LONG-TERM NEURODEVELOPMENTAL OUTCOME AFTER FETAL THERAPY

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Long-term neurodevelopmental outcome after fetal therapy

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PART I

GENERAL INTRODUCTION

General Introduction

An increasing number of fetal diseases are being detected prior to birth due to major improvements in prenatal ultrasound examinations and the wide implementation of screening programs.[1] For various diseases, fetal therapy may be a life-saving option or an alternative to postnatal treatment, to prevent permanent organ damage including the developing fetal brain.
A major breakthrough in fetal therapy was the introduction of intrauterine blood transfusion (IUT) for severe fetal anemia. This intervention was first described in the early 1960s by Sir William Liley in New Zealand.[2] Since then, fetal therapy has gradually evolved and resulted in a dramatic increase in overall survival in several fetal diseases. Throughout the world, specialized fetal therapy centers were initiated and a new medical discipline commenced. In the Netherlands, fetal surgical interventions were concentrated in one center, The Leiden University Medical Center (LUMC). The LUMC is a tertiary medical center which serves as the national referral center for fetal therapy.

Intrauterine transfusion in fetal anemia
The technique of the first IUT was based on intraperitoneal blood transfusion. In the next decades, the technique evolved to an intravascular approach. The first intravascular IUTs in the Netherlands were performed in 1986. Indications for IUT mainly include fetal anemia due to hemolytic disease caused by Rhesus or Kell alloimmunization. Alloimmune hemolytic disease results from maternal alloimmunization to red cell antigens, for which mother and fetus are incompatible. Maternal antibodies pass the placenta into the fetal circulation and cause destruction of fetal red cells. The resulting progressive hemolysis leads, if left untreated, to severe fetal anemia, fetal hydrops and perinatal death.[3] Nowadays, perinatal survival rates after IUT for severe fetal anemia exceed 95% in experienced centers.[4] Other, non-immune, indications for IUT include parvovirus B19 infection or chronic or acute fetal maternal hemorrhage (FMT). Approximately 30 fetuses are treated annually with IUT at our center. With an average of 3 transfusions per fetus, up to a 100 transfusions are performed per year.

Fetoscopic laser surgery in monochorionic twin pregnancies
The other major intervention in fetal therapy, besides IUT, is related to fetal interventions in complicated monochorionic (MC) twin pregnancies. MC twins share their placenta and their blood circulation is connected by vascular anastomoses at the placental surface. Placental vascular anastomoses allow acute or chronic inter-twin blood transfusions between the circulation of the two fetuses. Imbalanced inter-twin blood flow can lead to severe complications such as twin-twin transfusion syndrome (TTTS).

In TTTS, imbalanced blood flow from one twin (the donor) to the other twin (the recipient), results in hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient twin. The first treatment of choice in TTTS is fetoscopic laser coagulation of placental vascular anastomoses. In the Netherlands, the first fetoscopic laser surgery was performed at the LUMC in the year 2000. Nowadays, around 60 MC twin pregnancies are treated annually at our center with fetoscopic laser surgery, with an overall survival rate of 74%.[5] In a recently described complication in MC twins named twin anemia-polycythemia sequence (TAPS), several interventions can be considered including IUT or fetoscopic laser surgery. The optimal treatment of TAPS remains to be determined.

Fetoscopic surgical interventions in complicated MC twin pregnancies include, besides laser coagulation of placental vascular anastomoses, also selective feticide through umbilical cord coagulation or radiofrequency ablation (RFA). In specific complicated MC pregnancies, selective feticide via cord occlusion or RFA can be offered as an alternative management option. Indications include twin reversed arterial perfusion (TRAP) sequence, selective intrauterine growth restriction (sIUGR), monoamniotic twin pregnancies or severe discordant congenital anomalies. Perinatal survival rates following selective feticide vary between 65% and 92%, depending on indication and technique.[6]

Long-term neurodevelopmental outcome after fetal therapy

With an increasing number of children being born alive after fetal therapy, attention is shifting from short-term outcome and perinatal survival to long-term outcome and neurodevelopmental morbidity. However, data on long-term neurodevelopmental outcome after fetal therapy remain scarce. Long-term neurodevelopmental outcome studies are costly and difficult to perform and therefore hard to realize. In addition, follow-up studies after fetal therapy are hampered by the rarity of these fetal diseases. Nevertheless, long-term follow-up studies are of paramount importance to determine optimal fetal management. Follow-up studies may provide clinicians better insights into the long-term neurodevelopmental outcome and quality of survival in children after fetal therapy. Detailed and adequate information on long-term outcome is also required to improve the quality of antenatal parental counseling using evidence-based information.

Long-term follow-up studies with emphasis on child motor, cognitive and socio-emotional development are essential for conducting future randomized controlled trials in all fields of fetal therapy, in order to implement new or modified techniques. This requires cooperation between obstetricians, neonatologists, child psychologists and other experts in the field of early human development in order to look beyond perinatal survival as well as cooperation between international fetal therapy centers to

obtain reliable data with large enough case series with sufficient power. Large enough case series enable research on potential risk factors for adverse long-term outcome. It is important to continuously assess child development including formal psychological testing and standardized measures of well documented psychometric quality, with increasing reliability of results with increasing age of surviving children following fetal therapy.

The aim of this thesis is to improve our knowledge on the long-term neurodevelopmental outcome in children treated with fetal therapy and to identify potential risk factors for adverse long-term outcome.

Outline of this thesis

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PART II

INTRAUTERINE TRANSFUSION IN FETAL ANEMIA

Chapter 1

Long-term neurodevelopmental outcome after intrauterine transfusion for fetal anemia: A systematic review

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Abstract

The long-term neurodevelopmental outcome of children born after intrauterine blood transfusion (IUT) for red cell alloimmunization is considered favorable. Severe hydrops has been identified as a strong predictor for neurodevelopmental impairment. However, the long-term outcome of survivors of IUT for congenital Parvovirus B19 infection and fetomaternal hemorrhage is not well known. Limitations of the follow-up studies to date are small sample size, lack of controls, unclear criteria for impairment and lack of standardized developmental tests. Future research should take in to account more subtle impairments, since cognitive functioning < - 1 SD, behavioral and learning problems already have a significant impact on care requirements and future socio-economic potential. A better understanding of the effect of IUT and fetal anemia on child development over time will allow more accurate parental counseling and targeted interventions to optimize child development when needed.

Background

Fetal anemia can have either an immune or non-immune cause. Maternal red blood cell alloimmunization is the most common cause of immune fetal anemia. Alloimmunization results from prior contact with an antigen, for which mother and fetus are incompatible, either through fetomaternal transfusion or prior blood transfusion.[1] This triggers the formation of immunoglobulin G antibodies that are able to cross the placenta into the fetal blood circulation, causing hemolysis.

Non-immune fetal anemia has many causes including congenital Parvovirus B19 infection and fetomaternal hemorrhage (FMH). Fetal anemia due to congenital Parvovirus B19 infection results from crossing of the placental barrier of the virus and inhibition of fetal erythropoiesis by infection of erythroid precursor cells.[2] Fetal anemia in FMH results from the passage of (acute or chronic) fetal blood into the maternal circulation, due to placental abnormalities, maternal trauma or invasive obstetrical procedures.[3;4]

When untreated, fetal anemia may result in cardiac failure, hydrops, hypovolemic shock, fetal or neonatal death, neurologic injury or cerebral palsy (CP).[1;3] The mainstay to correct fetal anemia is intrauterine intravascular blood transfusion (IUT). IUT can be considered a safe procedure with a relatively low procedure-related complication rate and a low perinatal loss rate.[5] Perinatal survival rates after IUT nowadays exceed 90%.[5;6] Although advances in techniques allow even moribund and severely anemic fetus to survive, severe anemia or a prolonged hydropic state may lead to neurodevelopmental impairment (NDI).[7;8] Hence, the outcome of antenatal management must be assessed not only by survival but also by long-term neurodevelopmental outcome.[9]

We performed a systematic review of the literature on the long-term neurodevelopmental outcome in children treated with IUT for fetal anemia, secondary to maternal alloimmunization, Parvovirus B19 infection and FMH. The prevalence and nature of favorable and adverse neurodevelopmental outcome in light of methodological strengths and weaknesses of studies will be highlighted. In addition, risk factors for NDI are discussed. The aim of the review was to identify important areas for future research.

Methods of the Review

A systematic literature search was utilized to retrieve the studies and articles for this review. An electronic MEDLINE literature search was performed using the following mesh terms: Fetal Erythroblastosis, Intrauterine, Time, Prognosis, Epidemiologic Studies, Human Development, Neurobehavioral Manifestations and Morbidity. The computer aided search was limited to English, French, Dutch and German language

articles and included the period from 1981 to January 2011, since intrauterine intravascular transfusions are performed since 1981. All reference lists of primary articles and reviews were examined to search for additional references. Then, a manual search of identified articles was conducted. If needed, authors were contacted for further information. The following inclusion criteria were applied: children treated with intrauterine intravascular transfusion for fetal anemia secondary to maternal alloimmunization, congenital Parvovirus B19 infection or fetomaternal hemorrhage, assessment of neurodevelopmental outcome and the conduct of statistical tests. The methodological quality of each selected study was assessed independently by two reviewers (JK and EL). The following exclusion criteria were applied: case reports, dissertations, qualitative studies, book chapters, guidelines and commentaries.

Results

We found no other review article or meta-analysis focusing solely on the long-term neurodevelopmental outcome in children treated with IUT for anemia due to maternal red cell alloimmunization, congenital Parvovirus B19 infection and FMH. In over 30 years, we identified only 11 studies that met our inclusion criteria (9 on maternal alloimmunization and 2 on congenital Parvovirus B19 infection). Beyond case reports, research on long-term neurodevelopmental outcome following FMH is limited to 3 small series (31, 26 and 15 children).[10;11] Since no IUTs were performed to correct anemia, these series were not included for review. Our selected studies are summarized, in chronological order, in Tables 1 (red cell alloimmunization) and 2 (Parvo B 19 infection).

Table 1 Long-term neurodevelopmental outcome in children treated with IUT for maternal red cell alloimmunization.

Author, year	Outcome measure	CP	NDI	Methodological comments
Doyle, 1993[12]	Bayley Scales	2.6% (1/38)	7.9% (3/38)	Controls not contemporaneous, transfusion group better SES
Stewart, 1994[9]	Cattel Test	no (0/8)	no (0/8)	Insufficient information on patients and methods, insufficient power
Janssens, 1997[13]	Van Wieghen, POPS, Gesell Schedules, Denver Screening Test	4% (3/69)	10.1% (7/69)	Wide age range of the children
Hudon, 1998[14]	Gesell Schedules, McCarthy Scales	4.5% (1/22)	n.a.	No controls, high lost to follow-up rate, no formal criteria NDI, insufficient power
Grab, 1999[15]	School Performance	no (0/35)	n.a	No controls, no neurodevelopmental tests
Farrant, 2001[16]	Neurodevelopmental questionnaire	3.3% (1/30)	n.a	No controls, insufficient information patients and methods, no neurodevelopmental tests
Harper, 2006[17]	Differential Ability Scales, Wide Range Assessment, Gordon Diagnostic System	6.2% (1/16)	12.5% (2/16)	Insufficient power
Weisz, 2009[18]	Neurodevelopmental questionnaire	no (0/40)	n.a	No controls, no neurodevelopmental tests, no formal criteria NDI
Lindenburg, 2011[19]	Touwen, Bayley Scales, Wechsler Scales	2.1% (6/291)	4.8% (14/291)	No controls, wide age range of the children
Total		**2.4% (13/549)**	**4.9% (27/549)**	

Table 2 Long-term neurodevelopmental outcome in children treated with IUT for parvovirus B19 infection.

Author, year	Outcome measure	CP	NDI	Methodological comments
Dembinski, 2002[20]	Griffiths Test, Snijders Oomen Intelligence Test, Kaufman Battery	no (0/20)	no (0/20)	No controls, high lost to follow up rate
Nagel, 2007[8]	Bayley Scales, Snijders Oomen Intelligence Test	6.25% (1/16)	12.5% (2/16)	No controls, insufficient power
Total		**2.7% (1/36)**	**5.5% (2/36)**	

CP is Cerebral Palsy; NDI is Neuro Developmental Impairment which is defined as CP, cognitive functioning or developmental delay (< 2SD), blindness or deafness; n.a. is not available; POPS is Project on Preterm and Small for Gestational Age Infants in the Netherlands 1983.

Methodological Issues

The overall quality of the 11 selected studies is suboptimal. All studies concern small single center follow-up studies, including between 8 and 69 children, except for a recent study including 291 children for follow-up.[19] Only 4 studies included controls, to validate their outcome against healthy children, children with similar neonatal problems, or children diagnosed with fetal anemia who did not receive IUTs. Both interval and timing of follow-up range considerably between studies that is, from 1984-1990 to 1988-2008 and the children are tested as young as one month old to 16 years of age. All studies are cross sectional in design and, therefore, do not allow for observation of development over time. In 3 follow-up studies the children were not individually investigated with formal psychological testing. Criteria for NDI were not consequently described and neither was the way impairment was 'measured'. In general, the outcome measures were able to identify major neurological deficits, but the more subtle abnormalities like behavioral problems or learning difficulties, which have a significant impact on care requirements, were likely overlooked.[21] Abovementioned methodological issues and heterogeneity make the selected studies difficult to compare and, as a consequence, knowledge on the neurodevelopmental outcome of these children remains limited. Knowledge on the long-term development of children after IUT over time is however necessary. The main findings of each follow-up study are listed below, in chronological order.

