contribute to neonatal and long-term neurodevelopmental outcome and in order to compare outcome data from different TTTS series.

Our study also shows that reasons for intentional demise were related to a suspected unfavorable or poor prognosis in the majority of cases.(7, 8, 13) In about half of the pregnancies in which selective fetal reduction was carried out, laser coagulation was the planned treatment initially, but turned out to be impossible to perform during the procedure due to either fetal positions or impaired visibility caused by intra-amniotic bleeding. These cases represent 4.3% of the total number of pregnancies in which laser was the intended treatment. Because leaving TTTS untreated exposes both fetuses at great risk for severe complications including death, selective feticide is usually chosen in these situations to give at least one twin a reasonable chance of disease-free survival.

In one TTTS pregnancy, the occurrence of complications combined with the fact that the pregnancy was unwanted to begin with, led to the decision to terminate the pregnancy. In five TTTS pregnancies, parents opted for selective fetal reduction as a primary treatment, in order to optimize the chances of at least one healthy child, instead of laser coagulation of the vascular anastomoses. In these five pregnancies, there were no (severe) anomalies or growth restrictions in either of the co-twins at the time of treatment decision.

Reasons for withholding or withdrawing neonatal intensive care treatment were all related to unfavorable prognosis. In almost half of the 38 cases of intentional neonatal demise, the decision to stop treatment was made because

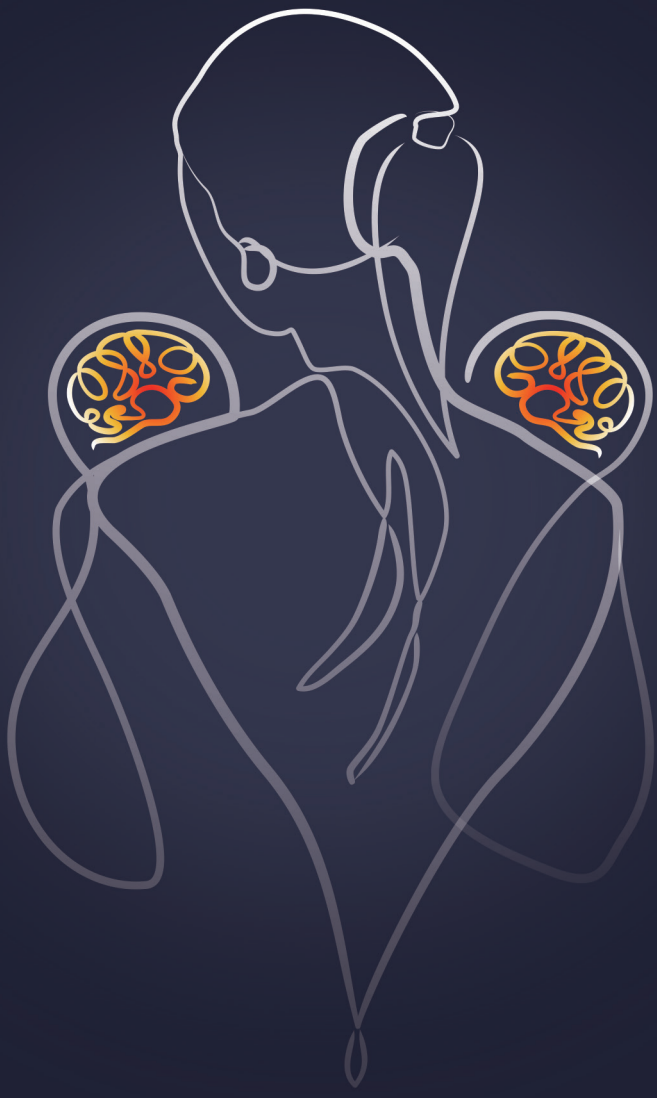
of severe cerebral injury, of which the majority was acquired antenatally (72.2% of neonates with severe cerebral injury, versus 27.8% acquired postnatally), comparable to what we previously reported.(15) Termination of treatment in neonates with severe cerebral injury strongly affects the long-term neurodevelopmental outcome of a cohort of TTTS survivors. Other complications leading to withdrawal of NICU care were either directly related to TTTS or TTTS treatment (pulmonary hypoplasia after oligo- or anhydramnios, severe hydrops, extreme prematurity after PPROM), to severe prematurity, or to perinatal asphyxia.

In a recent study, we showed a significant improvement in survival and long-term neurodevelopmental outcome in TTTS cases treated our center during the past decade.(17) We hypothesized that improvement in long-term outcome may partly be due to increased awareness and detection of fetal or neonatal cerebral injury and associated increased intentional demise. However, as shown in this study, the rates of intentional fetal and neonatal demise in the three consecutive 5-year subcohorts between 2000 and 2014 were not different. Other factors, including improvement of obstetric and neonatal care for TTTS mothers and infants, could have contributed to the improvements in outcome.

In conclusion, intentional fetal and neonatal demise occurred in 9.8% of TTTS affected fetuses in our center. This high rate of intentional demise may result in a relatively favorable short-term and long-term neurodevelopmental outcome in survivors of TTTS.

References

1. Lutfi S, Allen VM, Fahey J, O'Connell CM, Vincer MJ. Twin-twin transfusion syndrome: a population-based study. Obstet Gynecol. 2004;104(6):1289-97.
2. Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, et al. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. American Journal of Obstetrics and Gynecology. 2008;199(5).
3. Akkermans J, Peeters SH, Klumper FJ, Lopriore E, Middeldorp JM, Oepkes D. Twenty-Five Years of Fetoscopic Laser Coagulation in Twin-Twin Transfusion Syndrome: A Systematic Review. Fetal Diagn Ther. 2015;38(4):241-53.
4. Huber A, Diehl W, Bregenzer T, Hackeloer BJ, Hecher K. Stage-related outcome in twin-twin transfusion syndrome treated by fetoscopic laser coagulation. Obstet Gynecol. 2006;108(2):333-7.
5. Lopriore E, Oepkes D, Walther FJ. Neonatal morbidity in twin-twin transfusion syndrome. Early human development. 2011;87(9):595-9.
6. van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal diagnosis and therapy. 2013;33(2):81-9.
7. Chmait RH, Kontopoulos EV, Korst LM, Llanes A, Petisco I, Quintero RA. Stage-based outcomes of 682 consecutive cases of twin-twin transfusion syndrome treated with laser surgery: the USFetus experience. Am J Obstet Gynecol. 2011;204(5):393 e1-6.
8. van Klink JM, Slaghekke F, Balestriero MA, Scelsa B, Introvini P, Rustico M, et al. Neurodevelopmental outcome at 2 years in twin-twin transfusion syndrome survivors randomized for the Solomon trial. Am J Obstet Gynecol. 2016;214(1):113 e1-7.
9. Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstetrics and gynecology. 2011;118(5):1145-50.
10. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine. 2004;351(2):136-44.
11. Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, et al. Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet (London, England). 2014;383(9935):2144-51.
12. Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. The Cochrane database of systematic reviews. 2014;1:CD002073.
13. Chmait RH, Kontopoulos EV, Jackson M, Horenstein J, Timor-Tritsch I, Quintero RA. Selective Reduction Using Intravascular Potassium Chloride Injection after Laser Surgery for Twin-Twin Transfusion Syndrome. Fetal Diagn Ther. 2015.
14. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 1999;19(8 Pt 1):550-5.
15. Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2012;120(1):15-20.
16. de Laat MW, Wiegerinck MM, Walther FJ, Boluyt N, Mol BW, van der Post JA, et al. [Practice guideline 'Perinatal management of extremely preterm delivery']. Nederlands tijdschrift voor geneeskunde. 2010;154:A2701.
17. van Klink JM, Koopman HM, van Zwet EW, Middeldorp JM, Walther FJ, Oepkes D, et al. Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. Am J Obstet Gynecol. 2014;210(6):540 e1-7.



Part SIX

Summary and discussion

Summary

Twin-twin transfusion syndrome or TTTS is a rare condition that complicates about 1 in 10 monochorionic twin pregnancies. Monochorionic twins share a single placenta, which nearly always contains vascular anastomoses connecting the two fetal circulations. TTTS occurs when there is a unidirectional net transfusion of blood between the twins through these anastomoses. As a result, the twin losing blood volume, known as the donor, becomes hypovolemic and oliguric. Eventually, the donor twin may become 'stuck' in the membranes due to severe oligohydramnios. Conversely, the twin receiving excessive blood flow, known as the recipient, experiences volume overload, resulting in polyuria and polyhydramnios. This presentation on prenatal ultrasound is referred to as Twin Oligohydramnios Polyhydramnios Sequence (TOPS) and it is the hallmark of TTTS diagnosis. Untreated TTTS invariably leads to serious complications for both twins, including severe brain injury, neurodevelopmental delays, and death. However, with timely diagnosis and appropriate treatment, the outcomes for TTTS are significantly improved. The appropriate treatment for TTTS is fetoscopic laser coagulation of the vascular anastomoses, a minimally invasive surgical procedure that was developed in the 1990s. In this procedure, a fetoscope, containing a tiny camera and laser fibre, is introduced into the amniotic sac of the recipient, where the connecting blood vessels between the twins are coagulated with laser to prevent further unequal blood flow. Laser surgery has improved perinatal survival rates as well as neonatal and long-term neurodevelopmental outcomes. The Leiden University Medical Center (LUMC) serves as the Dutch national referral center for fetal therapy and fetoscopic laser surgery was introduced here in the year 2000. Given the ongoing developments in the fields of monochorionic twin complications, fetal therapy, and neonatology over the last decades, the evaluation of neonatal and long-term outcomes after laser treatment for TTTS remains of utmost importance.

This thesis deals with matters of the brain in TTTS, as we investigated brain injury and neurodevelopmental outcome in TTTS fetuses, neonates and children. **Part one** of the thesis introduces the subject by providing a detailed case description of a twin pair affected by TTTS, where the recipient twin suffered severe brain injury resulting in long-term neurological sequelae. A review of the literature on TTTS with a special focus on neonatal cerebral injury and long-term neurodevelopment is described in **part two**. In **part three**, brain injury in TTTS is examined through studies exploring the incidence, types, and risk factors of brain injury in fetuses and neonates. The long-term neurodevelopmental outcomes of children affected by fetal and neonatal brain injury after TTTS are reported. Further insights into long-term outcomes are provided in **part four**, which includes two studies

using standardized tests to assess both mild and severe neurodevelopmental impairment (NDI), as well as behavioral problems in TTTS survivors. Finally, **part five** of the thesis delves into the rates of intentional fetal and neonatal demise in TTTS following the introduction of fetoscopic laser therapy at the LUMC.

Chapter 1 provides a review of the literature summarizing the latest insights regarding TTTS. It covers knowledge gained from placental injection studies, prenatal management including advancements in laser techniques, as well as fetal and neonatal complications, with extra attention given to brain injury and long-term neurodevelopmental outcome. The review concludes with suggestions for targets for further improvements in the management of the disease, emphasizing the importance of long-term follow-up for TTTS survivors, as well as the centralization of care in specialized fetal therapy centers.

In *chapter 2*, we assessed the incidence and risk factors for severe brain lesions in a case-control study of monochorionic twins with TTTS treated with fetoscopic laser surgery. We used dichorionic twins matched for gestational age as a control group and analyzed postnatal cranial ultrasonography results. We included 267 TTTS neonates treated between 2004 and 2011, matched with 267 dichorionic controls from the same period. The incidence of severe cerebral injury in the TTTS group and control group was 9% and 7%, respectively. This difference was not statistically significant. The analysis of potential risk factors revealed that only gestational age at birth was independently associated with an increased risk for severe cerebral injury. More than half of brain lesions in the TTTS group were detected within 24 hours after birth, compared to only 17% in the dichorionic control group. We report the striking finding that four recipients in our TTTS group suffered an arterial stroke in the territory of the left middle cerebral artery (MCA), whereas no donors and none of the dichorionic twins experienced this rare complication. We concluded that the risk of severe brain injury in TTTS treated with laser is comparable to a matched dichorionic control group and is independently associated with prematurity. However, compared to dichorionic twins, brain injury in TTTS is significantly more likely to occur before birth.

In *chapter 3*, our aim was to investigate brain injury in more detail by examining different types of brain injury in TTTS treated with laser surgery occurring before and after birth, the imaging modalities employed, and the long-term neurodevelopmental outcomes associated with various types of brain injuries. We again studied potential risk factors for brain injury. We categorized brain injuries into eight predefined groups, divided into 'diffuse'

and 'focal' categories. The study cohort consisted of 466 TTTS pregnancies treated between 2010 and 2020. Brain MRI was performed in only 3% of pregnancies and 4% of neonates; therefore, the majority of findings were based on fetal and neonatal brain ultrasonography. Brain injury was diagnosed in 2% of TTTS fetuses and 5% of neonates, with all predefined injury groups present. Among cases with fetal brain injury, fetal death (spontaneous or intentional) occurred in 69%. In neonates with a postnatal diagnosis of brain injury, the neonatal mortality rate was 22%. Neurodevelopmental impairment (NDI) was present in 31% of long-term survivors with brain injury. We identified two risk factors for brain injury: recurrent TTTS/post-laser twin anemia polycythemia sequence (TAPS) and lower gestational age at birth. A novel finding of this study was that cerebellar hemorrhages were a quite frequent finding, both antenatally and postnatally. We suspect that this is due to better recognition resulting from advancements in imaging techniques, as well as the increased survival of extremely preterm infants, who are at the highest risk for this complication in the neonatal period. In conclusion, based on the mix of diffuse and focal brain injuries found in fetuses and neonates, various mechanisms are likely responsible for the occurrence of brain injury in TTTS treated with laser surgery, and the presence of brain injury is associated with a high likelihood of NDI or death. Cases with recurrent TTTS or post-laser TAPS and/or lower gestational ages at birth are at increased risk of brain injury. Additionally, the true incidence of brain injury remains uncertain due to the limited use of MRI. Because of the risk of cerebellar hemorrhage in TTTS, special attention to the fossa posterior is advised.

In *chapter 4*, we examined the incidence of NDI in TTTS survivors treated with laser surgery from 2011 to 2014 and compared their outcomes to a previous cohort treated from 2008 to 2010. Neurological, cognitive, and motor development of the children were evaluated at two years of age using the Bayley Scales of Infant and Toddler Development (third edition), and risk factors associated with Bayley-III scores were determined. Our findings showed that severe NDI was observed in 3% of survivors in the new cohort compared to 6% in the previous cohort, although this difference did not reach statistical significance. Similarly, the rate of disease-free survival, defined as survival without severe NDI, did not significantly differ between the two cohorts. Mild NDI, defined as cerebral palsy GMFCS grade 1 and/or a cognitive or motor composite score between 1 standard deviations (SD) and 2 SD below the mean, was found in 23% of children in the new cohort. Low birth weight and being small for gestational age (SGA) were independently associated with lower cognitive scores, while severe cerebral injury was related to lower motor scores. Importantly, children with severe NDI were born at or after 32 weeks

of gestation in 53% of cases and had no evidence of cerebral injury on cranial ultrasound in 59% of cases. This suggests that neither gestational age above 32 weeks nor the absence of cerebral injury necessarily preclude severe NDI. We conclude that despite advancements in TTTS management, outcomes seem to have plateaued. Low birth weight, SGA, and severe cerebral injury remain significant risk factors for poor neurodevelopmental outcomes in children treated with laser surgery for TTTS. While the incidence of severe NDI has decreased, mild NDI is common in TTTS survivors. These mild problems should not be overlooked, as they can interfere with daily life across the lifespan, including school functioning, learning and everyday activities.

In *chapter 5*, we aimed to fill in part of the knowledge gap concerning the milder long-term consequences of TTTS. This study was the first to examine behavioral outcomes of twins who underwent fetoscopic laser coagulation treatment. Behavioral assessments at the corrected age of two years using the Child Behavior Checklist (CBCL) were evaluated in over 400 TTTS survivors treated at the LUMC between 2008 and 2015. The CBCL is a tool used to assess behavioral and emotional problems in children that is completed by parents or caregivers. The results of the CBCL provide scores on two broadband scales: internalizing problems (such as anxiety and depression), and externalizing problems (such as aggression and rule-breaking behavior), as well as a total problems score. Key findings from our study revealed that behavioral problems were reported in 8% of TTTS survivors, without a difference between donors and recipients. This incidence is comparable with that observed in the general population. Furthermore, the incidence of both cognitive and motor impairment was increased among children with behavioral problems. A higher level of maternal education was associated with fewer behavioral problems in their children. Since a considerable proportion of mothers in the study had a high level of education compared to the general population, and since the children included in the study had a higher birth weight and were more often born at term compared to those lost to follow-up, the incidence of behavioral problems may have been underestimated. While assessing behavioral problems at such an early age does not predict all problems that may manifest later in life, early identification and intervention for behavioral problems in TTTS survivors, particularly those with severe developmental delays, may be crucial for improving long-term outcomes.

The study outlined in *chapter 6* of this thesis aimed to assess the incidence and reasons behind intentional fetal and neonatal demise in TTTS managed at the LUMC between 2000 and 2014. The rationale for this study was the hypothesis that the observed improvement in long-term neurodevelopmental outcomes could be attributed, at least in part, to heightened awareness and

detection of cerebral injury due to growing expertise in TTTS management, potentially leading to an increase in intentional demise over time. Intentional demise was defined as termination of pregnancy, selective fetal reduction, or withdrawal of neonatal intensive care. Our findings indicated that a considerable portion of TTTS families were affected by some form of intentional demise, occurring in 17% of TTTS pregnancies and affecting 10% of fetuses/neonates. Comparison across three consecutive 5-year sub-cohorts failed to reveal a difference in the rates of intentional demise over time. The main reasons for intentional demise were complications or technical challenges during the laser procedure, severe fetal abnormalities, and significant neonatal complications arising from TTTS and/or prematurity. Severe brain injury was the reason for intentional demise in 22% (24/110) of fetuses and neonates. In essence, the decisions surrounding intentional demise were driven by poor prognoses, underscoring their potential impact on long-term neurodevelopmental outcome. Overall, this study sheds light on the determinants of intentional fetal and neonatal demise in TTTS cases in The Netherlands.

General discussion & future directions

Twin-twin transfusion syndrome (TTTS) was first described by the German obstetrician Friedrich Schatz in 1875 as a complication specific to identical twins, caused by a shared circulation between the two fetuses.(1) He demonstrated intertwin anastomoses by injecting placental vessels with colored solutions and was the first to realize their potentially harmful effects.

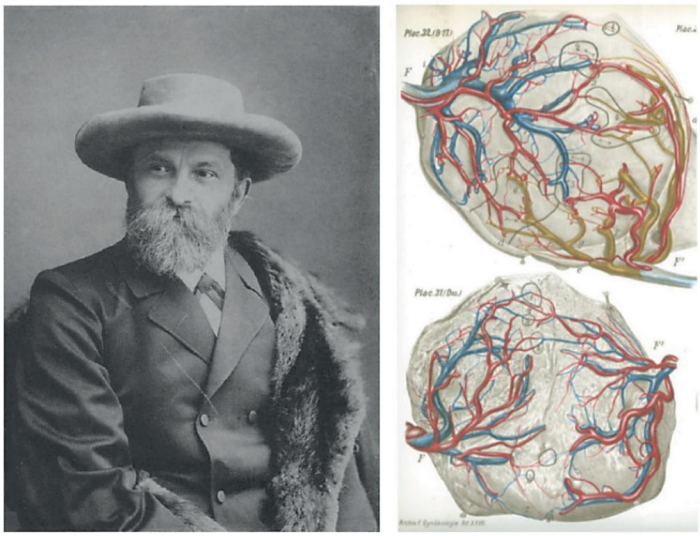


Figure 1. *Left:* Friedrich Schatz, late 19th century (2) *Right:* Schatz's drawings of placental vascular anastomoses in monochorionic twin placentas, injected with colored solutions (3)

For a long time, TTTS was a diagnosis with very little hope of a favorable outcome. A revolutionary new therapy was developed in the late 20th century and fetoscopic laser coagulation of the vascular anastomoses has become the preferred treatment for TTTS.(4) Fetoscopic laser surgery has been demonstrated to be the best available therapy in terms of perinatal survival rate, gestational age at delivery, and the risk of brain injury and long-term neurodevelopmental impairment (NDI).(5-8) Perinatal survival rates have continued to improve in recent decades, possibly related to the ongoing refinement of laser techniques, as well as the growing experience and awareness within the fields of fetal therapy and neonatology.(9) Consequently, the majority of fetuses now survive TTTS and go on to lead full lives. That does not mean that those lives are not influenced by their prenatal history,

as TTTS infants remain susceptible to complications. Fetal and neonatal morbidities due to TTTS are not fully prevented by laser surgery and include cerebral, cardiovascular, renal, intestinal and hematologic disease, as well as rare occurrences such as in utero acquired limb ischemia and amniotic band syndrome.(10) Brain injury may be caused by prematurity, TTTS, or both, increasing the risk of long-term NDI. The primary objective in every TTTS pregnancy should be survival of both twins without the occurrence of long-term NDI.

The aim of this thesis was to provide insight into the risks of fetal and neonatal brain injury, along with the long-term neurodevelopmental outcomes of survivors of TTTS in the contemporary era of fetoscopic laser surgery. To achieve this aim, we conducted a series of studies examining fetal, neonatal, and pediatric follow-up data from the Leiden University Medical Center (LUMC).

Brain injury

Incidence

The ever-growing experience in the management of monochorionic pregnancies in general and TTTS in particular have led to a slow but steady decline in the incidence of brain injury in this potentially catastrophic disease. While the introduction of laser surgery as the primary treatment for TTTS clearly marks the beginning of the fall in brain injury incidence, further developments in the management of TTTS pregnancies and in neonatology have likely contributed to its further decrease.(9) Reported rates of brain injury after treatment with laser surgery vary between studies due to inconsistencies in definitions and screening regimens, and range from 3 to 16%.(7, 11) Our cerebral injury study outlined in *chapter 2* was performed in the largest cohort of TTTS survivors treated with laser surgery to date and we uniquely used a control group of dichorionic twins matched for gestational age. We compared the incidence of severe cerebral injury on neonatal cranial ultrasound scans and found that the risk of brain injury was 9% in the TTTS neonates after laser surgery performed between 2004 and 2011, which was not different from the 7% incidence found in the dichorionic twins. Compared to an earlier cohort from our center however, the incidence had clearly dropped, being 14% in TTTS neonates treated between 2002 and 2005.(12) This trend continued in our latest study which evaluated brain injury and detected an overall incidence of brain injury of 6% in liveborn neonates with known neuroimaging results

(*chapter 3*). These numbers are in line with other more recent reports.(13, 14) Some authors have looked exclusively at fetal brain injury, eliminating the influence of prematurity on perinatal brain damage. The results from our *chapter 3* study align with a recent systematic review, both showing a 2% incidence of fetal brain lesions.(15) Most studies in this review, as well as our own study, rely predominantly on the use of ultrasound and only partly on MRI. Therefore the true incidence of fetal brain injury in TTTS is probably underestimated, as MRI has been shown to have additional value in determining fetal brain abnormalities, including subtle injuries and disturbed brain growth.(16) Studies using MRI exclusively have reported antenatal brain injury in TTTS fetuses treated with laser surgery in 2-11% of fetuses.(17-19)

Risk factors and proposed mechanisms

Brain lesions in TTTS patients can be acquired both antenatally and postnatally. The most important risk factor for fetal and neonatal brain injury is to not be treated with laser surgery.(7) This information may seem irrelevant in the current era because laser surgery is now the primary treatment performed for TTTS. However, it is important for neonatologists to realize that laser treatment is not always available or possible for several potential reasons, including late presentation, technical impossibility to perform the procedure or fetal distress requiring prompt cesarean section. Preterm TTTS infants not treated with laser surgery have a significantly higher risk of mortality and severe neonatal morbidity, including severe cerebral injury.(20) Fetuses not treated with laser are exposed to hemodynamic imbalance, anemia or polycythemia for a longer period of time and they are born while still in an unstable hemodynamic state. A principal factor contributing to preterm brain injury in general is the inadequate cerebral autoregulation present in the immature brain. Severe hemodynamic instability is therefore very likely to result in perinatal brain injury in these babies. This thesis deals with TTTS patients who were treated with laser surgery. Other authors have found increased rates of fetal brain injury in cases with recurrent TTTS or TAPS after laser surgery, both complications seen as a result of residual anastomoses.(17) We were able to confirm this finding in *chapter 3*. In this study, we analyzed both fetal and neonatal brain injury and aimed to identify risk factors for brain injury. Recurrent TTTS or post-laser TAPS resulted in a three-fold higher brain injury risk in this large cohort of over 900 TTTS fetuses. Our findings emphasize the need for ultrasonographic follow-up after laser surgery.