Neurodevelopmental Outcome after Maternal Red Cell Alloimmunization

The first report on long-term neurodevelopment was published in 1993 by Doyle et al.[12] With formal psychological testing and clearly described criteria for NDI, Doyle found no impairment at 2 years of age in 92% (3/38) of children transfused in utero. The transfusion group compared favorably with both high risk survivors of very low birth weight (VLBW) and low risk children of normal birth weight. However, the low risk group was not contemporaneous with the transfusion group.

With little information on their patients and methods, Stewart et al. reported no difference in neurodevelopment at 18-24 months between 8 children with Rhesus disease treated with IUT and 8 children with Rhesus disease not treated with IUT.[9] Neither group was different in terms of neurodevelopmental outcome from the general population. Such a small sample size evidently lacks statistical power to find differences. Furthermore, age range of follow up was 18-24 months, which is often too early for accurate assessment of CP or severe developmental delay.

Janssens et al. found normal neurodevelopment in 89.9% (62/69) of children after IUT (range 6 months to 6 years of age).[13] With well-defined criteria for NDI, the 69 children compared favorably with children of very low birth weight and/or small for gestational

age, 10.1% versus 18% respectively. In contrast to the findings by Doyle et al.[12], the transfusion group compared less favorably with healthy controls, 10.1% versus 6% respectively. Unfortunately, no p-values were reported. The discrepancy between Doyle et al. and Janssens et al. is probably to the much larger sample size in the latter.

Hudon et al. reported neurodevelopmental scores within average at 9-62 months in 33 children, with no differences in children with or without a history of hydrops ($P = .72$).[14] One child presented with CP (1/22) and one child with bilateral deafness (1/21). One of the major limitations of this study was the relatively large loss-to follow-up (82.5%, 33/40). This may have biased results, as chances for adverse outcome are generally higher in the group that is initially lost to follow up.[22]

Grab et al. found no moderate or severe neurologic impairment at 6 years of age in 35 children, including 7 cases with fetal hydrops at initial transfusion.[15] Fetuses with hydrops at initial transfusion tended to have a higher perinatal mortality and had a significant higher rate of preterm delivery ($P = .03$). At follow-up, survivors were not individually investigated nor tested for neurodevelopment. Conclusions were solely based on questionnaires that were completed by the child's primary care providers, a method known for underreporting of affected children.[17]

Farrant et al. performed a follow-up study in 36 children treated with IUT.[16] One child was born prematurely following death of a co-twin from twin-twin-transfusion syndrome and had CP and developmental delay, with an abnormal cranial ultrasound. Although 2 other children had an abnormal cranial ultrasound, no other child had neurodevelopmental impairment. Again, no child was individually investigated and tested for neurodevelopment. Of note, little information on the patients and methods was reported e.g., the age of assessment of the children was not recorded.

In 16 children with a history of hydrops, Harper et al. reported major neurological morbidity in 12.5% (2/16) of children.[17] The neuropsychological assessment represented a balance of tasks to identify general and, even, subtle neuropsychological deficits. Except for a measure of attention, neurologic and neuropsychological outcome was similar to their unaffected siblings. According to the authors the significant difference was not verified by clinical and classroom behavioral observations that were required for confirmation of the diagnosis of attention deficit hyperactivity disorder. Six of the sixteen survivors (37.5%) had a minor physical or neurologic finding. However, like previous studies, this study was underpowered.

Weisz et al. concluded that 85% of 40 children treated with IUT for fetal anemia reached satisfactory motor milestones according to age.[18] Abnormal motor development by the age of 1 year was observed in 15% (6/40) and abnormal cognitive development in children aged ≥ 1 year in 13.5% (5/37), with no differences in children with a history of mild, moderate or severe anemia. However, the authors did not specify what they

considered 'abnormal' and, like Grab[15] and Farrant[16], obtained their data not from the children individually with formal psychological testing but from their parents using a computerized questionnaire.
Recently, Lindenburg et al. performed a large long-term follow up study of 291 children treated with IUT secondary to maternal alloimmunization between 1992 and 2008.[19] The primary objective was to assess the incidence of NDI, a composite outcome defined as the presence of at least one of the following: abnormal neurological outcome (CP), cognitive developmental test score < -2 SD, bilateral blindness or bilateral deafness requiring amplification. All children were tested at a median age of 8.2 years. Severe developmental delay was detected in 3.1% (9/291) of children, with a 4.8% incidence of NDI (14/291). Despite the notable large sample size, it concerns a single centre study with a considerable large follow-up interval. Moderate developmental delay (< -1 SD) was detected in 14.4% (42/291) of children.
Overall, when the results of the above mentioned follow-up studies are pooled together, the rate of CP and NDI is 2.4% (13/549) and 4.9% (27/549), respectively.

Neurodevelopmental Outcome after Parvovirus B19 infection
Dembinski et al. found neurodevelopmental scores within 2 SD of a normal population in 20 children at 13 months to 9 years of age transfused for Parvovirus B19 induced anemia and fetal hydrops.[20] Although children with neurodevelopmental scores of < -1 SD fall within 2 SD of a normal population, these children are at risk for difficulties in school functioning. Nevertheless, the number and characteristics of these children were not further addressed. As only 20 out of 31 children (65%) were seen for testing, the study was limited by a high loss to follow-up rate.[5]
Nagel and colleagues described 16 survivors at a median age of 4 years of which 5 children (32%) demonstrated neurodevelopmental delay on formal psychological tests (mild delay n=3, severe delay n=2).[8] Two children had minor congenital defects. Five children were <18 months at the time of testing and thus too young for reliable assessment. In this study, adverse outcome was not related to severity of anemia and acidemia, and the authors suggest congenital Parvovirus B19 infection itself might cause CNS damage.[5] This hypothesis needs further study. Once more, low statistical power was the main limitation of this study.
Overall, when the results of the 2 follow-up studies are pooled together, the rate of CP and NDI is 2.7 (1/36) and 5.5% (2/36), respectively.

Risk Factors for Neurodevelopmental Impairment
Lindenburg et al. showed severe hydrops to be a strong pre-operative predictor of NDI[19], while the majority of studies could not confirm such a relationship.[12-15] Other risk factors concerned the number of IUTs received, severe neonatal morbidity and parental

education[19], again in contrast to the majority of studies.[8;12-15;20] According to Janssens et al. the probability that neurologic abnormalities would occur was significantly greater when perinatal asphyxia had been present ($P < 0.05$) and with a lower cord hemoglobin level at birth ($P = .03$).[13] The discrepancy in outcome is due to the considerable difference in sample size between the studies. Sample sizes were often too small to detect a significant effect of risk factors on the outcome measures.
On the whole, risk factors were not clearly defined or registered in all studies and were recorded at different points in time, which make comparison difficult. Furthermore, the risk factors were studied in light of not so subtle outcome measures e.g., CP and cognitive functioning < -2 SD. Risk factors for an adverse outcome might become more apparent with more sensitive outcome measures such as cognitive functioning < -1 SD, which already has a large impact on care and educational requirements of children.

Discussion

Despite the use of intrauterine transfusion for fetal anemia for 3 decades, knowledge on the long-term neurodevelopment of children treated with IUT is limited. The long-term neurodevelopmental outcome of children born after IUT for red cell alloimmunization is considered to be favorable. Severe hydrops has been identified as a strong predictor for NDI. However, the long-term outcome of survivors of IUT for congenital Parvovirus B19 infection and fetomaternal hemorrhage is not well known.
Few follow-up studies have been performed, commonly with sample sizes lacking the power to detect adverse neurological outcome. Since follow-up studies are restricted by the relative rarity of the disease and treatment, multicenter efforts are of utmost importance to increase sample size. However, with a large enough sample size any effect can be found statistically significant. Therefore, an equal emphasis should be placed on the clinical relevance of outcome: whether or not a child perceives impairment at an individual level and when to intervene as a clinician according to that individual perception.
In order to facilitate communication, replication and collaboration between research groups it is necessary to be transparent, specific and uniform with respect to study design and outcome. Uniform ante-, peri- and neonatal characteristics should be recorded, at fixed time points. Imaging of the brain of the fetus as well as the neonate and child should be performed. It is important to continuously assess neurodevelopment of the children, at fixed points in time, with formal psychological testing and standardized measures of well documented psychometric quality, for instance at 4 years, 8 years, 12 years and at 16 years of age. Table 3 represents a proposition for future research.

Table 3 A proposition for future research: Assessment according to age in years.

Fetus	Neonate	2	5	8	10	12	14	16 years
Brain development: cerebral imaging								
		Senses: hearing test, vision test **Cognitive functioning**: Bayley scales/ Ages and Stages Questionnaire, Wechsler scales						
			Physical functioning: Touwen Neurological Examination, CP (Gross Motor Function Classification System) **School functioning**: special education, number of grades below age-appropriate level					
				Neuropsychological functioning: learning, language, executive functioning, attention, visual spatial abilities, memory, fine motor development **Psychosocial functioning and behavior**: internalizing and externalizing behavior, Quality of Life, Achenbach System, Vineland Adaptive Behavioral Scales **Developmental problems**: Attention deficit, hyperactivity, autism spectrum			

CP is Cerebral Palsy.

Wechsler scales are reliable and valid measures of cognitive functioning in (young) children.[23;24] Beyond a full scale intelligence quotient, one can take into account the abilities that make up a full scale score, such as verbal skills, visual spatial skills and processing speed. For example, a certain child has an average full scale IQ score of 91, with a high-average verbal IQ score of 109, but a performance IQ score of 75. If we just look at the average full scale score, the child's difficulties with visual spatial tasks will be overlooked. The child will not be scored as 'impaired' in the research to date, but in practice the child will encounter considerable difficulties at school or in daily life which are not recognized with the current criteria for an adverse outcome.

To enable valid comparisons between follow-up studies, uniform criteria for an adverse outcome are indispensable. However, the outcome measures to date are not able to identify subtle abnormalities and should therefore be supplemented with a more sensitive definition of impairment: cognitive functioning test score < -1 SD, the presence of a learning problem, mild or moderate motor problems, symptoms of pervasive developmental disorder, attention deficit and/ or behavioral problems. These 'subtle abnormalities' already have a significant impact on care and educational requirements and affect the future socioeconomic potential of a child.[21] Since subtle abnormalities might also include increased behavior or social emotional problems, questionnaires that cover psychosocial and behavioral functioning should be included to obtain a full image of the child at different ages and stages. Up till now, these measures are lacking in the long-term follow-up of children treated with IUT for fetal anemia.

A better understanding of the impact of IUT on child development over time, based on standardized outcome measures, will allow more accurate counseling of parents and targeted interventions to optimize development in these children when needed.

Key Guidelines:

- Long-term neurodevelopmental outcome after IUT for maternal red cell alloimmunization is considered favorable.
- Severe hydrops is a risk factor for an adverse outcome after IUT for maternal red cell alloimmunization, as well as neonatal morbidity, the number of IUTs received and parental education.
- Neurodevelopmental outcome after IUT in congenital Parvo B19 infection and FMT is not well known.

Research Directions:

- Long-term follow-up studies after IUT in congenital Parvo B19 infection and FMT are urgently needed.
- To enable valid comparisons between follow-up studies, uniform and well described criteria for an adverse outcome are indispensable.
- Future research should take in to account more subtle abnormalities, since cognitive functioning < -1SD, behavioral problems and learning difficulties already have a significant impact on care requirements and the child's future socio-economic potential.

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Chapter 2

Health-related quality of life and behavioral functioning after intrauterine transfusion for alloimmune anemia

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Abstract

Objective
To assess health-related quality of life (HRQOL) and behavioral functioning in children and adolescents treated before birth with intrauterine transfusions for alloimmune anemia.

Study design
Cross-sectional cohort study conducted at the Dutch referral center for the management of fetal alloimmune anemia. Follow-up data were obtained from 285 children at a mean age of 10.5 years (range, 3-21.5 years) with a response rate of 544/563 questionnaires (97%). Child-, adolescent- and parent-rated HRQOL was evaluated with the TNO AZL Child/Adult Quality of Life Questionnaire (TACQOL/TAAQOL). Parents reported on behavioral functioning with the Strengths and Difficulties Questionnaire (SDQ). Scores were compared to Dutch norm data.

Results
Significantly lower scores were reported by parents of children 6-11 years compared with Dutch norms on 3 scales: cognitive-, social functioning and positive emotions ($P < .00$, $P = .02$, $P = .04$). In children aged 8-11 the cognitive functioning scale score was significantly lower compared with Dutch norms ($P = .01$). The children aged 12-15 reported higher scores on negative emotions ($P = .02$). When corrected for multiple testing, only the parent-rated cognitive functioning scale remained significant ($P < .001$). Regarding the HRQOL scores of adolescents ≥ 16 years, no differences were detected. Overall, behavioral difficulties were reported in 37/246 (15%) children 3-16 years, and were associated with maternal educational levels ($P < .001$).

Conclusion
Parents reported lower scores on cognitive functioning in their children aged 6-11 years compared to norms. Behavioral difficulties were more prevalent than norms, and were associated with maternal educational level. Overall, in the majority of survivors long-term outcome following IUT for alloimmune anemia appears favorable.

Introduction

Fetal and neonatal hemolytic disease results from maternal alloimmunization to red cell antigens, for which mother and fetus are incompatible. Maternal immunoglobulin G antibodies pass the placenta into the fetal circulation causing destruction of fetal red cells. The resulting progressive fetal anemia leads, if left untreated, to fetal hydrops and perinatal death. The mainstay to correct fetal anemia is intrauterine intravascular blood transfusion (IUT). IUT is considered a safe procedure with a relatively low procedure-related complication rate and a low perinatal loss rate.[1;2] In experienced centers, perinatal survival rates exceed 95%.[3;4]

As perinatal survival is improving, one of the concerns is that this could lead to an increase in children with long-term impairments. The incidence of long-term impairment in children treated with IUT ranges from 4.5 to 12%.[5-12] Our previous study in the current cohort, including 291 children, found a 5% incidence of long-term neurodevelopmental impairment after IUT for alloimmune anemia.[13] Nevertheless, even in children without obvious impairments, subtle problems may occur including health-related quality of life (HRQOL) issues or increased behavioral difficulties.[14]

The aim of this study was to assess quality of life and behavioral functioning in children and adolescents treated before birth with IUT for severe alloimmune hemolytic disease, and to compare the outcomes with Dutch norm data.

Methods

This cross-sectional cohort study was conducted at the Leiden University Medical Center (LUMC), the national referral center for the management and treatment of fetal alloimmune anemia. All children and adolescents who received IUT for alloimmune anemia between 1988 and 2008 at the LUMC, and participated in the LOTUS study were eligible.[15] Our previous study in this cohort, focused on the long-term neurodevelopmental outcome.[13] Patients with severe congenital anomalies unrelated to intrauterine anemia or treatment were excluded. Invitation letters were distributed by mail, and if families consented, questionnaires were sent out and collected during a follow-up visit at our outpatient clinic or by mail. The study was approved by the ethics committee of the LUMC and written informed consent was obtained.

The following perinatal and neonatal data were collected retrospectively from the LUMC Rhesus database and medical records: hemoglobin level at IUT, severity of fetal anemia[16], presence and severity of fetal hydrops[17], number of IUTs, gestational age at birth, severe neonatal morbidity, and perinatal asphyxia. Severe neonatal morbidity was defined as

the presence of 1 or more of the following: respiratory distress syndrome, necrotizing enterocolitis ≥ grade 2, sepsis, and/or severe cerebral injury. Perinatal asphyxia was defined as 3 or more of the following 5 criteria: non-reassuring cardiotocogram, umbilical cord arterial pH <7.10 and base excess ≥16 mmol/L or lactate >10 mmol/L, Apgar score <5 at 5 minutes after birth, failure of spontaneous breathing at 5 minutes after birth, and onset of multiple organ failure.
Maternal education was recorded and divided into 3 levels: low (primary school), average (secondary school) and high (higher vocational school and university). Ethnicity was recorded as one or both parents with Dutch nationality or non-Dutch nationality. Long-term neurodevelopmental impairment was evaluated in our previous study in this cohort and defined as at least one of the following: cerebral palsy, severe motor and or cognitive delay (< -2SD), bilateral blindness and/or deafness.[13]

Measures

Two questionnaires were used to assess HRQOL: The Netherlands Organisation for Applied Scientific Research-Academisch Ziekenhuis Leiden (TNO-AZL) Child Quality of Life questionnaire (TACQOL) and the TNO-AZL Adult Quality of Life questionnaire (TAAQOL).[18-21]

TACQOL The TACQOL is a generic HRQOL instrument and consists of a parent form (PF) for parents of children aged 6-11 years and a child form (CF) for children aged 8-15 years.[18;19;19;22] HRQOL is defined as the combination of Health Status (HS) and the affective evaluation of problems in HS. The TACQOL contains 7 scales with 8 items each: physical complaints, motor functioning, autonomy, cognitive functioning, social/ peer functioning, positive- and negative emotional functioning. To each item-pair a score ranging from 0-4 is administered with scale scores ranging between 0-32. On the positive- and negative emotional functioning scales, a score ranging from 0-2 is administered with scale scores ranging from 0-16. Higher scores indicate better HRQOL. The psychometric properties are satisfactory and norm data from the general Dutch population are available.[18;19;19;22]

TAAQOL The TAAQOL addresses HRQOL in adolescents and adults aged ≥ 16 years and includes 45 items, divided into 12 scales: gross motor functioning, fine motor functioning, pain, sleeping, cognitive functioning, social functioning, daily activities, sexuality, vitality, positive, depressive and aggressive emotions.[20;21] A single score is given for each item-pair (HS and the affective evaluation of problems in HS) and for each item in the vitality, positive, depressive and aggressive emotions scales. Scores for each scale range from 0 to 100, with higher scores indicating better HRQOL. The

psychometric properties are satisfactory and TAAQOL norm data from the general Dutch population are available.[20]

Behavioral functioning Behavioral functioning was assessed with the Strengths and Difficulties Questionnaire Parent Form (SDQ-PF), a 25-item behavioral screening questionnaire to identify problems in children 3-16 years.[23;24] The SDQ contains 25 items: 20 items across 4 problem scales (emotional symptoms, conduct, hyperactivity and peer problems) and a 5-item pro-social behavior scale. For each item a score is administered from 0-2 (not true, somewhat true, and certainly true) with scale scores ranging 0-10. Higher scores indicate more problems, except for the pro-social behavior scale where higher scores indicate more strengths. Summing the scores from the 4 problem scales yields a total difficulties score ranging 0-40. The psychometric properties of the SDQ are acceptable.[25] Dutch norm data are available for boys and girls separately (aged 8-16 years) as well as preliminary cut-off values for the general Dutch population.[24;26;27] A total difficulties score of ≥ 14 indicates behavioral difficulties, representing 10% of the general Dutch population.[27]

Statistical analyses

The Statistical Package for Social Sciences version 20.0 was used for statistical analyses (IBM, Armonk, NY, USA). Descriptive results for nominal variables are presented as number of cases and percentages. Data are reported as mean ± SD for continuous variables and median (range) for variables with a non-normal distribution. Differences between participants and non-participants were analyzed using independent *t* tests and Chi square tests. The TACQOL-CF was split in to 2 age groups, 8-11 and 12-15 years, since the scale structure and the reliability for the autonomy and social functioning scales proved to be less satisfactory for the children 12-15 years.[19] The autonomy scale was deleted and the social functioning scale replaced with the peer functioning scale for this age group. To compare HRQOL and SDQ scores of children treated with IUT with mean Dutch norm data, independent *t* tests were used. Effect sizes of group differences (Cohen's d) were computed by dividing the mean differences by the SD of the norm group. Effect sizes ≥ 0.80 were considered large.[28] Due to multiple testing, Bonferroni correction was applied, and a $P < 0.001$ level was considered statistically significant. Total behavioral difficulties for the children aged 3-16 years (scores ≥ 14) were analyzed using a binomial test.

The following potential risk factors associated with HRQOL and total behavioral difficulties were studied in univariable linear regression models: severity of fetal anemia (Z-hemoglobin), fetal hydrops at IUT, number of IUTs, gestational age at birth, severe neonatal morbidity, perinatal asphyxia, gender, maternal education and ethnicity.[13;29]

A multivariable linear regression model included all variables that showed significant association with HRQOL and total behavioral difficulties in the univariable analysis. Results are expressed as regression coefficient B with 95% confidence interval (CI). Since the HRQOL questionnaires lack a total HRQOL score, risk factor analyses was planned only for the subscales that showed a significant difference ($P < 0.001$) between the IUT group and Dutch norm data.

Results

A flow diagram of study participants is shown in the Figure.

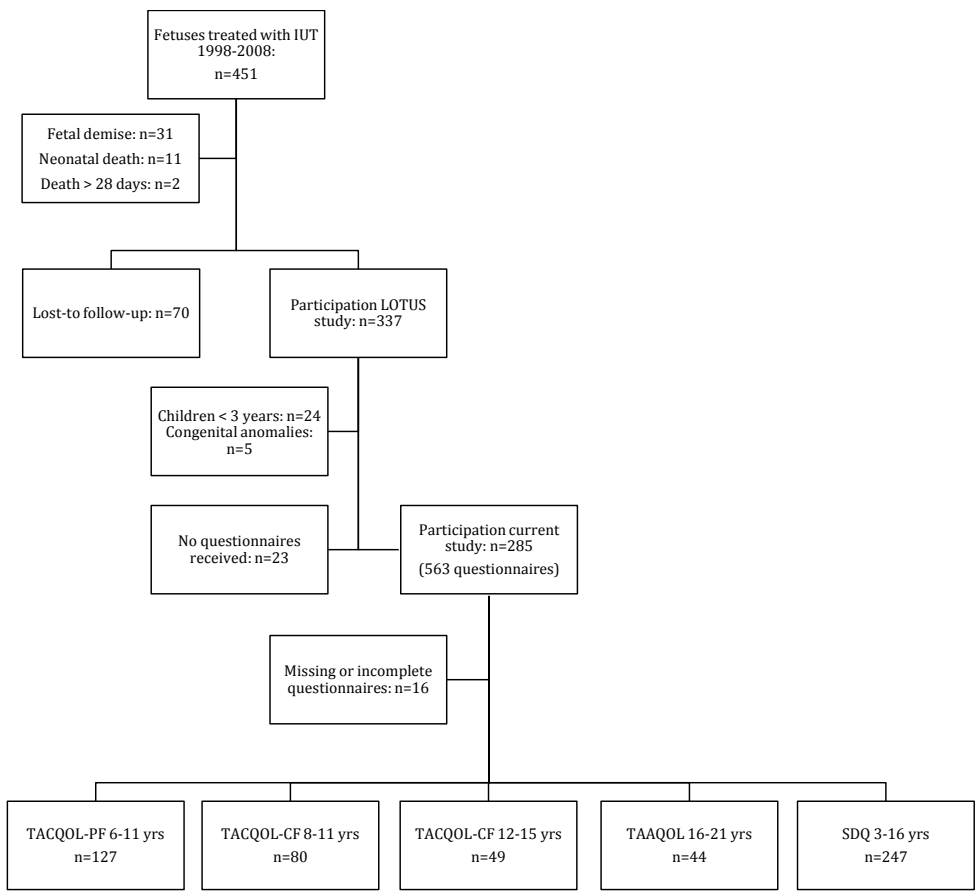

Figure Flow diagram of study participation.
TACQOL, TNO AZL Child Quality of Life questionnaire; PF, Parent Form; yrs, years; CF, Child Form; TAAQOL, TNO AZL Adult Quality of Life Questionnaire; SDQ, Strengths and Difficulties Questionnaire.

Between 1988 and 2008, 451 fetuses were treated with IUT for alloimmune anemia at our center. Of the 337 children participating in the initial LOTUS study, five (1%) children were diagnosed with severe congenital anomalies unrelated to intrauterine anemia or treatment, and were excluded from further analysis. Due to the age range of the questionnaires, 24 (7%) children under the age of 3 were excluded from the current study. Of 23 (7%) children no questionnaires were received. Follow-up data were obtained from 285/308 (93%) children and adolescents, with a total response rate of 547/563 (97%) questionnaires consisting of 127 TACQOL Parent Forms age 6-11 (2 missing), 80 TACQOL Child Forms age 8-11 (6 missing), 49 TACQOL Child Forms age 12-15 (1 missing), 44 TAAQOL questionnaires age ≥ 16 (2 missing) and 247 SDQs age 3-16 (5 missing). Detailed information on the baseline characteristics of the 285 participants is presented in Table 1.

Table 1 Baseline characteristics of the participants and lost to follow-up group.