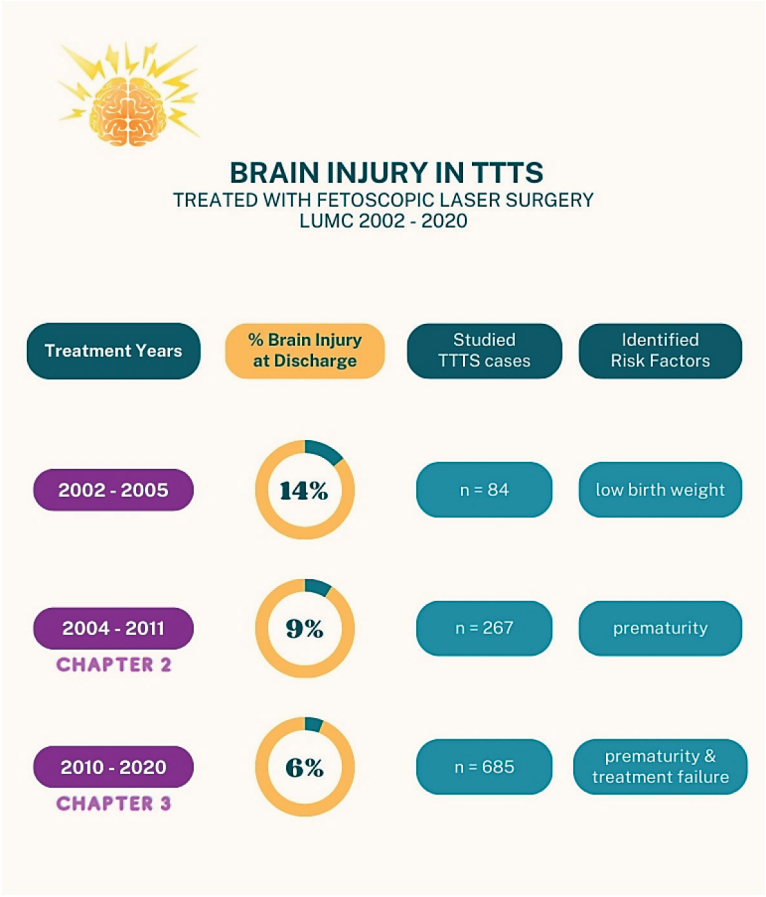


Figure 2. Overview of the incidence of brain injury at discharge in liveborn TTTS infants treated at the LUMC in the past two decades.(12, 21, 22)

Treatment failure: recurrent TTTS or post-laser TAPS

As the majority of TTTS survivors are born preterm, their risk for prematurity-related cerebral injury is also increased. Of the factors identified to have an association with brain injury in TTTS survivors, prematurity is indeed the most important one.(11, 14) The findings presented in *chapters 2 and 3* confirm prematurity as the most important factor associated with neonatal brain injury. Other factors that could potentially be related to the risk of brain injury, including donor or recipient status, TTTS stage and gestational age at laser surgery, were not related to the risk of cerebral injury. The fact that prematurity

is the principal factor related to brain injury in TTTS survivors does not mean that all brain injury is prevented if we can just get these pregnancies to last until term. A proportion of brain injury that is diagnosed after birth, actually has its origin in the fetal period. This statement is based on the observations in *chapter 2* that, although the incidence of cerebral injury in TTTS survivors treated with laser was comparable to the dichorionic twin control group, TTTS neonates were eight times more likely to already have these lesions on their first cranial ultrasound scan performed within 24 hours after birth. A similar observation was made in an earlier report from our center, in which the control group consisted of monochorionic twins without TTTS.(12) Also, several cases with brain injury diagnosed after birth, presented in *chapter 3*, were consistent with ischemic events that occurred during the fetal period, based on the type and timing of injuries.

Types of brain injury

Although many previous authors have described brain injury occurring in the context of TTTS, the pathophysiologic mechanisms behind most injuries have still not been fully elucidated. Most studies have only reported severe intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL) as outcome measures in TTTS infants.(5, 23, 24) In fetal neuroimaging studies, reported brain lesions include diffuse lesions linked to early in utero hypoxia-ischemia, like multicystic encephalomalacia, cystic PVL, cerebral atrophy and even migrational and gyrational disorders.(25) Focal lesions, including IVH and focal infarctions are also described. More recent studies using MRI have reported additional brain abnormalities in TTTS, including sinovenous thrombosis, milder forms of white matter injury and significant differences in biometric measurements of different brain areas in TTTS fetuses.(17-19, 26-29) Details about types and timing of different brain injuries can give us more insight into the mechanisms behind their occurrence, but the way we plan, assess and report on neuroimaging results is crucial for the interpretation of the data we acquire. In *chapter 2* of this thesis, a remarkable finding was that four former recipients in the TTTS group suffered middle cerebral artery (MCA) strokes, whereas this abnormality was not detected in donors or in infants from the dichorionic control group. In *chapter 3*, we detected two additional cases of MCA stroke in recipients and again, none in donors. The diagnosis was made on fetal imaging in one of these cases and in the other case, cranial ultrasound on the first day of life showed a large area of tissue loss in the left MCA territory, implying that an MCA stroke must have occurred weeks earlier when the patient was still in the womb. Although Benders et al. had previously established that TTTS is an important risk factor for arterial

stroke in preterm infants, no details about mode of treatment for TTTS nor donor/recipient status were given for the stroke cases in their study.(30) In their large fetal MRI study, Stirnemann et al. also found two cases of MCA stroke in recipients, and none in donors.(17) Furthermore, an earlier fetal brain imaging study by Quarello reported one recipient to have a 'unilateral clastic brain lesion', which is probably an arterial stroke too.(26) After the completion of our neuroimaging study, we found another similar patient: Rosie, who was also a former recipient and whose injury, based on the timing of detected abnormalities, probably originated before or around laser surgery. Although a widening of her left lateral ventricle had been noted on ultrasonography nine days after laser surgery, it was not clear at that point how serious Rosie's injury really was. Her case demonstrates the importance of repeated neuroimaging in TTTS fetuses throughout the pregnancy in order to timely diagnose potentially devastating injuries. In the literature about cerebral injury in TTTS, we did not find any cases of arterial stroke in donor twins. Thus, specifically recipient twins appear to be at risk for this type of brain injury in TTTS. The exact mechanism of arterial ischemic stroke in recipient twins remains uncertain, but we hypothesize that it may be related to vascular sludging due to polycythemia and hyperviscosity. Another possibility is that fetal volume overload and hypertension due to TTTS lead to endovascular changes that increase the risk for stroke. Others have suggested that thrombo-embolic phenomena resulting from the TTTS disease process or fetoscopic laser surgery may be responsible for focal ischemic damage in TTTS fetuses.(31)

When we investigated the types of brain injury in TTTS fetuses and neonates treated with laser surgery in *chapter 3*, one other type of injury stood out. We detected four antenatal and four postnatal cases with cerebellar hemorrhage (CBH), while only one other report in the literature had previously described a single fetal TTTS case with this type of injury.(27) An association between CBH and fetal anemia with intrauterine blood transfusions (IUT) has been reported in two small case series.(32, 33) We suspect that the increased recognition of CBH is due to advancements in prenatal and postnatal neuroimaging techniques. Our findings implicate that the cerebellum deserves special attention in TTTS patients both antenatally and postnatally.

We attempted to improve our understanding of the mechanisms behind brain injury in TTTS by dividing the types of brain injury into two groups: 'diffuse' and 'focal' types of brain injury. The reason behind this division was that cerebral hypoperfusion and anemia probably cause diffuse, symmetrical brain injury such as multicystic encephalomalacia and cystic PVL, whereas hyperviscosity due to polycythemia and possibly thrombo-emboli would most likely lead to focal brain injury. This division is of course somewhat

artificial and far from perfect, as for instance IVH and CBH are characterized as focal injury types, while it is well-known that fluctuations in cerebral blood flow combined with impaired cerebral autoregulation are an important underlying mechanism for the occurrence of IVH and CBH in preterm infants. Although diffuse injury types were more common prenatally and in donors, the numbers of patients with brain injury were too small to detect any certain risk profiles for specific injury types.

Use of magnetic resonance imaging

In our institution, brain MRI is not routinely performed in TTTS fetuses or (preterm) neonates. In our fetal and neonatal neuroimaging study described in *chapter 3*, we established that fetal brain MRI was performed in 3% of TTTS pregnancies between 2010 and 2020. In the same period, just 4% of surviving TTTS neonates underwent brain MRI in the neonatal period or at term-equivalent age (TEA). While recent MRI studies have demonstrated the potential additional value of fetal MRI over neurosonography alone, the question remains whether routine fetal MRI in TTTS is warranted, especially in cases with seemingly uncomplicated, successful laser treatment and normal fetal neurosonography findings.(34) An important consideration here is that the ideal timing of MRI for the detection of fetal brain injury is often in the late second or early third trimester. This is because laser surgery is generally performed in the second trimester and there should be an interval of several weeks between surgery and MRI to allow for the optimal detection of cystic lesions following ischemic events. In the Netherlands, legal restrictions limit the possibility of termination of pregnancy or selective fetal reduction after 24 weeks of gestation. Consequently, conducting an MRI at this stage would typically be too late to contribute to informed decisions regarding the (dis-) continuation of the pregnancy. However, even in these cases, a fetal brain MRI can provide professionals and parents with more certainty about the type and suspected severity of brain injury in the fetus, allowing for more detailed counseling and sometimes, reassurance. In our neuroimaging study, we identified an ex-donor who was diagnosed with bilateral perisylvian polymicrogyria on a postnatal MRI scan. This MRI was made after suspicions of abnormal gyration seen on postnatal, but not prenatal, cerebral ultrasound. This case exemplifies a scenario in which a fetal MRI scan would likely have detected the abnormality, allowing for informed preparation of the parents regarding this severe brain abnormality. Furthermore, this diagnosis at an earlier stage might have influenced decisions regarding delivery and NICU management. The same holds true for Rosie's case: a fetal MRI would have led to an earlier diagnosis, better equipping her parents for the journey ahead.

Whether an earlier diagnosis would have influenced treatment decisions made by obstetricians or neonatologists in her case is unsure, given that laser surgery was performed at 21 weeks, and an MRI would likely have been scheduled after 24 weeks of gestation. In our view, the findings from our studies suggest that while we lack sufficient data to strongly recommend routine fetal brain MRI in TTTS treated with laser surgery, lowering the threshold for fetal MRI is likely wise, particularly in cases with incomplete laser surgery or when changes in fetal brain morphology are observed during repeated ultrasound examinations.

There is something else we need to consider. While ultrasound is primarily suited for the detection of overt brain lesions, image quality has significantly improved in recent years and newer ultrasound machines now offer increasingly detailed images. In postnatal neurosonography, the use of additional acoustic windows provides the opportunity to visualize areas of the neonatal brain that are difficult to assess through the anterior fontanelle, such as the posterior fossa. Owing to these advancements, more abnormalities can be detected by ultrasound than before. Similarly, the field of fetal neurosonography has been evolving. "Advanced neurosonography" involves generating images of the fetal brain in the coronal and sagittal planes, in addition to the 'basic' axial plane assessment that has been widely used in clinical practice as well as in research. This technique significantly improves the detection of fetal brain anomalies, according to a study of fetuses at risk for acquired brain lesions.(35) In our center, this technique has become standard for the evaluation of TTTS fetuses only a few years ago. Previously, fetal brain ultrasound screening was limited to the axial plane, and additional planes were only evaluated when abnormalities were suspected. The results of our neuroimaging study in *chapter 3* have to be interpreted with this in mind; had we consistently applied advanced neurosonography to all TTTS cases, as is our current practice, we might have detected more brain lesions antenatally.