	Participants (n = 285)	**Lost to follow-up (n = 93)**	***P***
Gestational age at first IUT, mean ± SD (range), weeks	28 ± 5 (17-36)	27 ± 5 (16-35)	.096
Hemoglobin at first IUT, mean ± SD (range), g/dL	3.4 ± 1.5 (0.7-8.2)	3.4 ± 1.5 (1.1-7.4)	1.00
Number of IUTs per fetus, mean ± SD (range)	3 ± 1 (1-6)	3 ± 1.5 (1-8)	1.00
Rhesus D alloimmunization, n (%)	233 (82)	82 (88)	.199
Rhesus c, n (%)	16 (6)	1 (1)	.084
Kell, n (%)	31 (11)	9 (10)	.848
Other, n (%)	5 (2)	1 (1)	1.00
Hydrops, n (%)	77 (26)	30 (32)	.354
Mild, n (%)	55 (19)	17 (18)	.880
Severe, n (%)	22 (8)	13 (14)	.097
Gestational age at birth, mean ± SD (range), weeks	36 ± 2 (28-39)	36 ± 2 (29-38)	1.00
≤ 32 weeks, n (%)	7 (3)	6 (7)	.095
32-35 weeks, n (%)	49 (17)	15 (16)	.875
≥ 35 weeks, n (%)	229 (80)	72 (77)	.555
Male, n (%)	148 (52)	49 (53)	.905
Exchange transfusions per neonate, median (IQR)	1 (0-2)	1 (0-2)	.601
Top-up transfusions per neonate, median (IQR)	2 (1-3)	1 (1-2)	.202
Severe neonatal morbidity, n/N (%)	26/285 (9)	1/23 (13)	.465
Dutch ethnicity, n/N (%)	259/285 (91)	18/23 (79)	.067
One parent Dutch, n/N (%)	8/285 (3)	1/23 (4)	.507
Non-Dutch, n/N (%)	18/285 (6)	4/23 (17)	.069
Maternal education			
Low, n/N (%)	85/282 (30)	6/23 (26)	.815
Average, n/N (%)	115/282 (41)	10/23 (44)	.828
High, n/N (%)	82/282 (29)	7/23 (30)	1.00

IQR, interquartile range

No significant differences in antenatal and neonatal characteristics were found between the participants and the lost to follow-up group. Mean gestational age at first IUT of the study group was 28 ± 5 weeks (range: 17-36) and the mean number of IUTs per fetus 3 ± 1 (range: 1-6). The majority was treated for Rhesus D alloimmunization (233/285, 82%). Mean gestational age at birth was 36 ± 2 weeks (range: 28-39). The mean age of the children and adolescents at follow-up was 10.5 ± 4.7 years (range: 3.0-21.5). Neurodevelopmental impairment was evaluated in our previous study in this cohort and diagnosed in 14/285 (5%) of participants in the current study.

Health-related Quality of Life (TACQOL and TAAQOL)

Table 2 shows the scale scores indicating HRQOL for the parent- and child forms. Significantly lower scores were reported by parents of children 6-11 years compared with the Dutch norm group on 3 scales: cognitive functioning, social functioning and positive emotions ($P < .00$, $P = .02$ and $P = .04$ respectively). In children aged 8-11 only the cognitive functioning scale score was significantly lower compared with the Dutch norm group ($P = .01$). The children aged 12-15 reported higher scores on the negative emotions scale compared to Dutch norms ($P = .02$). When corrected for multiple testing, only a difference with medium effect size was detected for the parent-rated cognitive functioning scale ($P < .00$). Table 3 shows the scale scores indicating HRQOL for the adolescent forms. No significant differences were detected between the adolescents ≥ 16 years treated with IUT and Dutch norms. Excluding the cases with neurodevelopmental impairment from all HRQOL analyses did not alter the results.

Potential risk factors were entered in a univariable regression analysis to assess the association with the parent-rated cognitive functioning scale (Table 4). No significant association was detected between potential risk factors and the cognitive functioning scale.

Table 2 Mean HRQOL scores according to parent- and child forms compared with Dutch norm references.

	Parent Form 6-11 (n = 127)	**Dutch norm (n = 1700)**	***d***	***P***	**Child Form 8-11 (n = 80)**	**Dutch norm (n = 1094)**	***d***	***P***	**Child Form 12-15 (n = 49)**	**Dutch norm (n = 1252)**	***d***	***P***
Physical complaints	27.4 (3.9)	27.1 (4.0)	0.08	.469	25.7 (4.9)	24.9 (5.1)	0.16	.208	24.4 (4.9)	23.7 (5.4)	0.13	.357
Motor functioning	30.6 (2.5)	30.8 (2.6)	0.08	.434	29.9 (2.9)	29.8 (3.2)	0.03	.764	28.9 (4.4)	29.8 (3.3)	0.27	.066
Autonomy	31.2 (2.0)	31.2 (1.7)	0.00	.958	31.4 (1.4)	31.2 (1.9)	0.11	.359				
Cognitive functioning	27.3 (5.0)	29.0 (3.8)	0.45	.000**	27.1 (4.6)	28.4 (3.9)	0.33	.005*	27.2 (4.7)	27.6 (4.1)	0.09	.559
Social / Peer functioning	29.3 (3.5)	29.9 (2.5)	0.24	.015*	29.3 (2.8)	29.7 (2.8)	0.14	.259	30.5 (4.0)	31.1 (2.9)	0.21	.162
Positive emotions	14.4 (2.2)	14.8 (2.0)	0.20	.042*	13.8 (2.5)	13.6 (2.5)	0.08	.467	13.3 (2.2)	13.0 (2.8)	0.11	.448
Negative emotions	11.8 (2.6)	11.5 (2.4)	0.13	.147	11.9 (2.6)	11.6 (2.7)	0.11	.425	12.5 (1.9)	11.6 (2.6)	0.35	.020*

Values are mean ± standard deviation (SD), effect size (d) and *P.*
*Difference at $P < .05$; **Difference at $P < .001$; Bonferroni correction for multiple testing applied.
TACQOL, TNO AZL Child Quality of Life questionnaire.

Table 3. Mean HRQOL scores of adolescents treated with IUT aged 16-21 years compared with Dutch norm references

TAAQOL subscales	IUT-group (n = 44)	Dutch norm (n = 179)	*d*	*P*
Gross motor functioning	95.2 (10.2)	92.8 (16.5)	0.15	.361
Fine motor functioning	98.6 (7.6)	97.1 (10.7)	0.14	.401
Cognition	78.7 (25.5)	84.2 (20.6)	0.27	.130
Sleep	76.6 (27.7)	76.3 (23.4)	0.01	.949
Pain	81.3 (25.1)	81.1 (20.4)	0.00	.961
Social contacts	89.3 (17.5)	89.8 (16.3)	0.03	.876
Daily activities	87.9 (20.1)	81.8 (22.9)	0.27	.104
Sexuality	92.1 (18.0)	92.1 (18.7)	0.00	.992
Vitality	67.3 (23.0)	65.1 (21.8)	0.10	.559
Positive emotions	74.4 (22.1)	73.4 (19.1)	0.05	.758
Depressive emotions	79.9 (23.0)	79.0 (17.9)	0.05	.774
Aggressive emotions	83.5 (23.8)	84.7 (17.8)	0.07	.701

Values are mean ± standard deviation (SD), effect size (d) and *P.*
TAAQOL, TNO AZL Adult Quality of Life Questionnaire.

Table 4. Analysis of potential risk factors associated with HRQOL cognitive functioning scale

Characteristics	Univariate analysis B (95% CI)	*P*
Hydrops, severe	1.43 (-1.99 – 4.86)	0.409
Z-Hemoglobin (SDs)	0.33 (-0.12 – 0.77)	0.151
Number of IUTs	-0.11 (-0.80 – 0.59)	0.760
GA at birth	-0.16 (-0.70 – 0.39)	0.572
Perinatal asphyxia, yes	1.20 (-3.84 – 6.23)	0.638
Severe neonatal morbidity, yes	0.03 (-3.11 – 3.16)	0.988
Gender, male	0.57 (-1.24 – 2.38)	0.535
Maternal education, low	-0.66 (-2.58 – 1.25)	0.493
Average	1.67 (-0.17 – 3.51)	0.076
High	-1.16 (-3.12 - 0.80)	0.244
Dutch ethnicity,	-1.43 (-4.33 – 1.47)	0.331
One parent Dutch	-2.94 (-7.95 – 2.07)	0.248
Non-Dutch	3.35 (-0.03 – 6.73)	0.052

Values are regression coefficient B (95%CI) and *P*.
*Difference at $P < .001$.

Behavioral Functioning (SDQ)

Mean SDQ scores of the children treated with IUT compared to Dutch norms (8-16 years), stratified for boys and girls, are presented in Table 5. No differences between boys treated with IUT compared to norms were detected. Parents of girls treated with IUT reported higher means on emotional symptoms, peer problems and total difficulties. After correction for multiple testing ($P < .001$), no differences were detected. Overall, for the children 3-16 years, 37/246 (15%) parents reported behavioral problems above the total difficulties cut-off point (≥ 14). This is significantly higher than the 10% reported in both literature and the general Dutch population ($P < .001$).[27] The results were not affected after excluding the cases diagnosed with neurodevelopmental impairment.

Potential risk factors were entered in a univariable regression analysis to assess the association with total behavioral difficulties (Table 6). Low maternal education was associated with higher total difficulties scores (B 2.72, 95% CI 1.28 – 4.16, $P < 0.000$) and high maternal education was associated with lower total difficulties scores (B -2.73, 95% CI -4.16 - -1.30, $P < 0.000$).

Table 5 Behavioral functioning of boys and girls aged 8-16 years treated with IUT compared with Dutch norm references aged 8-16 years.

	Boys				**Girls**			
	IUT-group	**Dutch norm**			**IUT-group**	**Dutch norm**		
SDQ-Parent Form	**(n = 69)**	**(n = 146)**	***d***	***P***	**(n = 74)**	**(n = 154)**	***d***	***P***
Total Difficulties	8.6 (6.3)	7.8 (5.5)	0.15	0.344	7.5 (5.3)	5.6 (4.8)	0.40	0.007*
Emotional symptoms	2.3 (2.3)	1.8 (1.9)	0.26	0.094	2.6 (2.5)	1.7 (1.8)	0.50	0.002*
Conduct problems	1.4 (1.6)	1.3 (1.6)	0.06	0.669	0.8 (1.2)	0.8 (1.2)	0.00	1.000
Hyperactivity	3.2 (2.6)	3.3 (2.9)	0.03	0.807	2.6 (2.4)	2.1 (2.3)	0.22	0.131
Peer problems	1.7 (2.0)	1.4 (1.7)	0.18	0.255	1.5 (1.8)	0.9 (1.4)	0.43	0.006*
Prosocial behavior	7.9 (2.3)	8.2 (1.6)	0.19	0.268	8.7 (1.7)	8.8 (1.4)	0.07	0.639

Values are mean ± standard deviation (SD), effect size (d) and *P*.
*Difference at $P < .05$; Difference at $P < .001$; Bonferroni correction for multiple testing applied.
SDQ, Strengths and Difficulties Questionnaire.

Table 6. Analysis of potential risk factors associated with behavioral difficulties

Characteristics	Univariate analysis B (95% CI)	*P*
Hydrops, severe	0.25 (-2.28 – 2.78)	0.844
Z-Hemoglobin (SDs)	0.00 (-.01 – 0.02)	0.682
Number of IUTs	-0.28 (-0.78 – 0.22)	0.268
GA at birth	0.19 (-0.24 – 0.62)	0.384
Perinatal asphyxia, yes	0.98 (-2.60 – 4.57)	0.591
Severe neonatal morbidity, yes	0.09 (-2.10 – 2.28)	0.937
Gender, male	0.98 (-0.36 – 2.33)	0.152
Maternal education, low	2.72 (1.28 – 4.16)	0.000*
Average	0.05 (-1.34 – 1.43)	0.950
High	-2.73 (-4.16 - -1.30)	0.000*
Dutch ethnicity,	-0.92 (-3.28 – 1.43)	0.443
One parent Dutch	2.75 (-1.59 – 7.10)	0.214
Non-Dutch	0.16 (-2.57 – 2.88)	0.911

Values are regression coefficient B (95%CI) and *P*.
*Difference at $P < .001$.

Discussion

This is the first study evaluating HRQOL and behavioral functioning in children and adolescents treated before birth with IUT for alloimmune anemia. The results of this study demonstrate that, differences were shown between the parent-rated IUT group and the norm population on cognitive, social and emotional domains. When corrected for multiple testing, only a difference was detected for the parent-rated cognitive functioning scale. Behavioral difficulties were more prevalent, and were associated with maternal educational levels.

Interestingly, the children did not self-report more difficulties with cognitive function than norms. Discrepancies between parent- and child-reports have been documented frequently and do not imply that either reporter is inaccurate.[30;31] Lack of agreement between parents and children on certain domains of HRQOL may result from different expectations, experiences and perceptions of the same situation.[32]

Parents reported more behavioral difficulties compared to literature and norms.[26;27;33] More problems were reported if mothers had a low educational level. Socio-demographic factors, including socio-economic status and parental educational level, are well-known predictors of subsequent child outcomes including cognitive development, HRQOL and socio-emotional and behavioral functioning.[34-36] Of the 37 children with behavioral difficulties, four (11%) were also diagnosed with neurodevelopmental impairment.[13]

Excluding the children with neurodevelopmental impairment did not affect the results. It is important to mention that the Strengths and Difficulties Questionnaire (SDQ) is a screening questionnaire and an elevated score does not equate to the actual presence of a behavioral disorder. The SDQ primarily aims to draw the attention of professionals to the potential presence of behavioral problems. Furthermore, the SDQ is not yet validated for all age ranges in the Dutch population. Further study regarding potential behavioral disorders are required to investigate the clinical significance of this finding.
Unfortunately, 21% (93/451) of the children were lost to follow-up, possibly because of the long time lapse since IUT treatment. However, comparison of the antenatal and perinatal characteristics showed no differences between the study group and lost to follow-up-group. Furthermore, it is likely that there are differences between our study group and the general Dutch population. Most importantly, the mean gestational age at birth was 36 weeks (late-preterm) in our study group versus 37-40 weeks of gestation in the general Dutch population. Studies have shown that children and adolescents born late preterm are at increased risk of cognitive, behavioral and emotional problems.[37] However, others suggest that children born mild to moderate premature do not experience an overall lower HRQOL.[38] Neurodevelopmental impairment was evaluated in our previous study in this cohort and the vast majority (95%) of children treated with IUT had normal neurodevelopmental functioning.[13] Several risk factors were found to be associated with neurodevelopmental impairment including fetal hydrops, number of IUTs, prematurity (<32 weeks), severe neonatal morbidity and parental education. In this study, only maternal educational level was associated with behavioral difficulties. Excluding the children with neurodevelopmental impairment did not alter our results. The pathophysiological mechanism causing long-term impairment in severely anemic and hydropic fetuses is not known. Hypoxic injury related to severe anemia or hydrops may result in cerebral injury which could lead to altered or impaired neurologic development.
To conclude, the current study shows that the majority of parents and children perceived child HRQOL following IUT to be within the normal range. Only a difference on cognitive functioning was shown between the IUT group and the norm population. According to our screening questionnaire, parents reported more behavioral difficulties compared to norms associated with lower maternal educational levels. Decreased cognitive functioning may refer to learning difficulties, attention deficit problems and/or difficulties in the interaction with peers. Possible clinical and longer term implications of increased behavioral difficulties are problems in adult life related to adaptation issues. Both cognitive and behavioral difficulties are known to have an impact on future socioeconomic potential. This study is however the first on HRQOL and behavioral functioning of children and adolescents after IUT, and results need to

be interpreted with care. The clinical significance of our findings should be validated by further study, as assessment of HRQOL and socioeconomic status in adulthood will provide a clearer representation of the long-term functional outcomes following IUT. Future studies should include adequate controls matched for important perinatal (e.g. gestational age at birth) and demographic variables (e.g. socioeconomic status). We believe that long-term follow-up should be an integrated component of IUT treatment using standardized psychometric measures with the ability to address more subtle problems alongside obvious neurodevelopmental impairments.

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Chapter 3

Immunoglobulins in neonates with Rhesus hemolytic disease of the fetus and newborn: Long-term outcome in a randomized trial

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Abstract

Objective

Prophylactic intravenous immunoglobulin (IVIg) does not reduce the need for exchange transfusion nor the rates of other adverse neonatal outcomes in neonates with Rhesus hemolytic disease of the fetus and newborn (Rhesus HDFN) according to our randomized controlled trial analysis. Our objective was to assess the long-term neurodevelopmental outcome in the children included in the trial and treated with either IVIg or placebo.

Methods

All families of children included in the trial were asked to participate in this follow-up study. Long-term neurodevelopmental outcome in children at least two years of age was assessed using standardized tests. Primary outcome was the incidence of neurodevelopmental impairment defined as at least one of the following: cerebral palsy, severe cognitive and/or motor developmental delay (a test score <-2SD), bilateral deafness or blindness.

Results

Sixty-six of the 80 children (82.5%) who were recruited in the initial randomized controlled trial participated in the follow-up study. Children were assessed at a median age of 4 years (range: 2-7 years). The median cognitive score in the IVIg group was 96 (range: 68-118) and 97 (range: 66-118) in the placebo group ($P = 0.79$). There was no difference in the rate of neurodevelopmental impairment between the IVIg and placebo groups (3% (1/34) versus 3% (1/32), $P = 1.00$).

Conclusions

Long-term neurodevelopmental outcome in children treated with intravenous immunoglobulin was not different from children treated with placebo. Standardized long-term follow-up studies with large enough case series and sufficient power are needed to replicate these findings.

Introduction

Rhesus hemolytic disease of the fetus and newborn (Rhesus HDFN) results from maternal red-cell alloimmunization against red blood cells antigens for which mother and fetus are incompatible. Rhesus HDFN can lead to perinatal death if fetal anemia is left untreated and can cause severe hyperbilirubinemia in neonatal survivors.[1]

Conventional postnatal treatment consists mainly of intensive phototherapy (PT), exchange transfusions (ET) and top-up red blood cell transfusion. ET is necessary in Rhesus HDFN when intensive PT fails and serum bilirubin levels approach the threshold for ET. However, ET is an invasive procedure and is associated with several adverse events.[2]

Treatment with intravenous immunoglobulin (IVIg) combined with PT has been suggested as an alternative therapy to reduce the need for ET. In a recent randomized double-blind placebo-controlled trial we found that prophylactic treatment with IVIg did not reduce the need for ET in children with Rhesus HFDN.[3] The effect of IVIg in children with Rhesus HDFN on the long-term neurodevelopmental outcome has not yet been studied.

The primary aim of this study was to evaluate the long-term neurodevelopmental outcome in all children with Rhesus HDFN included in the randomized controlled trial and treated with either IVIg or placebo. Our secondary aim was to assess the presence of allergies and susceptibility to ear-, nose- and throat infections. In a follow-up study in children after antenatal treatment for fetal/neonatal alloimmune thrombocytopenia (FNAIT), Radder et al found significantly less ear-, nose- and throat problems in those exposed to IVIg treatment compared to children not exposed to maternal IVIg treatment.[4]

Patients and Methods

This follow-up study was performed at the Leiden University Medical Center, the Netherlands, national referral center for the management, intrauterine and perinatal treatment of Rhesus HDFN. Parents of all children with Rhesus HDFN included in the randomized controlled trial and treated with IVIg (n=41) or placebo (n=39) between 2006 and 2010 were asked to participate in this follow-up study. The trial was initiated to investigate whether prophylactic use of IVIG reduces the need for exchange transfusions in neonates with Rhesus HDFN.[3] After stratification for treatment with intra-uterine transfusion (IUT), neonates were randomized for IVIg or placebo. Informed consent was obtained from all families. The long-term neurodevelopmental outcome in 20 of these

children was previously reported in a large follow-up study after IUT (LOTUS study).[1] All families were asked to visit our outpatient department for neurodevelopmental examination. Families who were unable to travel to our outpatient department, were visited at home.

Primary outcome was a composite outcome termed neurodevelopmental impairment (NDI) defined as at least one of the following: cerebral palsy, severe cognitive delay (a test score <-2 Standard Deviation (SD)), severe motor delay (a test score <-2 SD), bilateral deafness requiring hearing amplification and/or bilateral blindness. Secondary outcome measures in the current study were the presence of allergies and susceptibility to ear-, nose- and throat infections. Parents were questioned about the presence of recurrent ear-, nose- and throat infections, hospitalization and required surgery.

Cognitive development in children aged 2 to 3 years was assessed according to the Dutch version of the Bayley Scales of Infant and Toddler Development, 2nd edition (BSID-II).[5] BSID-II provides a mental development index (MDI) and a psychomotor development index (PDI). Children between 3 and 7 years of age were examined with the Dutch version of the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III). WPPSI-III provides a full scale Intellectual Quotient (IQ) score and subsection scores for Verbal IQ and Performance IQ.[6] BSID and WPPSI scores follow a normal distribution curve with a mean score of 100 and a standard deviation of 15. A score of 70-84 indicates mild delay (<-1 SD) and a score <70 indicates severe delay (<-2 SD). Parents of the children were still blinded at the time of assessment, and will be informed after the completion of this follow-up study. Socio-economic status of the parents was determined according to the Dutch Sociaal en Cultureel Planbureau, and was registered as high, average or low.[7]

Statistical analysis

Categorical variables were compared using the chi-square test or Fisher's exact test as appropriate. Student-t-test and Mann-Whitney test were used for continuous variables. A P value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, Il, USA).

Results

All families of the 80 children included in the trial were approached to participate in this follow-up study. Fourteen children (18%) could not be assessed since parents declined consent or due to loss of contact information, of which 7/41 in the IVIg group and 7/39 in the placebo group. No significant differences in baseline characteristics and

socio-economic status were found between the included and lost-to-follow-up group. Complete follow-up data were obtained from 66 (83%) children by a visit at our outpatient department (n = 57) or a visit at home (n = 9). A flow diagram of study participants is shown in the Figure. The baseline characteristics of the IVIg and placebo groups were similar (Table 1). Detailed information on the long-term neurodevelopmental outcome of the children included for follow-up in both groups, overall and after stratification for treatment with or without IUT, is presented in Table 2. Median age at follow-up assessment was 4.0 (range: 2 - 7) years in the IVIg group and 4.1 (range: 2 - 7) years in the placebo group. The incidence of NDI in children treated with IVIg was 3% (1/34) compared to 3% (1/32) in the placebo group (P = 1.00). NDI in the two children was due to severe cognitive delay (cognitive score = 66 and 68). Median cognitive score in children in the IVIg group was 96 (range: 68 - 118) compared to 97 (range: 66 - 118) in the placebo group (P = 0.79). Mild cognitive delay (< -1 SD) was detected in 18% (6/34) and 16% (5/32) in the IVIg group and placebo group, respectively (P = 0.83). None of the children had cerebral palsy, bilateral blindness or deafness. Similar results were obtained for the sub-groups of children after stratification for treatment with or without IUT.

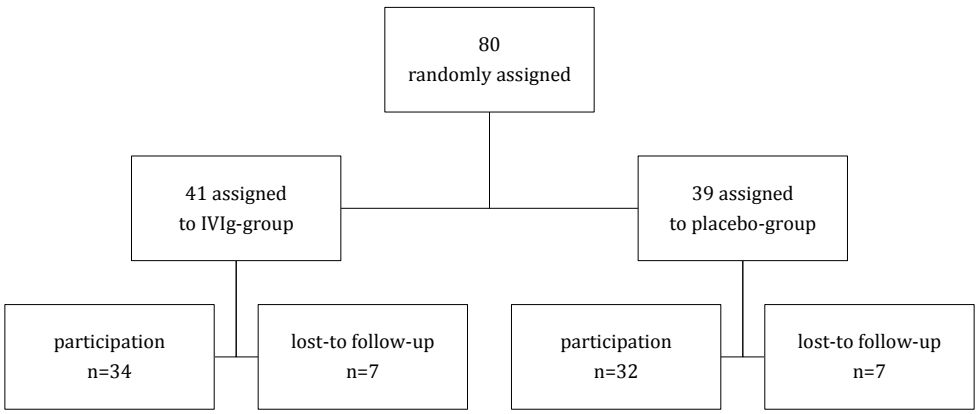

Figure Flow diagram of study participants.

Table 1 Baseline characteristics of the children eligible for follow-up.

	IVIg group (*N* = 41)	**Placebo group (*N* = 39)**	***P***
Gestational age at birth, mean ± SD, weeks	36.7 ± 1.0	36.5 ± 0.6	0.23
Birth weight, mean ± SD, grams	2994 ± 485	2953 ± 424	0.68
Male, *n* (%)	29 (71)	25 (64)	0.53
Neonates treated with intrauterine transfusions, *n* (%)	27 (66)	26 (67)	0.94
Number of IUTs per neonate, median (range)	2 (0-4)	3 (0-6)	0.48
Neonates treated with exchange transfusions, *n* (%)	7 (17.1)	6 (15.4)	0.84
Phototherapy, mean ± SD, days	4.7 (1.8)	5.1 (2.1)	0.34

No differences in the occurrence of allergies and ear-, nose- and throat infections between the IVIg and placebo groups was detected. The incidence of allergies in children treated with IVIg was 12 (4/34) compared to 19 (6/32) in those treated with placebo (P = 0.51). Recurrent ear-, nose-, and throat infections were present in 7/34 (21%) children treated with IVIg versus 9/32 (28%) children treated with placebo (P = 0.48).

Table 2 Long-term neurodevelopmental outcome in the IVIg and placebo groups and according to stratification for IUT

	Total group (*N* = 66)			**IUT group (*N* = 44)**			**no-IUT group (N = 22)**		
	IVIg (*n* = 34)	**Placebo (*n* = 32)**	***P***	**IVIg (*n* = 22)**	**Placebo (*n* = 22)**	***P***	**IVIg (*n* = 12)**	**Placebo (*n* = 10)**	***P***
Age at follow-up, median (range), years	4.0 (2.0-7.0)	4.1 (2.0-7.0)	0.67	3.1 (2.0-5.1)	4.0 (2.0-7.0)	0.46	5.0 (3.1-7.0)	5.0 (4.0-7.0)	.97
Cognitive score, median (range)	96 (68-118)	97 (66-118)	0.79	95 (76-118)	96 (66-111)	0.87	97 (68-108)	96.5 (79-118)	.58
Mild delay, *n* (%)	6 (18)	5 (16)	0.83	5 (23)	4 (18)	0.71	1 (8)	1 (10)	.97
Neurodevelopmental impairment, *n* (%)	1 (3)	1 (3)	1.00	0 (0)	1 (5)	0.32	1 (8)	0 (0)	.77

Discussion

This is the first and largest randomized controlled study to date on long-term neurodevelopmental outcome in children with Rhesus HDFN treated postnatally with IVIg or placebo. We were able to follow-up 83% children who participated in the initial randomized controlled trial and found no difference in long-term neurodevelopmental outcome between children treated with either IVIg or placebo. The incidence of NDI was 3% in children treated with IVIg compared to 3% in children who received placebo. After stratification for treatment with or without IUT, similar results were obtained. Analysis from our initial randomized controlled trial showed that IVIg had no beneficial effect on the short-term outcome. In addition, this study suggests that IVIg does not have a beneficial effect on the long-term neurodevelopmental outcome.