Neurodevelopmental outcome

Incidence

Based on the table on the following page, it is evident that several colleagues in the field have assessed the long-term neurodevelopmental outcomes following laser treatment for TTTS. Nevertheless, inclusion criteria, follow-up rates, age at follow-up and methodologies vary among studies, complicating the interpretation and comparison of results. The organization of a structured long-term follow-up program is a challenging task, requiring financial resources,

Table 1. Studies of NDI and cerebral palsy (CP) in TTTS treated with laser surgery (8, 13, 36-49)

Reference	Country	Age (y)	Patients	NDI %	CP %
Sutcliffe 2001	UK	2	66	9	9
Banek 2003	Germany	2-3	89	11	11
Graef 2006	Germany	2-4	167	8	6
Lenclen 2009	France	2	88	11	10
Lopriore 2009	Netherlands	2	278	18	6
Salomon 2010	France	5	73	-	12
Gray 2011	Australia	2-4	113	12	4
Graeve 2012	Germany	4-10	151	9	-
Van Klink 2014	Netherlands	2	155	6	3
McIntosh 2014	Australia	1-3	50	4	2
Vanderbilt 2014	USA	2	100	4	3
Tosello 2014	France	0-5	35	-	6
Korsakissok 2018	France	2-7	58	9	5
Schou 2019	Denmark	1-4	86	10	9
Chmait 2019	USA	2	99	4	3
Spruijt 2019	Netherlands	2	258	3	2
Overall				**9% (151/1741)**	**5% (93/1695)**
Range				**3-18%**	**2-12%**

adequate space, and highly trained professionals from different professional backgrounds. Throughout the years, we have consistently emphasized the importance of long-term follow-up for all children treated with fetal therapy, however we continuously face these same challenges, much like many of our colleagues in the field. In the Netherlands, a national guideline for the long-term follow-up of various groups of NICU graduates provides recommendations for multidisciplinary follow-up assessments. These include infants born before 30 weeks gestation or with a birth weight <1000 grams, those born SGA and with a birth weight <1500 grams, infants diagnosed with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia, and children who underwent fetal surgery. All children belonging to one of these groups are scheduled for follow-up appointments at 6, 12, and 24 months corrected age, as well as 5.5 and 8 years uncorrected age.(50) *Chapter 4* of this thesis describes the long-term follow-up study we performed to

determine the incidence of severe NDI among a large group of survivors after TTTS treatment with laser surgery between 2011 and 2014, comparing neurodevelopmental outcome at the corrected age of 24 months with the outcomes of a previous cohort from our center. For this study, we collected the neurodevelopmental assessments made using the Bayley Scales of Infant and Toddler Development. Despite noting that the absolute risk of severe NDI was 3% in the most recent cohort, compared to 6% in the previous one, we could not prove a significant improvement between the two time periods. Still, this may be viewed as a continuation of the positive trend observed in our center since the introduction of laser surgery.(43) Nevertheless, it is important to acknowledge that a neurodevelopmental evaluation at the age of 2 years can only partly predict (severe) impairment at a later age. This point was recently confirmed in a follow-up study from our group, which examined neurodevelopmental outcome in preterm TTTS survivors at the age of 5.5 years. This study revealed that severe NDI was present in 12% of these school-aged children, compared to only 3% when the same children were assessed at the corrected age of 2 years.(51) Although there is some overlap between the cohorts, an important distinction between the recent 5.5-year follow-up study and the study outlined in *chapter 4* lies in the composition of the cohorts. The 5.5-year follow-up cohort consisted only of preterm and/or small for gestational age (SGA) TTTS survivors, resulting in a lower mean gestational age of approximately 30 weeks and one-third of the study participants being classified as SGA. Preterm and SGA infants have consistently been invited for all follow-up visits, whereas there have been periods of time when financial and staffing constraints led to a higher loss-to-follow-up rate among (near-) term and appropriate for gestational age (AGA) TTTS survivors. In contrast, the cohort described in *chapter 4* describes a consecutive cohort of all TTTS survivors, with a mean gestational age of nearly 33 weeks and only 9% classified as SGA. This discrepancy indicates an underlying increased risk of NDI in the 5.5-year follow-up study, despite the prevalence of severe NDI at the age of two years appearing similar between both studies.

Risk factors for NDI

Fetoscopic laser treatment not only mitigates the risk of brain injury but also diminishes the risk of long-term NDI in survivors.(8, 40) Among the predictors of neurodevelopmental delay in children who underwent laser surgery for TTTS in utero are low gestational age at birth, low birth weight, higher TTTS stage and severe cerebral injury.(8, 13, 37, 40, 43-45, 48, 52) Studies into the comparative risk of NDI between donors and recipients consistently show a similar level of risk for both twins. Most studies have focused either on

assessing the risk of brain injury or on reporting long-term outcomes. As mentioned previously, there is a considerable degree of variation among studies regarding the definition of brain injury. Some studies report even minor abnormalities, while others focus solely on severe injuries associated with a high risk for long-term impairment, such as cystic PVL and high-grade IVH. Little is known about the long-term consequences of minor brain abnormalities reported in TTTS, a gap in knowledge that is particularly pronounced for prenatally detected injuries. Ideally, studies should integrate neuroimaging findings with long-term neurodevelopmental data to enable the accurate evaluation of the ramifications of specific types of brain injuries, whether minor or major. This is what we did in our neuroimaging study in *chapter 3*, where we not only established the incidence and types of brain injury but also documented the available long-term outcome data for all children affected by fetal and neonatal brain injury. Our results confirm the robust association between brain injury and subsequent NDI, with approximately one-third of children with brain injury manifesting severe NDI by the age of two years. Our study showed a very low survival rate among cases with fetal brain injury, mostly due to treatment decisions made because of fetal neuroimaging findings. The surviving children exhibited mild brain abnormalities and indeed had favorable neurodevelopmental outcomes. Nonetheless, we stress the importance of reporting the long-term outcomes of such cases—a practice lacking in the existing literature.

In *chapter 4*, we found that low birth weight and being classified as SGA are independently associated with lower cognitive scores on the Bayley Scales of Infant and Toddler Development. Moreover, severe brain injury had a significant negative effect on motor but not cognitive Bayley scores. While these risk factors demonstrated associations with neurodevelopmental outcomes, the association between gestational age at birth and cognitive Bayley scores did not reach statistical significance. This was probably caused by the fact that several children with severe NDI were born at gestational ages surpassing 32 weeks. Furthermore, although severe cerebral injury significantly increases the risk for NDI, the opposite is not always true: several children with severe NDI had had normal findings on neonatal cranial ultrasound examinations. These results once again emphasize the need for long-term follow-up for all TTTS survivors, independent of gestational age at birth and neonatal neuroimaging findings.

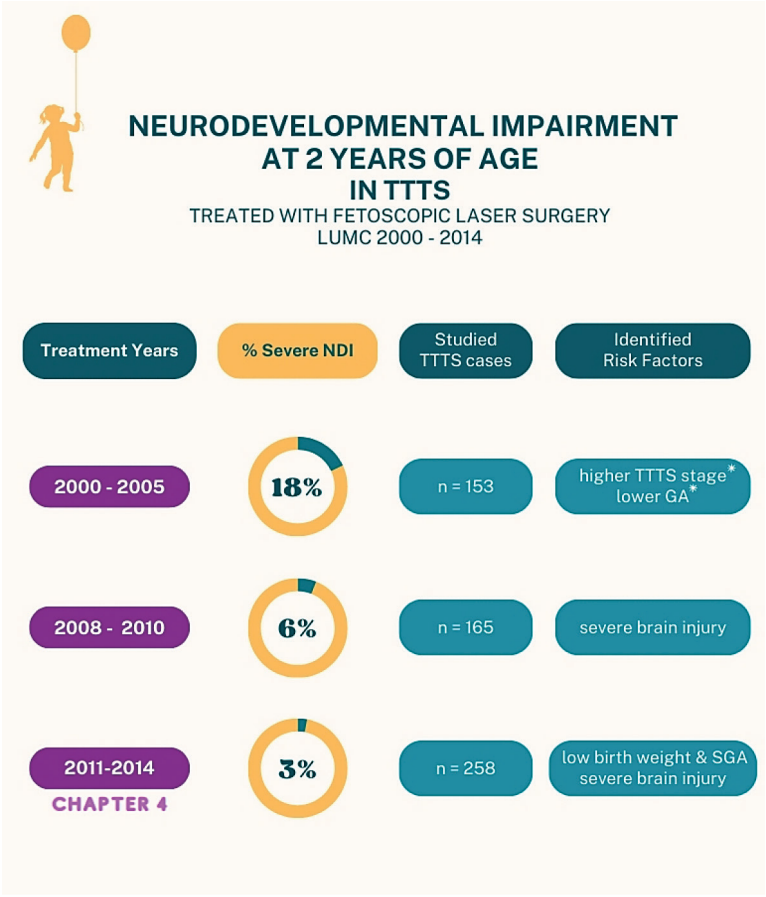


Figure 3. Overview of the incidence of severe NDI in TTTS survivors treated at the Leiden University Medical Center (43, 49)

* only trend toward an association of NDI with TTTS stage and GA (p = 0.08 for both)
GA, gestational age; SGA, small for gestational age

Mild or minor NDI

Owing to the improved survival rates and long-term neurodevelopmental outcomes observed in twins affected by TTTS, it is only logical that we delve a little deeper by examining not only severe neurological deficits, but also the incidence of mild impairments. Previous studies on the long-term outcomes after TTTS with laser surgery have reported instances of mild NDI with varying definitions and under various terminologies, including minor

neurological deficiencies, moderate impairment, or borderline development. Its incidence is reported to range between 8 and 29%.(13, 38, 42, 44) In *chapter 4* of this thesis, mild NDI was investigated in the largest cohort of TTTS survivors treated with laser surgery to date. We defined mild NDI as any of the following: cerebral palsy grade 1 according to the Gross Motor Functioning Classification System (GMFCS), or Bayley cognitive or motor composite score between 1 SD and 2 SD below the mean. The confirmed high rate of mild NDI of 23% underscores the importance of the use of standardized tests. These tests can assist in identifying the strengths and weaknesses in the development of each individual child, thereby aiding parents, teachers, and caregivers in finding the appropriate professional support, if needed.

Our study in *chapter 5* was the first-ever study to investigate behavioral outcome in TTTS survivors treated with laser surgery. Similar to those in *chapter 4*, the investigations in *chapter 5* took place when the children were 2 years old, corrected for prematurity. We demonstrated that the risk of behavioral problems at this age is comparable to the general population, but increased in children with cognitive as well as motor developmental delays. The risk of behavioral problems was mitigated by a higher level of maternal education. The 5.5-year follow-up study from our group described previously also included behavioral assessment at the school-age visit and found a higher rate of behavioral issues of 14% at the age of 5.5 years in preterm and SGA TTTS survivors.(51) Once more, these findings confirm that, while a thorough neurodevelopmental assessment around the age of two can detect the majority of children with severe impairments, the same cannot be said for mild impairments, particularly cognitive deficits, and behavioral issues.