The incidence of severe developmental delay in both groups (3% and 3%) seems in line with the incidence of severe delay in the general population (2.3%).[8] None of the children had cerebral palsy, bilateral blindness or deafness. The incidence of NDI found in this study was slightly lower compared to a large follow-up study performed recently by our study group in 291 children with Rhesus HDFN (LOTUS study). In this large cohort, the incidence of NDI was 5%. However, all children included in the LOTUS study were treated with IUT for fetal anemia, and in 26% of cases fetal hydrops was present. As shown in the LOTUS study, fetal hydrops is an important risk factor for adverse neurodevelopmental outcome. In the cohort of children included in the current study, only 4 children had mild fetal hydrops and none of them developed severe developmental delay.

Our findings also suggest that treatment with IVIg in the neonatal period does not seem to have harmful effects on the long-term outcome, which is in accordance with previous studies. In a randomized controlled trial of 82 infected neonates treated with IVIg prophylaxis in addition to antibiotics, von Muralt et al found no evidence of harmful effects on neurodevelopmental outcome.[9] In another study, Ahmed et al (2011) used IVIg therapy during pregnancy in eight patients with pemphigus vulgaris. No developmental or behavioral abnormalities were detected in children born after a pregnancy treated with IVIg, with a mean follow-up period of 7 years.[10] These studies, including ours, may however be too small to reliably determine the effect of IVIg on the long-term outcome.

The secondary objective of this study was to evaluate the incidence of allergies and ear-, nose- and throat infections in both groups. Comparison between both groups revealed no association between IVIg and allergies and ear-, nose- and throat infections. Not much is known on the association between IVIg in the perinatal period and the development of allergies and ear-, nose and throat problems at a later age. IgG appears to be involved in the regulation of IgE-mediated immediate-hypersensitivity reactions, allergies.[11] In a

previous study in 48 children after antenatal treatment for fetal/neonatal alloimmune thrombocytopenia (FNAIT), Radder et al found significantly less ear-, nose- and throat problems in those exposed to IVIg treatment compared to children not exposed to maternal IVIg treatment.[4] However, in our group, IVIg was given only once compared to repeated weekly administration of IVIg in the FNAIT group.

The most important limitation of our study is the relatively incomplete follow-up. We were not able to examine fourteen children (18%) due to loss of contact address or parents' decline to participate. However, comparison of baseline characteristics between the study- and the lost-to-follow-up group showed no significant differences, assuming little bias. Lastly, our randomized controlled study was designed to detect a difference on the short-term outcome (namely the use of ET in the neonatal period) and was not designed to detect a difference on the long-term neurodevelopmental outcome. Our conclusions may thus be limited by the relatively small sample size and power.

Conclusion

We found no differences in long-term neurodevelopmental outcome in children with Rhesus HDFN treated with IVIg compared to placebo. Standardized long-term follow-up studies with large enough case series and sufficient power are needed to replicate these findings.

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PART III

FETOSCOPIC LASER SURGERY IN TWIN-TWIN TRANSFUSION SYNDROME

Chapter 4

Long-term neurodevelopmental outcome in monochorionic twins after fetal therapy: A systematic review

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Abstract

Monochorionic (MC) twins are at risk for several disorders, including twin-twin transfusion syndrome (TTTS), Twin Reverse Arterial Perfusion (TRAP) and selective intrauterine growth restriction (sIUGR). Several fetal interventions, such as serial amnioreduction (AR), fetoscopic laser coagulation of placental anastomoses (FLC) and selective feticide have led to improved perinatal morbidity and mortality rates. Nevertheless, the rate of cerebral lesions in monochorionic twins after fetal therapy appears to be high. Follow-up studies show a high incidence of cerebral palsy (CP) and neurodevelopmental impairment (NDI). We performed a systematic review on the long-term neurodevelopmental outcome in MC twins with TTTS following AR and FLC and MC twins following selective feticide of the co-twin due to TTTS, TRAP, sIUGR and congenital anomalies.

Introduction

Due to placental vascular anastomoses, monochorionicity subjects twins to specific complications, in particular Twin-Twin Transfusion Syndrome (TTTS), selective Intrauterine Growth Restriction (sIUGR) and Twin Reverse Arterial Perfusion (TRAP). In addition, congenital anomalies are more common in monochorionic (MC) twins compared to dichorionic (DC) twins. Studies show a 3- to 4-fold higher risk of neurologic morbidities in MC twins compared to DC twins mainly due to a higher incidence of prematurity and very low birth weight[1;2].

TTTS complicates approximately 10% of MC twin pregnancies and develops typically between 15 and 26 weeks of gestation[3]. It results from shunting of blood from one twin, the donor, to the other twin, the recipient, through placental vascular anastomoses. Serial amnioreduction (AR) and fetoscopic laser coagulation of placental anastomoses (FLC) are the two main treatment options in chronic TTTS. According to a Cochrane systematic review, FLC results in higher survival rates and better neonatal outcomes than AR[4]. With higher survival rates, an increasing number of follow-up studies in TTTS survivors are being published, shedding more light on neurodevelopmental outcome in the long-term.

In cases where severe complications lead to the impending death of one twin, selective feticide can be offered, to prevent exsanguination of the surviving twin into the dead twin[5]. Perinatal survival rates after selective feticide using cord occlusion techniques are reported to be high[3], but information on the long-term neurodevelopmental outcome in survivors is limited.

The current systematic review focuses on the long-term neurodevelopmental outcome in MC twins with TTTS following AR and FLC and MC twins following selective feticide of the co-twin due to TTTS, TRAP, sIUGR and congenital anomalies.

Methods of the Review

A systematic literature search was utilized to retrieve the studies and articles for this review. An electronic MEDLINE, Embase and Cochrane Library literature search was performed using the following MESH terms: Monochorionic, amnioreduction, fetoscopic laser coagulation, selective feticide, cord occlusion, intrauterine, time, prognosis, epidemiologic studies, human development, neurobehavioral manifestations and morbidity. The computer aided search included the period from 1980 to April 2011. All reference lists of primary articles and reviews were examined to search for additional references. Then, a manual search of identified articles was conducted. If

needed, authors were contacted for further information. Studies were included if the following criteria were present: the inclusion of MC twins treated for TTTS with AR and/or FLC, MC twins treated with selective feticide of the affected co-twin, long-term neurodevelopmental outcome, standardized measures and the conduct of statistical tests. The methodological quality of each selected study was assessed independently by two reviewers (JvK and EL). The following exclusion criteria were applied: case reports, reviews, dissertations, book chapters, guidelines and commentaries.

Results

We found no review article or meta-analysis that focused specifically on the long-term neurodevelopmental outcome in children with TTTS treated with AR and/or FLC and neither on the long-term neurodevelopmental outcome after selective feticide of the affected co-twin. We identified 20 studies that met our inclusion criteria of which 8 on AR, 6 on FLC, 1 on AR and FLC, 2 reports on a RCT comparing AR with FLC[6;7], and 3 on selective feticide. Our selected studies are summarized, in chronological order, in Tables 1 (AR), 2 (FLC) and 3 (Feticide). Neurodevelopmental impairment (NDI) was determined on the basis of the presence of cerebral palsy (CP), cognitive/mental/developmental test scores < - 2SD, bilateral blindness, or deafness requiring amplification with hearing aids.

Long-term outcome in TTTS treated with serial amnioreduction

Ten studies on TTTS following AR have been published to date (see Table 1). The reported incidence of CP after AR ranges from 13 to 23%, except for two studies, reporting a 5% (2/42) and 6% (3/52) incidence of CP[8;9]. These exceptions are probably due to underreporting, since the diagnosis of CP in these studies was solely based on clinical records in the first, while only children born very preterm underwent neurologic examination in the latter.

The incidence of NDI in TTTS after AR varies considerably, ranging from 6% to 26%[8-15]. This large discrepancy is probably due to considerable differences in methodology between the studies and heterogeneity within the case series, such as TTTS stage at the time of intervention or gestational age at birth. In the majority of studies, case series were small, ranging from 20 to 52 children, and did not include appropriate comparison groups. As a consequence, studies were unable to assess whether NDI was due to confounders such as TTTS stage, prematurity or low birth weight[8]. Finally, not all studies included developmental tests[8;10;14;15].

According to Reisner et al. 21% (4/19) of the children had suspected CP at < 18 months and 15% (3/20) had confirmed CP at ≥ 18 months after AR for 'stuck twin syndrome'[15]. However, Reisner et al. did not employ developmental tests and their outcome did not include NDI.

The long-term outcome according to Mari et al. included the diagnosis of CP and was reported in 5% (2/42) of children[8]. One other child was diagnosed with multicystic encephalomalacia, which is a significant risk factor for developing CP. No developmental tests were used. Among the cases in which both twins had clinically normal development, 27% (9/34) required therapy because of mild speech and/or motor delay.

With formal psychological testing and a clear description of NDI, Cincotta et al. reported CP in 13% (3/23) and NDI in 22% (5/23) of survivors at the age of at least 2 years (2-4.5 years)[13]. Of note, all these children had abnormal brain scans. Periventricular leukomalacia and cerebral atrophy were seen in 17% of survivors, but in none of the gestation matched twin controls (P = .03).

Both Haverkamp et al. and Frusca et al. classified the results of their neurological and psychomotor examinations into 3 groups that is, normal development, minor disabilities such as 'clumsy' coordination problems and mild mental retardation, and major impairment that is, CP and severe developmental delay[11;12]. Haverkamp et al. found minor NDI in 33% (13/40) and NDI in 23% (9/40), while Frusca et al. found minor NDI in 16% (5/31) and NDI in 26% (8/31) of TTTS survivors.

At a mean age of 6.2 years at follow up, Lopriore et al. found an incidence of 26% (5/19) of CP[10]. Five children with CP needed special education or were ≥ 1 grades below the appropriate school level for their age and were therefore considered to have an abnormal mental development as well. In the group of children without CP or NDI, 22% (5/23) required speech therapy.

Dickinson et al. reported CP in 6% (3/49) of TTTS survivors[9]. At a median age of five years, NDI was present in 14% (7/49). Minor NDI was not reported. The authors complemented their developmental test with questionnaires on behavioral problems. Although the mean intelligent quotient was 8 points lower compared to a contemporaneous regional cohort, there were no differences in behavioral scores. However, this reassuring finding relates only to pre-school children. The authors suggest that future research should include school-aged children.

At 2 years, Lenclen et al. found minor and major NDI in 9.5% and 9.5% following AR (4/21), respectively[14]. Ages and Stages Questionnaires (ASQ) revealed lower scores in 21 AR survivors compared to 88 FLC survivors and 201 DC twins (P = .01) and ASQ domains were more often abnormal (P = .005).

Finally, Li et al. recently reported on the long-term outcome in 20 survivors treated with AR[16]. With a mean age at follow-up of 6.3 years (3-12 years), four children (20%)

had major NDI and two children (10%) had minor NDI. The children with NDI were delivered before 29 weeks of gestation.
When the results of the follow-up studies are pooled together, the overall rate of CP and NDI in TTTS following AR is 14% (46/322) and 20.5% (32/156), respectively (see Table 1).

Long-term outcome in TTTS treated with fetoscopic laser surgery
Eight follow-up studies in TTTS after FLC have been published to date (see Table 2). The reported incidence of CP and NDI following FLC ranges from 3 to 12% and 8 to 18%, respectively[17-22].
In 1999, De Lia et al. report a 5.4% (5/93) incidence of severe handicaps in TTTS survivors after FLC[19]. Mean age at follow-up was however 14 months (range 1 to 34 months), which is too early for accurate assessment of CP or developmental delay. In addition, no developmental tests were performed.
Sutcliffe et al. found a 9% (6/66) incidence of CP in TTTS survivors treated with laser[18]. However, follow-up was incomplete and in 47% (31/66) of survivors neurological outcome was assessed using information from a general practitioner. In the group assessed by a pediatrician, 14% (5/36) had CP. Although the children assessed by a pediatrician were also tested with a standardized developmental test (Griffiths' Scale), details on the number of children with severe developmental delay were not reported or scored as primary outcome.
Two large follow-up studies from Germany, reported an incidence of major neurological impairment of 11% (10/89) and 6% (10/167)[17;20]. In both studies, the definition of neurological impairment did not include severe developmental delay. Hence, children with severe developmental delay but without CP were not included in the group with major impairment.

Table 1 Long-term neurodevelopmental outcome in TTTS case series treated conservatively with AR.

Author, year	Outcome measure	CP	NDI	Methodological comments
Reisner, 1993[15]	Neurological examination	(7/39)	n.a.	No developmental tests, no controls, n = 19 < 18 months
Mari, 2000[8]	Clinical record, discussion parent or pediatrician, speech or physical therapy	5% (2/42)	n.a.	No developmental tests, inclusion of mild TTTS cases, follow-up based on clinical records/interviews, no controls, high NND rate (16%)
Cincotta, 2000[13]	Neurological examination, physiotherapy assessment, Griffiths Mental Development Scale	13% (3/23)	22% (5/23)	High NND rate (18%), the inclusion of gestation matched twin controls
Haverkamp, 2001[12]	Neurological examination, Denver Developmental Screening Test, Griffiths Mental Development Scale	23% (9/40)	23% (9/40)	High lost-to-follow-up rate (18%), incomplete follow-up, no controls
Frusca, 2003[11]	Neurological examination, Griffiths Mental Development Scale	16% (5/31)	26% (8/31)	35% (11/31) of children were < 2 years at time of follow-up, no controls
Lopriore, 2003[10]	Neurological examination, school functioning (mainstream vs. special education or ≥ 1 grades below age appropriate level)	21% (6/29)	n.a.	No developmental tests
Dickinson, 2005[9]	Neurological examination, General Health Questionnaire, Vineland Adaptive Behavioral Scales, Child Behavior Checklist, Bayley Scales, Stanford-Binet Intelligence Scale	6% (3/49)	14% (7/49)	Neurologic examination only in children born very preterm, minor NDI not reported, first report on behavioral outcome but only in pre-scholars, inclusion contemporaneous regional cohort
Lenclen, 2009[14]	Neurologic examination, Ages and Stages Questionnaire	19% (4/21)	n.a.	Children not individually investigated, preterm DC controls matched for GA at birth, inclusion of children following FLC
Salomon, 2010[7]	Neurologic examination, Ages and Stages Questionnaire, Wechsler Intelligence Scale for Children –IV, Goodenough Draw-a-Man-Test	13% (6/47)	n.a.	NDI not reported, high NND rate (22%), no controls, inclusion of children following FLC
Li, 2011[16]	Neurologic examination, Enjoji Development Scale, Wechsler Intelligence Scale for Children –R, Wechsler Preschool Primary Scale of Intelligence	23% (3/13)	23% (3/13)	Small study size, preponderance of mild TTTS cases
Total		**14% (46/322)**	**20.5% (32/156)**	

CP is Cerebral Palsy; NDI is Neuro Developmental Impairment which is defined as CP, severe developmental delay (< 2SD), blindness or deafness; NND is Neo Natal Death; DC is Di Chorionic.
Of note: The first part of the Eurofetus trial[6] is not included in this table because the children are more fully described in the follow-up of this trial[7].

Table 2 Long-term neurodevelopmental outcome in TTTS case series treated with laser surgery.

Author, year	Outcome measure	CP	NDI	Methodological comments
De Lia, 1999[19]	Neurological examination	3% (3/93)	n.a.	No developmental tests, mean age at follow-up 14 months (1-34), no controls
Sutcliffe, 2001[18]	Neurological examination, Griffiths Mental Development Scale	9% (6/66)	9% (6/66)	High lost-to-follow-up rate (19%), some families followed up by GP (47%), incomplete developmental tests (54%), no controls
Banek, 2003[17]	Neurological examination, Griffiths Mental Development Scale, Snijders-Oomen Intelligence test	11% (10/89)	n.a.	Severe developmental delay not included as criterion for major disability, no controls
Graef, 2006[20]	Neurological examination, Griffiths Mental Development Scale, Snijders-Oomen Intelligence test	6% (10/167)	8% (13/167)	Sub-optimal/incomplete use of developmental tests
Lenclen, 2009[14]	Neurological examination, Ages and Stages Questionnaire	10% (9/88)	n.a.	Children not individually investigated, preterm DC control subjects matched for GA at birth
Lopriore, 2009[21]	Neurological examination, Bayley scales	6% (17/278	18% (50/278	2 TTTS-pregnancies treated > 26 weeks' gestation
Salomon, 2010[7]	Neurological examination, Ages and Stages Questionnaire, Wechsler Intelligence Scale for Children-IV, Goodenough Draw-a-Man-Test	12% (9/73)	n.a.	NDI not reported, no controls
Gray, 2011[23]	Neurological examination, Griffiths scales, Bayley scales	4% (5/113)	12% (14/113)	Mixed developmental tests: Griffiths scales, second and third version of the Bayley scales, no controls
Total		**7.2% (69/967)**	**13.3% (83/624)**	

CP is Cerebral Palsy; NDI is Neuro Developmental Impairment which is defined as CP, severe developmental delay (< 2SD), blindness or deafness; NND is Neo Natal Death; GP is General Practitioner; GA is Gestational Age.
Of note: One follow-up study [22] in TTTS after laser is not included in this table because the included children are more fully described in the follow-up of this study [21].

In a long-term follow-up study in 88 TTTS survivors treated with laser, Lenclen et al. report NDI in 11% (10/88) (CP: n=9; blindness: n=1)[14]. ASQ scores were similar in FLS and DC children. As suggested by the authors, since ASQ was used as a screening tool to determine developmental outcome instead of a standardized developmental test performed by psychologists, developmental delay might have been underestimated.
Recently, 3 European fetal therapy centers (Barcelona, Leuven and Leiden) performed a multicenter follow-up study to investigate the risk factors for NDI in TTTS treated with FLC[22]. Long-term outcome data, including standardized developmental test results (Bayley scales), were collected in 278 TTTS survivors, the largest follow-up study in TTTS to date. CP was diagnosed in 6% of children (17/278; 95% CI 4%-10%). Severe mental developmental delay was diagnosed in 7% (19/278; 95% CI 4%-12%) and severe psychomotor developmental delay in 12% (34/278; 95% CI 9%-18%). Two children had bilateral blindness (1%) and two other had bilateral deafness (1%). Overall, the incidence of NDI was 18% (50/278; 95% CI 13%-24%). Minor NDI was not reported.
Gray et al. found a 4.4% (5/113) incidence of CP and NDI was diagnosed in 12.4% (14/113) of children[23]. Four children with CP also had severe cognitive impairment, whereas nine children without CP were diagnosed with severe cognitive impairment as well, according to Griffiths' and Bayley scales
When the results of these follow-up studies are pooled together, the overall rate of CP and NDI in TTTS following FLC is 7.2 % (69/967) and 13.3% (83/624), respectively (see Table 2). One follow-up study from Lopriore et al.[22] was excluded from this analysis because the included children were more fully described in the multicenter follow-up of this study[21].

Amnioreduction versus Fetoscopic Laser Coagulation: the Eurofetus Trial

The first part of the largest RCT to date, the Eurofetus trial, comparing the perinatal outcome in TTTS following AR or FLC was published by Senat et al. in 2004 and showed a significant benefit in the FLC group[6]. Children in the FLC group were more likely to be free of major neurologic complications compared to children in the AR group at 6 months of age, 52% (75/144) versus 31% (44/140), respectively (P = .003). The definition of major neurologic complications included the presence of severe intraventricular hemorrhage (grade III or IV), severe cystic periventricular leukomalacia (grade III or IV), blindness and/or deafness. However, reliable assessment of neurologic abnormalities requires a longer follow-up period than 6 months**.** Recently, the authors published the long-term outcome in a subgroup of children included in the trial and found similar rates of CP between the AR and the FLC group that is, 15% (6/41) and 13% (9/69), respectively (P = .12)[7]. Developmental outcome using ASQ at 24 months and 48 months was similar in both groups as well (P = .3 and P = .8). Only at 5 years of

age, the children in the FLC group had better ASQ scores compared to their peers in the AR group (261.3 ± 53.7 vs. 228.6 ± 79.1; *P* = .04). When assessed at 6 years of age with a more objective test (Wechsler Intelligence Scale for Children), again no difference was found between the groups (90.6 ± 19.9 vs. 91 ± 33.1; *P* = .27). These findings seem controversial considering the lower incidence of neurologic sequelae at 6 months of age in the FLC group and the outcomes of previous large case series (albeit not RCT)[6;14;20].
Several confounders may explain why FLC was not associated with a reduced risk of NDI compared to AR. First, although the mean total IQ was similar in both groups, the SD was much larger in the AR group (91 ± 33.1; a -1 SD score of 57.9). Presuming that IQ-scores are normally distributed, this suggests that a significant number of children in the AR group had severe developmental delay (IQ < 70; -2 SD). If this is the case, the rate of NDI could in fact be higher in the AR group. Unfortunately, a clear definition of NDI was not reported. Second, the most important reason for the absence of a difference in neurodevelopmental outcome is probably the extremely high rate of neonatal deaths (NND) in the AR group that is, 36% (26/73) of all live-born babies. Detailed information on the cause of death was not provided, but the high mortality rate was probably due to withdrawal of intensive-care-treatment in very preterm children with severe cerebral lesions. The missing information is crucial to put the results into perspective. As reported, 22% (20/93) of live-born children in the AR group had very severe cystic periventricular leukomalacia (grade III)[6]. A positive correlation between high NND rate and withdrawal of intensive-care-treatment in children with severe cerebral injury strongly influences the interpretation of the long-term outcome results. Had these severely damaged children survived, the rate of CP and/or NDI in the AR group would have been much higher.

Risk factors for Neurodevelopmental Impairment

Although several studies have found a trend towards an independent association between higher Quintero stage and NDI, statistical significance was not reached[22]. However, in a multivariate logistic regression model, both Gray et al. and Salomon et al. found more advanced Quintero stage to be a significant and independent risk factor for adverse outcome, (OR, 13.02; 95% CI, 1.92-88.33) and (OR, 3.23; 95% CI, 2.19-4.76; *P* < .001), respectively[7;23]. This may relate to the fact that in previous studies, stage IV TTTS represented a relatively small percentage of survivors, whereas these studies were able to include a relatively large percentage of severe cases[7].
Lopriore et al. found gestational age at birth independently associated with NDI (OR 1.33 for each week, 95% CI: 1.05 to 1.67, *P* = .016)[22]. This association has been confirmed by Lenclen et al. and Li et al.[14;16]. The 2 large follow-up studies from Germany reported that NDI was more likely in twins born < 32 weeks of gestation[17;20]. The association

between low gestational age at birth and NDI may not be surprising, as prematurity is a well-recognized major risk factor for adverse neurodevelopmental outcome[24]. Gray et al. could not confirm these results, however, on univariate analysis small for gestational age (SGA) twins were at increased odds for NDI[23]. When adjusting for Quintero stage, Salomon et al. could not confirm a relationship between advanced gestational age at FLC and neurodevelopmental outcome. This might be explained by a positive correlation between gestational age at intervention and Quintero stage[22].

Whether survival and long-term neurodevelopmental outcome in stage I TTTS is better following FLC compared to conservative management with AR is not clear. In a retrospective study in stage I TTTS, Lopriore et al. found a higher incidence of NDI in the conservatively managed group compared to the group treated with FLC, 23% (7/30) versus 0% (0/21), respectively (P = .03), emphasizing the urgent need for a RCT in low stage TTTS[25].

Finally, the majority of follow-up studies to date suggest that both donor and recipient twins are equally vulnerable to brain injury, since no difference in the rate of NDI between the donors en the recipients has been found[7;9;10;12;17;18;20-23].

Long-term outcome in MC twins following selective feticide of the co-twin

Neurodevelopmental follow up of the surviving twin after selective feticide of the co-twin is limited to only 3 small studies, with a range of included children from 6 to 67[26-28]. Details on the 3 follow-up studies are shown in Table 3.

After bipolar cord coagulation for life-threatening malformation in one twin, TRAP sequence and severe TTTS with cerebral abnormalities, Robyr et al. found normal follow-up in all survivors but one with 'some form of developmental delay' at 1.4 years[28]. Follow-up was performed on a clinical basis at a very young age (0-42 months) and explicit criteria for NDI were lacking. According to the authors, the main contributors for poor outcome were performance of the procedure before 18 weeks of gestation and preterm rupture of the membranes (PROM) leading to preterm delivery.

In the largest prospective study on cord coagulation to date, follow-up was available in 72 survivors, with 67 (93%) children at ≥ 1 year[26]. Cord coagulation was offered for TRAP, severe discordant anomaly, sIUGR and selected cases of TTTS. A pediatrician assessed fifty-five children at a median age of 1.3 years while in 12 children information was provided by the general practitioner. Mental and motor development was classified as normal, mild, moderate or severe delay with Bayley scales or Snijders-Oomen Intelligence test and Peabody test. Four children had mild cognitive and/or motor delay, and 1 was diagnosed with severe cognitive delay. All but one child with delay were born before 29 weeks. Three children were treated for TRAP after 23 weeks and 2 had grade II periventricular leukomalacia on early brain scan. Although grade II

leukomalacia indicates a risk of 60-70% of CP, no CP was reported. The neonatal course of the children < 1 year of age was uneventful. According to the authors the assessment of developmental outcome would have been more accurate if all children had been evaluated at the treatment centers with the same assessment tools.

In a small follow-up study by telephone in 6 cases at a mean age of 4 months (2.3-6.6 months), Moise et al. reported that all survivors did well after radiofrequency ablation of the co-twin for major fetal anomalies[27]. However, 1 child was still in the NICU at the time of reporting, but had not developed major complications.

NDI in the abovementioned case series was probably underreported, as case series were too small, developmental tests were not consequently employed and age at follow-up was too young to detect developmental delay which, in general, becomes more apparent at a later age. Developmental problems following selective feticide may arise from the underlying pathology, the type of procedure as well as from preterm delivery[26]. The series published to date are too small to assess the relative contribution of each of these factors.