Intentional demise

As we are well aware, TTTS is a condition characterized by significant mortality and morbidity rates, even in the era of fetoscopic laser surgery. While this surgical intervention aims to effectively eliminate the underlying cause of intertwin transfusion, it is not always feasible and may give rise to complications such as post-laser twin anemia polycythemia sequence (TAPS), preterm prelabor rupture of the membranes (PPROM), and (extremely) preterm birth. In circumstances where the prospect of a favorable outcome is limited due to severe feto-fetal transfusion at presentation, challenges in laser treatment or the emergence of post-surgery complications, discussions regarding end-of-life options become inevitable. These options may include termination of pregnancy (TOP) when prognosis is dire for both fetuses, selective fetal reduction (SFR) in cases where complications are limited to one twin or if technical challenges prevent successful laser surgery, or the decision

to withhold life-sustaining neonatal intensive care at birth, for instance when gestational age exceeds the legal termination limit. In some instances, NICU care may be withdrawn if severe complications arise in the neonatal period. While such decisions are unavoidable across fetal therapy centers and NICUs in different countries, cultural and legal factors shape the approach taken by clinicians and parents in addressing these delicate matters. End-of-life decisions during pregnancy and at the NICU impact morbidity and mortality rates, as children with the poorest prognoses do not survive when end-of-life decisions are made. We hypothesized that this phenomenon might partially explain the observed improvement in long-term neurodevelopmental outcomes over time. Are we becoming better at identifying children with unfavorable developmental trajectories, thus resulting in more frequent end-of-life decisions? In *chapter 6*, we examined the incidence of and reasons for what we termed 'intentional demise', a composite term encompassing TOP, SFR and withholding or withdrawal of NICU care. To our knowledge, no prior studies have reported rates of intentional demise in TTTS pregnancies. Our findings revealed that up to one in six women (17%) with a TTTS pregnancy experienced intentional fetal or neonatal demise, with this rate remaining consistent between 2000 and 2014. A frequent reason for intentional demise was severe brain injury, accounting for a total of 22% of cases of intentional demise. Brain injury was of antenatal origin in the majority of infants in whom NICU care was withheld or withdrawn for this reason. These findings hold significance for interpreting pregnancy outcomes and long-term neurodevelopmental trajectories following TTTS. We advocate for transparency regarding this sensitive topic in future publications on the outcome of TTTS pregnancies.

Future directions

While this thesis has shed more light on the incidence, timing, and long-term consequences of fetal and neonatal brain injury in TTTS, further questions remain to be answered, as is always the case in research.

Collaboration

To further improve outcomes for families affected by TTTS, collaboration between fetal and pediatric medicine specialists in future research endeavors is crucial. Challenges for fetal medicine primarily involve optimizing prediction and diagnosis of TTTS, along with refining technical aspects of laser surgery to minimize the risks of post-laser PPROM and residual anastomoses. Developments in fetal neuroimaging offer promise for improved detection of fetal brain injury. From a pediatric perspective, detailed neuroimaging and follow-up studies remain vital for understanding the significance of brain abnormalities diagnosed in utero or in the neonatal period. As with most things in life, timing is everything: follow-up studies should extend at least to school age in order to comprehensively assess the ongoing development in cognitive, motor, and psychosocial domains.

Neuroimaging

In many TTTS cases with brain injury, the exact timing and mechanisms of the injury remain unclear. Our research has shown that TTTS neonates are much more likely to exhibit brain injury diagnosed within 24 hours after birth (*chapter 2*), suggesting a higher incidence of antenatal injury compared to dichorionic twins. Future studies using repeated, advanced, multiplanar fetal neurosonography from the time of TTTS diagnosis until birth, along with standard third trimester fetal MRI, could greatly enhance our understanding of the mechanisms and timing of brain injury in TTTS fetuses. Ideally, advanced fetal neuroimaging should be followed by repeated neonatal cranial ultrasonography until term, as well as neonatal and/or term-equivalent MRI, to monitor the evolution of abnormalities throughout the brain's maturation process.

Given that the majority of studies investigating brain injury in TTTS to date lack specific descriptions of neuroimaging findings, discerning the true significance of such injuries across different studies poses a challenge. As established in *chapter 3* of this thesis, multiple TTTS survivors showed combinations of multiple types of injuries, making it challenging to categorize them for the sake of comparison between studies. The use of validated brain injury severity scores, such as the Kidokoro score for term-equivalent brain

MRI, could improve our comprehension of the severity of injuries in future studies on this subject.

Previous research consistently indicates that the risks of brain injury are equal for donors and recipients in TTTS. However, their intrauterine experience could be described as completely opposite from each other. Consequently, the mechanisms of brain injury likely differ for donors and recipients, especially when it occurs before or after incomplete laser surgery. In this thesis, we argue that MCA stroke appears to be a specific risk for TTTS recipients, although the exact mechanism of this injury remains uncertain. Future fetal and neonatal neuroimaging studies should prioritize investigating intertwin differences to better understand the processes underlying brain injury occurrence in TTTS donors and recipients. By unraveling these mechanisms, we may be able to develop targeted prevention strategies.

Long-term follow-up

As mentioned many times before, future neurodevelopmental follow-up studies in children born after fetal therapy for TTTS are indispensable for providing optimal care to these families. Future long-term follow-up studies focusing on children with both minor and major brain abnormalities may support the counseling of parents following the identification of brain injury through advanced fetal and neonatal neuroimaging techniques. Ensuring the inclusion of all TTTS infants in follow-up studies, rather than solely those deemed 'high risk,' is paramount.

Since the incidences of brain injury and severe NDI have notably declined over the past two decades, we have to redirect our focus and include milder forms of impairment in our follow-up studies. Particularly during school-age years and beyond, mild neurodevelopmental challenges and impaired psychosocial development, including behavioral problems, can profoundly impact children and their families.

A potential new direction of research could focus on the psychological well-being of families affected by TTTS and its effect on long-term developmental outcome in children. Studies have revealed that TTTS leads to severe anxiety and depression in the majority of mothers during and after pregnancy.(53) Moreover, perinatal anxiety has been identified as a significant predictor of adverse social-emotional development in children.(54) Future research exploring psychological support measures for families throughout pregnancy, delivery and infancy may help determine whether this effect can be alleviated.

Lastly, to establish the true effect of TTTS on fetal and neonatal brain injury as well as long-term neurodevelopmental outcomes, future research should ideally incorporate a control group of uncomplicated monochorionic twins.

Final conclusion

The findings of the studies conducted in this thesis, which investigated large consecutive cohorts of TTTS fetuses, neonates and children, indicate a decrease in the incidences of brain injury and neurodevelopmental impairment since the introduction of fetoscopic laser surgery. This decrease is accompanied by a shift towards milder forms of developmental issues. In the most recent decade, we may have reached the point where further improvement is hindered by two remaining risk factors. The first is prematurity, which we confirm as the most important factor associated with severe brain injury in infants affected by TTTS. Secondly, ongoing imbalanced intertwin transfusion through residual anastomoses after laser treatment, defined as recurrent TTTS or post-laser TAPS, poses a continued risk for the occurrence of brain injury. While prematurity is a risk factor for both severe brain injury and long-term impairment, our studies emphasize the necessity of neurodevelopmental follow-up for all TTTS survivors until at least school age, underlining that there is no such thing as a 'low risk' TTTS survivor. Much is still unknown concerning fetal and neonatal brain injury in TTTS, and we need to further increase our knowledge. It is our job to take care of the families affected by this devastating disease, and they deserve the best possible care based on the best possible research. Hopefully, for future generations, we can prevent TTTS from causing brain injury and neurodevelopmental sequelae or perhaps, even prevent TTTS altogether.

References

1. Schatz F. Eine besondere Art von einseitiger Polyhydramnie mit anderseitiger Oligohydramnie bei eineiigen Zwillingen. Archiv für Gynäkologie. 1882;19(3):329-69.
2. Prochownik N. Nachruf auf Friedrich Schatz. 1920;113.
3. Schatz F. Die Gefässverbindungen der Placentakreisläufe eineiiger Zwillinge, ihre Entwickelung und ihre Folgen. Archiv für Gynäkologie. 1886;27:1-72.
4. Glennon CL, Shemer SA, Palma-Dias R, Umstad MP. The History of Treatment of Twin-to-Twin Transfusion Syndrome. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2016;19(3):168-74.
5. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine. 2004;351(2):136-44.
6. Rossi AC, D'Addario V. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome: a metaanalysis and review of literature. American journal of obstetrics and gynecology. 2008;198(2):147-52.
7. van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E. Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal diagnosis and therapy. 2013;33(2):81-9.
8. Lenclen R, Ciarlo G, Paupe A, Bussieres L, Ville Y. Neurodevelopmental outcome at 2 years in children born preterm treated by amnioreduction or fetoscopic laser surgery for twin-to-twin transfusion syndrome: comparison with dichorionic twins. American journal of obstetrics and gynecology. 2009;201(3):291.e1-5.
9. Akkermans J, Peeters SH, Klumper FJ, Lopriore E, Middeldorp JM, Oepkes D. Twenty-Five Years of Fetoscopic Laser Coagulation in Twin-Twin Transfusion Syndrome: A Systematic Review. Fetal diagnosis and therapy. 2015;38(4):241-53.
10. Lopriore E, Oepkes D, Walther FJ. Neonatal morbidity in twin-twin transfusion syndrome. Early human development. 2011;87(9):595-9.
11. Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstetrics and gynecology. 2011;118(5):1145-50.
12. Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology. 2006;194(5):1215-20.
13. Korsakissok M, Groussolles M, Dicky O, Alberge C, Casper C, Azogui-Assouline C. Mortality, morbidity and 2-years neurodevelopmental prognosis of twin to twin transfusion syndrome after fetoscopic laser therapy: a prospective, 58 patients cohort study. Journal of gynecology obstetrics and human reproduction. 2018;47(10):555-60.
14. Vanderbilt DL, Schrager SM, Llanes A, Chmait RH. Prevalence and risk factors of cerebral lesions in neonates after laser surgery for twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2012;207(4):320.e1-6.
15. Sileo FG, Curado J, D'Antonio F, Benlioglu C, Khalil A. Incidence and outcome of prenatal brain abnormality in twin-to-twin transfusion syndrome: systematic review and meta-analysis. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2022;60(2):176-84.
16. Jarvis D, Mooney C, Cohen J, Papaioannou D, Bradburn M, Sutton A, et al. A systematic review and meta-analysis to determine the contribution of mr imaging to the diagnosis of foetal brain abnormalities In Utero. Eur Radiol. 2017;27(6):2367-80.
17. Stirnemann J, Chalouhi G, Essaoui M, Bahi-Buisson N, Sonigo P, Millischer AE, et al. Fetal brain imaging following laser surgery in twin-to-twin surgery. BJOG : an international journal of obstetrics and gynaecology. 2018;125(9):1186-91.

18. Aertsen M, Van Tieghem De Ten Berghe C, Deneckere S, Couck I, De Catte L, Lewi L. The prevalence of brain lesions after in utero surgery for twin-to-twin transfusion syndrome on third-trimester MRI: a retrospective cohort study. Eur Radiol. 2021;31(6):4097-103.
19. Hochberg A, Silber R, Avnet H, Rosen H, Katorza E, Hoffmann C, et al. Fetal and neonatal brain lesions following laser ablation for twin-to-twin-transfusion-syndrome as detected by pre- and post-natal brain imaging. Prenatal diagnosis. 2021;41(12):1531-40.
20. Lopriore EA, Slaghekke F, Verweij EJ, Haak MC, Middeldorp AJM, Lopriore E. Neonatal Outcome in Twin-to-Twin Transfusion Syndrome Not Treated with Fetoscopic Laser Surgery. Twin research and human genetics : the official journal of the International Society for Twin Studies. 2022;25(1):45-9.
21. Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2012;120(1):15-20.
22. Spruijt MS, van Klink JMM, de Vries LS, Slaghekke F, Middeldorp JM, Lopriore E, et al. Fetal and neonatal neuroimaging in twin-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2024.
23. Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, et al. Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. American journal of obstetrics and gynecology. 2007;196(5):450.e1-7.
24. Cincotta RB, Gray PH, Gardener G, Soong B, Chan FY. Selective fetoscopic laser ablation in 100 consecutive pregnancies with severe twin-twin transfusion syndrome. Aust N Z J Obstet Gynaecol. 2009;49(1):22-7.
25. Ascherl R, Sorge I, Thome U, Hirsch FW, Blaser A, Kiess W, et al. Severe gyration and migration disorder in fetofetal transfusion syndrome: two case reports and a review of the literature on the neurological outcome of children with lesions on neuroimaging. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2018;34(1):155-63.
26. Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2007;20(8):589-97.
27. Merhar SL, Kline-Fath BM, Meinzen-Derr J, Schibler KR, Leach JL. Fetal and postnatal brain MRI in premature infants with twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association. 2013;33(2):112-8.
28. Tarui T, Khwaja OS, Estroff JA, Robinson JN, Gregas MC, Grant PE. Altered fetal cerebral and cerebellar development in twin-twin transfusion syndrome. AJNR Am J Neuroradiol. 2012;33(6):1121-6.
29. Hecher K, Plath H, Bregenzer T, Hansmann M, Hackeloer BJ. Endoscopic laser surgery versus serial amniocenteses in the treatment of severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 1999;180(3 Pt 1):717-24.
30. Benders MJ, Groenendaal F, Uiterwaal CS, de Vries LS. Perinatal arterial stroke in the preterm infant. Seminars in perinatology. 2008;32(5):344-9.
31. Conte G, Righini A, Griffiths PD, Rustico M, Lanna M, Mackie FL, et al. Brain-injured Survivors of Monochorionic Twin Pregnancies Complicated by Single Intrauterine Death: MR Findings in a Multicenter Study. Radiology. 2018;288(2):582-90.
32. Simonazzi G, Bernabini D, Curti A, Bisulli M, Pilu G, Brill CB, et al. Fetal cerebellar damage in fetuses with severe anemia undergoing intrauterine transfusions. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(3):389-92.

33. Aziz NA, Peeters-Scholte CM, de Bruine FT, Klumper FJ, Adama van Scheltema PN, Lopriore E, et al. Fetal cerebellar hemorrhage: three cases with postnatal follow-up. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2016;47(6):785-6.
34. van Doorn M, Oude Rengerink K, Newsum EA, Reneman L, Majoie CB, Pajkrt E. Added value of fetal MRI in fetuses with suspected brain abnormalities on neurosonography: a systematic review and meta-analysis. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(18):2949-61.
35. van der Knoop BJ, Zonnenberg IA, Verbeke J, de Vries LS, Pistorius LR, van Weissenbruch MM, et al. Additional value of advanced neurosonography and magnetic resonance imaging in fetuses at risk for brain damage. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2020;56(3):348-58.
36. Sutcliffe AG, Sebire NJ, Pigott AJ, Taylor B, Edwards PR, Nicolaides KH. Outcome for children born after in utero laser ablation therapy for severe twin-to-twin transfusion syndrome. BJOG : an international journal of obstetrics and gynaecology. 2001;108(12):1246-50.
37. Banek CS, Hecher K, Hackeloer BJ, Bartmann P. Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2003;188(4):876-80.
38. Graef C, Ellenrieder B, Hecher K, Hackeloer BJ, Huber A, Bartmann P. Long-term neurodevelopmental outcome of 167 children after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2006;194(2):303-8.
39. Lopriore E, Ortibus E, Acosta-Rojas R, Le Cessie S, Middeldorp JM, Oepkes D, et al. Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2009;113(2 Pt 1):361-6.
40. Salomon LJ, Ortqvist L, Aegerter P, Bussieres L, Staracci S, Stirnemann JJ, et al. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. American journal of obstetrics and gynecology. 2010;203(5):444.e1-7.
41. Gray PH, Poulsen L, Gilshenan K, Soong B, Cincotta RB, Gardener G. Neurodevelopmental outcome and risk factors for disability for twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology. 2011;204(2):159 e1-6.
42. Graeve P, Banek C, Stegmann-Woessner G, Maschke C, Hecher K, Bartmann P. Neurodevelopmental outcome at 6 years of age after intrauterine laser therapy for twin-twin transfusion syndrome. Acta paediatrica (Oslo, Norway : 1992). 2012;101(12):1200-5.
43. van Klink JM, Koopman HM, van Zwet EW, Middeldorp JM, Walther FJ, Oepkes D, et al. Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology. 2014;210(6):540.e1-7.
44. McIntosh J, Meriki N, Joshi A, Biggs V, Welsh AW, Challis D, et al. Long term developmental outcomes of pre-school age children following laser surgery for twin-to-twin transfusion syndrome. Early human development. 2014;90(12):837-42.
45. Vanderbilt DL, Schrager SM, Llanes A, Hamilton A, Seri I, Chmait RH. Predictors of 2-year cognitive performance after laser surgery for twin-twin transfusion syndrome. American journal of obstetrics and gynecology. 2014;211(4):388 e1-7.
46. Tosello B, Blanc J, Haumonte JB, D'Ercole C, Gire C. Short and medium-term outcomes of live-born twins after fetoscopic laser therapy for twin-twin transfusion syndrome. Journal of perinatal medicine. 2014;42(1):99-105.
47. Schou KV, Lando AV, Ekelund CK, Jensen LN, Jorgensen C, Norgaard LN, et al. Long-Term Neurodevelopmental Outcome of Monochorionic Twins after Laser Therapy or Umbilical Cord Occlusion for Twin-Twin Transfusion Syndrome. Fetal diagnosis and therapy. 2019;46(1):20-7.

48. Chmait RH, Chon AH, Schrager SM, Llanes A, Hamilton AH, Vanderbilt DL. Neonatal cerebral lesions predict 2-year neurodevelopmental impairment in children treated with laser surgery for twin-twin transfusion syndrome. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2019;32(1):80-4.
49. Spruijt MS, Lopriore E, Tan R, Slaghekke F, Klumper F, Middeldorp JM, et al. Long-Term Neurodevelopmental Outcome in Twin-to-Twin Transfusion Syndrome: Is there still Room for Improvement? Journal of clinical medicine. 2019;8(8).
50. Rijken M, Werkgroep Landelijke Neonatale Follow-up. Aanbeveling Landelijke Neonatale Follow-up - NICU follow-up 2015.
51. Knijnenburg PJC, Spruijt MS, Jansen L, Rijken M, Tan R, Slaghekke F, et al. Neurodevelopmental Trajectories of Preterm Born Survivors of Twin-Twin Transfusion Syndrome: From Birth to 5 Years of Age. J Pediatr. 2022;240:51-7.e1.
52. Sananes N, Gabriele V, Weingertner AS, Ruano R, Sanz-Cortes M, Gaudineau A, et al. Evaluation of long-term neurodevelopment in twin-twin transfusion syndrome after laser therapy. Prenatal diagnosis. 2016;36(12):1139-45.
53. Falletta L, Fischbein R, Bhamidipalli SS, Nicholas L. Depression, anxiety, and mental health service experiences of women with a twin-twin transfusion syndrome pregnancy. Archives of women's mental health. 2018;21(1):75-83.
54. Polte C, Junge C, von Soest T, Seidler A, Eberhard-Gran M, Garthus-Niegel S. Impact of Maternal Perinatal Anxiety on Social-Emotional Development of 2-Year-Olds, A Prospective Study of Norwegian Mothers and Their Offspring : The Impact of Perinatal Anxiety on Child Development. Matern Child Health J. 2019;23(3):386-96.

Samenvatting

Tweelingtransfusiesyndroom, in de Engelstalige literatuur meestal twin-twin transfusion syndrome of TTTS genoemd, is een zeldzame aandoening die bij ongeveer 1 op de 10 monochoriale tweelingzwangerschappen voorkomt. Monochoriale tweelingen delen gezamenlijk één placenta en deze bevat vrijwel altijd vasculaire anastomosen, die de twee foetale circulaties met elkaar verbinden. TTTS ontstaat wanneer er netto een unidirectionele transfusie van bloed tussen de foetussen plaatsvindt via deze anastomosen. Als gevolg hiervan wordt de foetus die bloedvolume verliest, de donor genoemd, hypovolemisch en oliguur. Uiteindelijk kan de donorfoetus strak in de vliezen komen te zitten als gevolg van het ernstige oligohydramnion. In het Engels wordt deze foetus daarom soms de 'stuck twin' genoemd. Omgekeerd krijgt de foetus die teveel bloedvolume ontvangt, de ontvanger of recipiënt genoemd, last van een volume-overbelasting die resulteert in polyurie en een polyhydramnion. Deze combinatie van bevindingen op prenatale echografie wordt aangeduid als Tweeling Oligohydramnion Polyhydramnion Sequentie (TOPS) en dit is het hoofdkenmerk van de diagnose TTTS. Onbehandeld leidt TTTS onvermijdelijk tot ernstige complicaties voor beide foetussen, waaronder ernstige hersenschade, ontwikkelingsproblemen op de lange termijn en zelfs overlijden. Echter, een tijdige diagnose en de juiste behandeling kunnen de uitkomsten van TTTS sterk verbeteren. De beste behandeling voor TTTS is foetoscopische lasercoagulatie van de vasculaire anastomosen, een minimaal invasieve chirurgische ingreep die in de jaren 90 van de vorige eeuw werd ontwikkeld. Bij deze ingreep wordt een foetoscoop, die een kleine camera en laserfiber bevat, ingebracht in de vruchtzak van de ontvanger, waarna de verbindende bloedvaten tussen de foetussen worden gecoaguleerd met de laser om de disbalans in de bloedstromen een halt toe te roepen. Laserchirurgie verbetert de perinatale overlevingskansen, de neonatale uitkomsten én de lange termijn ontwikkelingsuitkomsten van kinderen met TTTS. Het Leids Universitair Medisch Centrum (LUMC) is het nationale verwijscentrum voor foetale therapie in Nederland en foetoscopische lasercoagulatie voor TTTS werd hier in het jaar 2000 geïntroduceerd. Gelet op de voortdurende ontwikkelingen binnen de behandeling van gecompliceerde monochoriale tweelingen, de foetale therapie en de neonatologie in de afgelopen decennia, blijft de evaluatie van de neonatale en lange termijn resultaten na laserbehandeling voor TTTS van groot belang.

Dit proefschrift behandelt 'hoofdzaken in tweelingtransfusiesyndroom': we beschrijven studies waarin hersenschade en lange termijn ontwikkelingsuitkomsten zijn onderzocht bij foetussen, neonaten en kinderen met TTTS. **Deel een** van het proefschrift introduceert het onderwerp met een gedetailleerde beschrijving van een casus van een tweelingpaar dat getroffen werd door

TTTS, waarbij de recipiënt ernstige hersenschade bleek te hebben, met gevolgen voor haar lange termijn ontwikkeling. Een review van de literatuur over TTTS met speciale aandacht voor neonatale hersenschade en lange termijn ontwikkeling wordt beschreven in **deel twee**. In **deel drie** wordt hersenschade bij TTTS onderzocht in studies die de incidentie, typen en risicofactoren voor hersenletsel bij foetussen en neonaten onderzoeken. Ook worden hier de ontwikkelingsuitkomsten van kinderen met foetale en neonatale hersenschade na TTTS beschreven. Meer inzicht in lange termijn resultaten wordt gegeven in **deel vier**, dat twee studies beschrijft die gestandaardiseerde tests hebben gebruikt om zowel milde als ernstige ontwikkelingsproblemen, evenals gedragsproblemen bij kinderen die TTTS overleefden te evalueren. Ten slotte gaat **deel vijf** van dit proefschrift in op de incidentie van 'intentioneel' foetaal en neonataal overlijden bij TTTS sinds de introductie van foetoscopische laserchirurgie in het LUMC.

Hoofdstuk 1 beschrijft een review van de literatuur waarin de laatste inzichten over TTTS worden samengevat. Het behandelt kennis opgedaan uit placenta-injectie studies, antenatale behandeling inclusief de verschillende ontwikkelingen in lasertechniek, evenals foetale en neonatale complicaties, met extra aandacht voor hersenschade en lange termijn ontwikkeling. De review wordt afgesloten met suggesties voor mogelijke richtingen om verdere vooruitgang te kunnen boeken in de behandeling van de ziekte, waarbij het belang van lange termijn follow-up voor TTTS patiënten wordt benadrukt, evenals de centralisatie van zorg in gespecialiseerde foetale therapiecentra.

In *hoofdstuk 2* worden de incidentie en risicofactoren voor ernstige hersenschade onderzocht in een case-control studie van monochoriale tweelingen met TTTS, behandeld met foetoscopische laserchirurgie. De TTTS-patiënten werden op basis van zwangerschapsduur gematcht met dichoriale tweelingen en de resultaten van postnatale hersenechografie werden vergeleken. In deze studie werden 267 TTTS-neonaten, behandeld tussen 2004 en 2011, geïncludeerd en gematcht met 267 dichoriale controlepatiënten uit dezelfde periode. De incidentie van ernstige hersenschade in de TTTS-groep en de controlegroep was respectievelijk 9% en 7%. Dit verschil was niet statistisch significant. De analyse van mogelijke risicofactoren laat zien dat alleen zwangerschapsduur bij geboorte onafhankelijk geassocieerd was met een verhoogd risico op ernstige hersenschade. Meer dan de helft van de hersenletsels in de TTTS-groep werd binnen 24 uur na de geboorte vastgesteld, vergeleken met slechts 17% in de dichoriale controlegroep. We beschrijven de opmerkelijke bevinding dat bij vier recipiënten in de TTTS-groep een arterieel ischemische stroke in het gebied van de linker arteria cerebri media werd vastgesteld, terwijl geen enkele donor en geen van

de dichoriale tweelingen deze zeldzame complicatie hadden. Geconcludeerd wordt dat het risico op ernstige hersenschade bij TTTS behandeld met laser vergelijkbaar is met een gematchte dichoriale controlegroep en dat vroeggeboorte de belangrijkste risicofactor is voor hersenschade. In vergelijking met dichoriale tweelingen treedt hersenschade bij TTTS significant vaker al vóór de geboorte op.

Het doel van de studie in *hoofdstuk 3* was om hersenschade in meer detail te onderzoeken, door de verschillende typen hersenschade bij met laserchirurgie behandelde TTTS foetussen en neonaten, de gebruikte beeldvormende modaliteiten, en de met deze hersenschade geassocieerde lange termijn ontwikkelingsuitkomsten te bestuderen. Ook werden opnieuw potentiële risicofactoren voor hersenschade onderzocht. De gevonden hersenafwijkingen werden gecategoriseerd in acht vooraf gedefinieerde groepen, die werden onderverdeeld in de categorieën 'diffuus' en 'focaal'. De onderzoekspopulatie bestond uit 466 TTTS-zwangerschappen behandeld tussen 2010 en 2020. Een MRI hersenen werd slechts bij 3% van de zwangerschappen en 4% van de neonaten verricht; derhalve is het grootste deel van de bevindingen gebaseerd op foetale en neonatale hersenechografie. Hersenschade werd gediagnosticeerd bij 2% van de TTTS-foetussen en 5% van de neonaten, waarbij alle vooraf definieerde typen hersenschade werden gevonden. Van alle foetussen met antenatale schade aan de hersenen trad (spontane of intentionele) foetale sterfte op in 69% van de gevallen. Onder neonaten met een postnatale diagnose van hersenschade was de neonatale mortaliteit 22%. Lange termijn ontwikkelingsproblemen waren aanwezig bij 31% van de overlevenden met hersenschade. In deze studie werden twee risicofactoren voor hersenschade geïdentificeerd: recidiverende TTTS/post-laser Tweeling Anemie Polycythemie Sequentie (TAPS) en een lagere zwangerschapsduur bij geboorte. Een nieuwe bevinding van deze studie was dat cerebellaire bloedingen, zowel prenataal als postnataal, in meerdere casus werden gevonden. Vermoedelijk is dit het gevolg van betere herkenning dankzij de vooruitgang in beeldvormende technieken, alsmede van de toegenomen overleving van zeer vroeggeboren neonaten, die het hoogste risico lopen op deze complicatie in de neonatale periode. Op basis van de resultaten wordt geconcludeerd dat verschillende mechanismen verantwoordelijk zijn voor het optreden van antenatale en postnatale hersenschade bij TTTS behandeld met laserchirurgie. De aanwezigheid van hersenschade is geassocieerd met een aanzienlijk risico op stoornissen in de lange termijn ontwikkeling of overlijden. Recidief TTTS/post-laser TAPS en een lagere zwangerschapsduur bij geboorte verhogen het risico op hersenschade bij de foetus of neonaat. De werkelijke incidentie van hersenschade in de onderzochte

populatie blijft onzeker vanwege het beperkte gebruik van MRI. Vanwege het risico op cerebellaire bloedingen bij TTTS wordt aanbevolen de fossa posterior bewust in beeld te brengen.

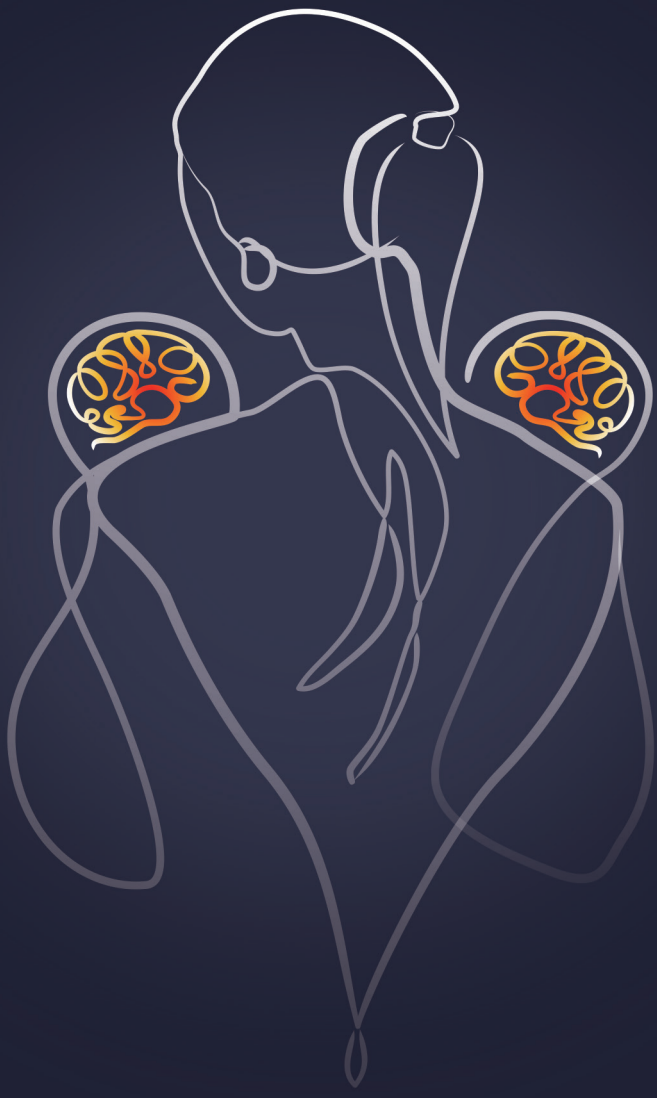
In *hoofdstuk 4* wordt de incidentie van lange termijn ontwikkelingsproblemen bij TTTS overlevenden, die tussen 2011 en 2014 met laserchirurgie werden behandeld, vergeleken met de uitkomsten van een eerder cohort, behandeld tussen 2008 en 2010. De neurologische, cognitieve en motorische ontwikkeling van deze kinderen werd op de leeftijd van 2 jaar onderzocht met behulp van de Bayley Scales of Infant and Toddler Development (derde editie), en risicofactoren geassocieerd met Bayley-III-scores werden onderzocht. In deze studie werden ernstige ontwikkelingsproblemen waargenomen bij 3% van de overlevenden in het nieuwe cohort vergeleken met 6% in het vorige cohort, hoewel dit verschil geen statistische significantie bereikte. Ook de ziektevrije overleving, gedefinieerd als overleving zonder ernstige ontwikkelingsproblemen, verschilde niet significant tussen de twee cohorten. Milde ontwikkelingsproblemen, gedefinieerd als cerebrale parese GMFCS graad 1 en/of een cognitieve of motorische score tussen -1 en -2 standaarddeviaties (SD) onder het gemiddelde, waren aanwezig bij 23% van de kinderen in het nieuwe cohort. Een (absoluut) laag geboortegewicht en een laag geboortegewicht voor de zwangerschapsduur (small for gestational age: SGA) waren onafhankelijk geassocieerd met lagere cognitieve scores, terwijl ernstige hersenschade gerelateerd was aan lagere motorische scores. Een belangrijke bevinding is dat kinderen met ernstige ontwikkelingsproblemen in 53% van de gevallen geboren werden na een zwangerschapsduur van tenminste 32 weken, en dat in 59% van de kinderen met ernstige ontwikkelingsproblemen geen ernstige hersenschade was gezien op hersenechografie. Dit suggereert dat noch een zwangerschapsduur boven de 32 weken, noch de afwezigheid van hersenschade op echo uitsluiten dat er later ernstige ontwikkelingsproblemen kunnen ontstaan. We concluderen dat ondanks de vooruitgang in de behandeling van TTTS, de resultaten een plateau lijken te hebben bereikt. Een laag geboortegewicht, SGA en ernstige hersenschade blijven significante risicofactoren voor problemen in de lange termijn ontwikkeling bij TTTS-overlevenden. Hoewel de incidentie van ernstige ontwikkelingsuitkomsten is gedaald, worden milde ontwikkelingsproblemen frequent gezien bij TTTS-overlevenden. Deze milde problemen moeten niet terzijde worden geschoven, aangezien ze verschillende aspecten van het leven, waaronder het functioneren op school, het leervermogen en vele dagelijkse activiteiten negatief kunnen beïnvloeden.

Het doel van *hoofdstuk 5* was om een deel van de kennislacune op het gebied van milde ontwikkelingsproblemen op de lange termijn op te vullen.

In deze studie werd voor het eerst het gedrag onderzocht van tweelingen die tijdens de zwangerschap een foetoscopische laserbehandeling hadden ondergaan. De gedragsonderzoeken van meer dan 400 TTTS-overlevenden, behandeld in het LUMC tussen 2008 en 2015 en verricht op de gecorrigeerde leeftijd van 2 jaar met behulp van de Child Behavior Checklist (CBCL), werden geëvalueerd. De CBCL is een instrument dat wordt ingevuld door ouders of verzorgers en waarmee gedrags- en emotionele problemen bij kinderen kunnen worden beoordeeld. De resultaten van de CBCL bestaan uit scores op twee brede schalen: internaliserende problemen (zoals angst en depressie), externaliserende problemen (zoals agressie en regelovertredend gedrag), evenals een totale probleemscore. Belangrijke bevindingen uit deze studie waren dat gedragsproblemen werden gerapporteerd bij 8% van de TTTS-overlevenden, zonder verschil tussen donoren en recipiënten. Deze incidentie is vergelijkbaar met de incidentie in de algemene populatie. Verder werd gezien dat de incidentie van zowel cognitieve als motorische ontwikkelings-problemen verhoogd was bij kinderen met problemen in het gedrag. Een hoger opleidingsniveau van de moeder bleek geassocieerd met een lagere kans op gedragsproblemen bij het kind. Aangezien een groot deel van de moeders in deze studie een hoog opleidingsniveau had vergeleken met de algemene populatie, en omdat de kinderen die in de studie werden geïncludeerd een hoger geboortegewicht hadden en vaker à terme waren geboren vergeleken met degenen die 'lost to follow-up' waren, is het mogelijk dat de incidentie van gedragsproblemen in deze studie wordt onderschat. Hoewel het beoordelen van gedragsproblemen op deze jonge leeftijd waarschijnlijk niet alle problemen voorspelt die zich later in het leven kunnen voordoen, kan vroege identificatie en interventie voor gedragsproblemen bij TTTS-overlevenden, met name degenen met een ernstige ontwikkelingsachterstand, cruciaal zijn voor het verbeteren van uitkomsten op de lange termijn.

De studie die in *hoofdstuk 6* van dit proefschrift wordt beschreven had tot doel om de incidentie en de redenen te beschrijven van 'intentionele' sterfte bij TTTS foetussen en neonaten, behandeld in het LUMC tussen 2000 en 2014. De rationale voor deze studie was de hypothese dat de waargenomen verbetering in lange termijn ontwikkelingsuitkomsten gedeeltelijk toegeschreven zou kunnen worden aan een toename van alertheid op en betere detectie van hersenschade dankzij de groeiende expertise in de behandeling van TTTS. Betere detectie van hersenschade zou kunnen kunnen leiden tot een toename van intentionele sterfte over de tijd. Intentionele sterfte werd gedefinieerd als een zwangerschapsafbreking, selectieve foetale reductie, of het niet starten, danwel staken van neonatale intensieve zorg. Deze studie laat zien dat een aanzienlijk deel van de vrouwen met een TTTS zwangerschap te maken kreeg

met een vorm van intentionele sterfte: dit kwam voor bij 17% van de zwangerschappen en 10% van de foetussen/neonaten. Wanneer drie opeenvolgende subcohorten van 5 jaar met elkaar werden vergeleken, werd geen verschil gevonden in de incidentie van intentionele sterfte over de tijd. De belangrijkste redenen voor intentionele sterfte waren complicaties of technische moeilijkheden tijdens de laserprocedure, ernstige foetale afwijkingen, en significante neonatale complicaties als gevolg van TTTS en/of prematuriteit. Ernstige hersenschade was de reden voor intentionele sterfte bij 22% (24/110) van de foetussen en neonaten. In essentie waren alle beslissingen rond intentionele sterfte gebaseerd op een verwachte slechte prognose. Dit betekent dat deze beslissingen inderdaad een mogelijk effect hebben op de lange termijn ontwikkelingsuitkomsten. Deze studie werpt licht op de determinanten van intentionele sterfte bij TTTS foetussen en neonaten in Nederland.



Appendices

Curriculum vitae
List of abbreviations
Publications
Dankwoord

Curriculum vitae

Marjolijn Spruijt werd op 23 november 1982 geboren in het Sint Radboud Ziekenhuis in Nijmegen, een half uur na haar tweelingbroer Thomas. Hoewel de antenatale echografie begin jaren '80 nog niet zo geavanceerd was, kon door het verschil in geslacht toch al voor de geboorte worden vastgesteld dat het een dizygote, en dus dichoriale tweeling moest betreffen.

In 2001 slaagde Marjolijn cum laude voor haar eindexamen gymnasium op de Nijmeegse Scholengemeenschap Groenewoud, waarna ze met haar studie geneeskunde mocht beginnen. De hoofdstad had een grote aantrekkingskracht op haar en zij koos dan ook voor de Universiteit van Amsterdam. In 2005 vertrok Marjolijn voor een wetenschapsstage naar Vancouver, Canada, waar ze onderzoek deed naar het geheugen voor gesproken taal bij pasgeborenen in het British Columbia Children's Hospital.

Na haar afstuderen aan de UvA in 2008 verhuisde Marjolijn naar Den Haag, om samen te gaan wonen met Pepijn én om haar carrière in de kindergeneeskunde te beginnen als arts niet in opleiding tot specialist (ANIOS) in het Westeinde ziekenhuis, en later in het Juliana Kinderziekenhuis. In 2011 ging ze als ANIOS aan de slag op de Neonatale Intensive Care Unit van het Leids Universitair Medisch Centrum (LUMC). In deze periode begon ze haar onderzoek naar de uitkomsten van kinderen met tweelingtransfusiesyndroom onder begeleiding van prof. dr. Enrico Lopriore. In het LUMC voltooide zij haar opleiding tot kinderarts in 2017 en haar fellowship neonatologie in 2019. Na een jaar als neonatoloog in het LUMC te hebben gewerkt, is Marjolijn sinds 2020 staflid neonatologie in het Radboudumc in Nijmegen.

Samen met hun kinderen Felix (2013) en Eva (2016) wonen Marjolijn en Pepijn in Berg en Dal.

List of abbreviations

AGA - appropriate for gestational age
ASQ - Ages and Stages Questionnaire
B - regression coefficient
Bayley-III - Bayley Scales of Infant and Toddler Development, third edition
CBCL - Child Behavior Checklist
CBH - cerebellar hemorrhage
CI - confidence interval
cUS - cranial ultrasonography
CP - cerebral palsy
DWI - diffusion-weighted imaging
DSM - Diagnostic and Statistical Manual of Mental Disorders
FLC - fetoscopic laser coagulation
GA - gestational age
GEE - Generalized Estimating Equations
GMFCS - Gross Motor Function Classification System
HIE - hypoxic-ischemic encephalopathy
HIFU - high-intensity focused ultrasound
IQR - interquartile range
IUFD - intrauterine fetal demise
IVH - intraventricular hemorrhage
LUMC - Leiden University Medical Center
MC – monochorionic
MCA-PSV - middle cerebral artery - peak systolic velocity
M-ABC - Movement Assessment Battery for Children
MoM - multiples of the median
MRI - magnetic resonance imaging
NDI - neurodevelopmental impairment
NICU - neonatal intensive care unit
OR - odds ratio
PHVD - post-hemorrhagic ventricular dilatation
PPROM - preterm prelabor rupture of membranes
PVHI - periventricular hemorrhagic infarction
PVL - periventricular leukomalacia
RCT - randomized controlled trial
SFR - selective fetal reduction
SGA - small for gestational age
SD - standard deviation
SPSS - Statistical Package for the Social Sciences

TAPS - twin anemia-polycythemia sequence
TEA - term-equivalent age
TOP - termination of pregnancy
TTTS - twin-to-twin transfusion syndrome
VM – ventriculomegaly
WISC - Wechsler Intelligence Scale for Children
WPPSI - Wechsler Preschool and Primary Scale of Intelligence
SWI - susceptibility-weighted imaging

Publications

Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology. 2012;120(1):15-20.

Spruijt MS, Tameeris E, Zhao DP, Middeldorp JM, Haak MC, Oepkes D, et al. Incidence and Causes of Intentional Fetal or Neonatal Demise in Twin-Twin Transfusion Syndrome. Fetal diagnosis and therapy. 2018;43(1):19-25.

Spruijt MS, Lopriore E, Tan R, Slaghekke F, Klumper F, Middeldorp JM, et al. Long-Term Neurodevelopmental Outcome in Twin-to-Twin Transfusion Syndrome: Is there still Room for Improvement? Journal of clinical medicine. 2019;8(8).

Brandsma FL, Spruijt MS, Rijken M, Tan R, Oepkes D, Lopriore E, et al. Behavioural outcome in twin-twin transfusion syndrome survivors treated with laser surgery. Archives of disease in childhood Fetal and neonatal edition. 2020;105(3):304-9.

Spruijt MS, Lopriore E, S JS, Slaghekke F, Van Klink JMM. Twin-twin transfusion syndrome in the era of fetoscopic laser surgery: antenatal management, neonatal outcome and beyond. Expert review of hematology. 2020;13(3):259-67.

Knijnenburg PJC, Spruijt MS, Jansen L, Rijken M, Tan R, Slaghekke F, et al. Neurodevelopmental Trajectories of Preterm Born Survivors of Twin-Twin Transfusion Syndrome: From Birth to 5 Years of Age. J Pediatr. 2022;240:51-7.e1.

Spruijt MS, van Klink JMM, de Vries LS, Slaghekke F, Middeldorp JM, Lopriore E, et al. Fetal and neonatal neuroimaging in twin-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2024;63(6):746-57.

Dankwoord

Om te zeggen dat het schrijven van dit proefschrift 'een hele bevalling' is geweest, is een understatement. Mijn beide bevallingen waren toch binnen 24 uur bekeken... Hoe anders was het met mijn promotieonderzoek. Na al die jaren ben ik trots op het eindresultaat. Ik wil een aantal mensen bedanken, zonder wiens steun dit proefschrift er niet zou zijn gekomen.

Ten eerste alle TTTS-kinderen en vooral hun ouders, bedankt voor jullie deelname aan onze studies. In het bijzonder de ouders van "Amy en Rosie" voor het delen van hun persoonlijke verhaal en foto's.

Beste Enrico, de impact van jouw aanmoediging en steun voor mij op klinisch én wetenschappelijk vlak mag niet worden onderschat, ze hebben mij dit proefschrift én de mooiste baan die je je maar kunt voorstellen opgeleverd. Ik kan je niet genoeg bedanken voor je vertrouwen, je humor en natuurlijk je geduld.

Lieve Jeanine, vanaf het eerste moment voelde het alsof wij op dezelfde golflengte zaten. Bedankt voor je onaflatende steun, je kritische blik, voor heel hard lachen en voor je vriendschap.

Beste Sylke, ik heb veel geleerd van jouw kennis en kunde op zowel wetenschappelijk als op klinisch gebied. Ik wil je bedanken voor je kritische blik op zowel inhoud als vorm, die mijn werk regelmatig beter heeft gemaakt, en daarnaast voor je positiviteit en vertrouwen.

Lieve neonatologen in het LUMC, bedankt voor de jarenlange fijne samenwerking en voor de ruimte die ik van jullie kreeg voor mijn onderzoek. De NICU in Leiden zal voor mij altijd een beetje als thuis voelen. Een speciaal bedankje voor professor Linda de Vries. Beste Linda, het was een eer en een genoegen om met je te mogen werken.

Beste perinatologen in het LUMC, bedankt voor jullie fantastische werk en enthousiaste bijdragen aan de studies in dit proefschrift. Jullie samenwerking met de neonatologie dient voor mij als voorbeeld van hoe het overal zou moeten.

Beste promovendi van het LUMC. Jullie waren nooit te beroerd om te helpen als ik ergens niet uitkwam met een database of wat dan ook. Bedankt!

Lieve leden van de staf neonatologie in het Radboudumc. In 2020 maakte ik de oversteek van west naar oost. Ik kwam uit een heel fijn team in Leiden, dus het was natuurlijk de vraag of de Leidse sfeer in Nijmegen geëvenaard zou kunnen worden. Gelukkig kan ik met volle overtuiging zeggen dat dat het geval is. Ik ben trots om deel uit te maken van ons team en heel blij met jullie als collega's. Bedankt voor de ruimte en tijd die jullie me hebben gegeven om mijn proefschrift af te maken. In het bijzonder wil ik Rosa, Marije en Milou bedanken. Dankzij jullie voelde Nijmegen al snel als een warm bad, bedankt voor jullie vriendschap en steun!

Dan mijn paranimfen. Lieve Stef, dat wij elkaar weer vaker kunnen zien beschouw ik als de kers op de taart van onze tocht terug naar het oosten. Niet dat onze vriendschap dat nodig had. Ik ben heel blij dat je naast me staat bij mijn verdediging, met zo'n powervrouw aan mijn zij hoef ik me nergens zorgen over te maken. Lieve San, hoewel ik je veel vaker zou willen zien, is ook onze vriendschap wat mij betreft sterker dan de afstand tussen ons. Sinds onze studie in Amsterdam hebben we veel life-events gedeeld, zelfs al zat jij in Groningen en ik in Den Haag. Wat heerlijk dat je er nu ook écht bij bent!

Speciaal wil ik Fenneke noemen. Lieve Fenk, dat jij met je spontaniteit en relativeringsvermogen tegenwoordig altijd om de hoek bent, heeft mijn leven zo verrijkt! Je hebt me echt gesteund én geïnspireerd om niet op te geven. Mede dankzij jou ligt dit boekje er nu eindelijk. Dank je wel lieve buuf.

Jammer genoeg zijn mijn ouders er niet meer om deze mijlpaal mee te maken. Ik wil ze bedanken voor mijn onbezorgde jeugd vol steun en liefde. Lieve papa, als dingen ingewikkeld worden probeer ik altijd aan jou te denken, want jij deed niet aan ingewikkeld, en dat helpt soms enorm. Lieve mama, wat had je hier graag bij willen zijn. Je bent mijn inspiratiebron geweest om mijn talenten te benutten. Zoals ik al schreef: je bent nog altijd bij me. Dus ook bij mijn verdediging.

Dan mijn rots in de branding. Lieve Pepijn, dat mijn proefschrift 'ineens' af was, leek voor jou eigenlijk geen verrassing, meer een vanzelfsprekendheid. Weten dat ik altijd op jou kan steunen maakt dat ik dit werk kan doen. Ik weet dat jij daar concessies voor doet. Bedankt voor je blinde vertrouwen in mij, voor alles wat je doet voor ons gezin en gewoon, bedankt dat je er bent.

Als laatste wil ik mijn kinderen bedanken. Lieve Felix en Eva, bedankt dat jullie zo fantastisch zijn, ik ben onnoemelijk trots op jullie!