Pooling the results of these follow-up studies, the overall rate of CP and NDI is 0% (0/109) and 2% (2/103), respectively (see Table 3).

Table 3 Long-term neurodevelopmental outcome in MC twins treated with selective feticide of the co-twin.

Author, year	Outcome measure	CP	NDI	Methodological comments
Robyr, 2005[28]	Clinical evaluation pediatrician, parental information	no (0/36)	2.7% (1/36)	No developmental tests, no specification of delay or criteria NDI, children assessed at very young age, no controls
Lewi, 2006[26]	Assessment neurologist, local pediatrician or GP, Bayley scales, Snijders-Oomen Intelligence test, Peabody	no (0/67)	1.5% (1/67)	Different assessment tools, no specification criteria for NDI, children assessed at a young age, no controls
Moise, 2008[27]	Contact by phone, medical records	no (0/6)	n.a.	No developmental tests, small case series, follow up at mean age of 4 months, NDI not reported, no controls
Total		**No (0/109)**	**2% (2/103)**	

CP is Cerebral Palsy; NDI is Neurodevelopmental Impairment which is defined as CP, severe developmental delay (< 2SD), blindness or deafness; GP is general practitioner.

Discussion and conclusions

According to current literature, the incidence of CP and NDI in TTTS treated with AR is high. The outcome of TTTS survivors treated with FLC appears to be more favorable. However, the Eurofetus RCT reported similar rates of impairment in children treated with AR or FLC. The results of this study were however limited and confounded by an extremely high rate of deaths in the neonates treated with AR, probably due to withdrawal of intensive care in neonates with severe cerebral injury.
Recent findings suggest an important prognostic value of Quintero staging. Increased disease severity, that is higher Quintero stage, may not only be associated with increased perinatal mortality, but also with long-term morbidity[7;22;29]. Timely detection and treatment of TTTS are warranted to both increase survival and improve chances for a better long-term outcome. A controversial issue in TTTS remains however the optimal treatment for Quintero stage I TTTS. Multicenter RCTs with appropriate long-term follow-up are required to determine optimum management. Both donor and recipient in TTTS seem equally vulnerable to impairment. Finally, and not surprisingly, low gestational age at birth is a major risk factor for adverse neurodevelopmental outcome. Although long-term outcome following selective feticide is considered favorable, research is very limited and there is an urgent need for accurate and large long-term follow-up studies.
Special care must be taken when comparing the results of the selected studies for review, as discrepancy may be due to different methodology, differences in neonatal death rates, considerable heterogeneity within the small case series and lack of uniform outcome criteria. All in all, regardless of antenatal treatment, all survivors are at risk for NDI and require long-term follow-up.

Key Guidelines:

- Long-term neurodevelopmental outcome in TTTS following serial amnioreduction is considered worse when compared to laser surgery.
- The first RCT to date could not confirm a difference in long-term outcome after amnioreduction and laser surgery.
- Higher Quintero stage may not only be associated with perinatal mortality, but also with long-term neurodevelopmental impairment.
- The optimal management in stage I TTTS remains to be determined.
- Low gestational age at birth is a major risk factor for adverse outcome.
- Long-term neurodevelopmental outcome after selective feticide is not well known.

Research Directions:

- Multicenter RCTs, including long-term follow-up, are required to determine the optimal management in stage 1 TTTS.
- Long-term follow-up after selective feticide is urgently needed.
- Uniform and clearly defined criteria for neurodevelopmental impairment, including formal psychological testing with standardized measures, are necessary.

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Chapter 5

Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: A systematic review and meta-analysis

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Abstract

Objective
To estimate the odds of severe cerebral injury and long-term neurodevelopmental impairment in monochorionic twins treated with amnioreduction versus laser surgery for twin-twin transfusion syndrome.

Methods
A systematic review and meta-analysis of studies on cerebral injury and long-term impairment after amnioreduction versus laser surgery were conducted. Odds ratios with their 95% confidence interval were computed.

Results
Electronic and manual search identified 63 references. Five studies were included for analysis. We found an ample seven-fold higher risk of severe cerebral injury in live-born children treated with amnioreduction compared to laser (OR 7.69, 95% CI 2.78-20.0, P = .00). In children surviving the neonatal period, the odds were three-times higher following amnioreduction (OR 3.23, 95% CI 1.45-7.14, P = .00). Although not significant, monochorionic twins treated with amnioreduction had higher odds of periventricular leukomalacia and intraventricular hemorrhage (OR 2.08, 95% CI .86-5.00, P = .10 and OR 3.56, 95% CI .82-14.29, P = .09). Unfortunately, there were insufficient long-term outcome data available to assess the odds of neurodevelopmental impairment.

Conclusion
Amnioreduction is associated with an increased risk of severe cerebral injury compared to laser surgery in twin-twin transfusion syndrome. Our study highlights a crucial lack of studies focusing on long-term neurodevelopmental outcome. Follow-up into childhood is indispensible to determine outcome in terms of cerebral palsy, cognitive and socio-emotional development.

Introduction

Twin-twin transfusion syndrome (TTTS) is a severe complication of monochorionic (MC) twin pregnancies resulting from shunting of blood from one twin (the donor) to the other twin (the recipient) through placental vascular anastomoses. The donor twin becomes hypovolemic and anuric with oligohydramnios. The recipient twin becomes hypervolemic and polyuric with polyhydramnios. TTTS severity can be staged I to V according to Quintero's classification system[1]. Serial amnioreduction of excessive amniotic fluid (AR) and fetoscopic laser coagulation of the placental vascular anastomoses (laser) are the two main treatment options in TTTS. There is extensive evidence that serial AR is associated with increased perinatal mortality when compared to laser surgery[2]. Reliable information on long-term impairment in survivors after both interventions is lacking[3].

The objective of the current systematic review and analysis was to estimate the odds of severe cerebral injury and long-term neurodevelopmental impairment in MC twins treated with serial AR compared to laser surgery for TTTS.

Methods

This systematic review was performed using PRISMA statement: preferred reporting items for systematic reviews and meta-analyses[4]. Inclusion criteria were formulated according to our pre-defined Patient-Intervention-Comparison-Outcome (PICO) question. The patients are live-born MC diamniotic twins with TTTS diagnosed using standard prenatal ultrasound criteria[5]. The intervention refers to serial AR and the comparison is fetoscopic laser coagulation of placental vascular anastomoses. The primary outcome entails severe cerebral injury and long-term neurodevelopmental impairment (NDI) with a follow-up period from pregnancy outcome to childhood:

1. Severe cerebral injury was defined as intraventricular hemorrhage (IVH) ≥ grade III[6], cystic periventricular leukomalacia (cPVL) ≥ grade II[7], ventricular dilatation ≥ 97th percentile[8], porencephalic cysts, arterial or venous infarction detected on cerebral imaging i.e., cranial ultrasound, Computed Tomography scan or Magnetic Resonance Imaging.
2. Neurodevelopmental impairment (NDI) was defined as cerebral palsy, bilateral blindness, bilateral deafness or cognitive developmental delay > 2 standard deviations (SD) below the population mean, diagnosed using standardized tests.

Due to an anticipated lack of randomized controlled trials, we included both randomized and non-randomized studies. Studies that did not match our PICO question were excluded. English language restrictions were applied.

Data Sources

An electronic literature search was performed with PubMed, MEDLINE and ISI Web of Science (WoS) up to March 2012. Table 1 presents the search strategies for PubMed that were subsequently adapted for use in MEDLINE and ISI WoS. To identify articles not captured by the electronic searches, we hand-searched reference lists of relevant studies.

Table 1. Search Strategies PubMed

Strategy	Mesh and entry terms
#1	("Fetofetal Transfusion"[Mesh] OR "Fetofetal Transfusion"[All Fields] OR "Twin Transfusion Syndrome"[All Fields] OR "Twin Transfusion Syndromes"[All Fields] OR "Twin Transfusion"[All Fields] OR "Twin Transfusions"[All Fields])
#2	("Amniocentesis"[Mesh] OR "Amniocentesis"[All Fields] OR "Amniocenteses"[All Fields] OR "Amnioreduction"[All Fields] OR "Amniodrainage"[All Fields])
#3	("Fetoscopy"[Mesh] OR "Fetoscopy"[All Fields] OR "Fetoscopic Surgeries"[All Fields] OR "Fetoscopic Surgical Procedures"[All Fields] OR "Intrauterine Laser Treatment"[All Fields] OR "Fetoscopic Laser Surgery"[All Fields] OR "In Utero Laser Ablation Therapy"[All Fields] OR "Laser Photocoagulation"[All Fields] OR "Laser Surgery"[All Fields] OR "Endoscopic Laser Surgery"[All Fields] OR "Laser Therapy"[All Fields])
#4	("Infant"[Mesh] OR "Infant"[All Fields] OR "Infant Development"[All Fields] OR "Child"[All Fields] OR "Child development"[All Fields] OR "Neurologic Injury"[All Fields] OR "Cerebral Damage"[All Fields] OR "Neurodevelopmental Outcome"[All Fields] OR "Neurodevelopment"[All Fields] OR "Developmental Follow-Up"[All Fields])
#5	#1 AND #2 AND #3 AND #4 Results: 25

= number.

Study Selection

Eligibility and methodological quality of the studies were assessed independently by the corresponding (JK) and last author (EL). The following data were extracted and tabulated: first author, year of publication, study design, country of origin, selection and allocation of patients, data collection, comparability of patients and controls, potential confounders, operationalization of primary outcome and outcome measurement, efforts to minimize bias, the incidence of severe cerebral injury and NDI in patients and controls (2x2 tables) and the length and completeness of follow-up. In case of overlap or duplications in patients between studies, the study with the best overall study quality was included for review. A randomized controlled trial was, a priori, considered the best study design. Disagreements regarding eligibility, methodological quality and data extraction were resolved by discussion and consensus between authors.

Statistical Analysis

To summarize the results of the selected studies, an Excel spreadsheet was used. We performed statistical analysis using Stata (StataCorp LP, College Station, Texas). Categorical or dichotomous data were meta-analyzed with odds ratios (ORs) and their 95% confidence intervals (CI), using 2x2 tables. We used the recommended method to add 0.5 where 2x2 tables contained cells with zero events, allowing continuity correction. Studies were a priori analyzed into two groups that is, studies including and studies excluding neonatal deaths in their outcome analysis. Heterogeneity between studies was examined with the inconsistency square (I^2) statistics, with between-study heterogeneity at $I^2 \geq 50\%$ and $P \geq .05$ [9]. In case of heterogeneity, a random effects model was used[10]. Otherwise, or in case of limited studies to reliably estimate between study variability, a fixed effect model was used. We performed meta-analyses and constructed forest plots to examine the effect of AR compared to laser surgery on severe cerebral injury with separate analyses for cPVL ≥ grade II and IVH ≥ grade III, and NDI with separate analyses for cerebral palsy, bilateral blindness, deafness and cognitive developmental delay. Publication bias was examined with the construction of a funnel plot and tested for asymmetry with the Egger test[11].

Results

Study identification

Combination of the 4 search strategies revealed 25 references in PubMed, 18 references in MEDLINE and 43 references in ISI WoS. A manual search revealed one additional study for consideration[12]. In total, after removal of duplicates, 63 references were screened. Figure 1 provides a flow diagram with the number of studies screened, assessed for eligibility and included for review according to our PICO question.

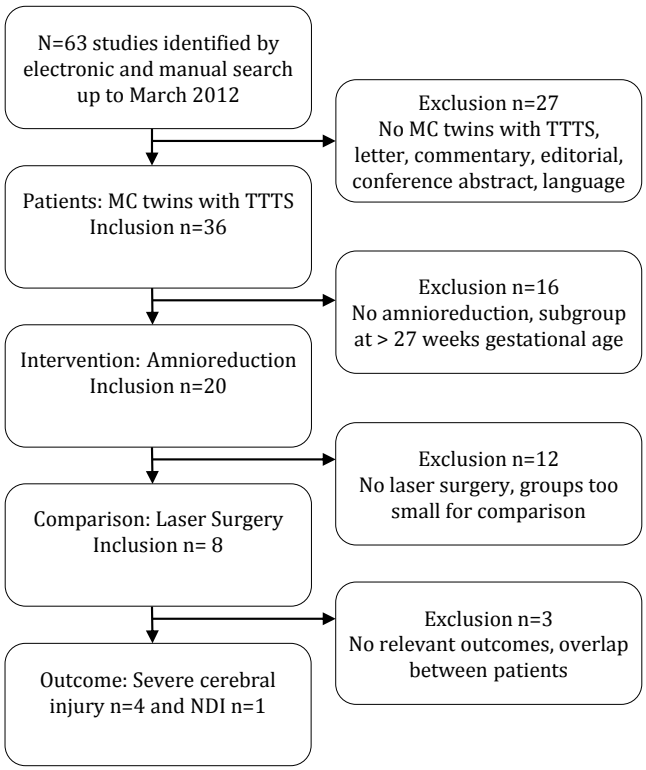

Figure 1. Flow diagram with the number of studies screened, assessed for eligibility and included for review with exclusion criteria according to our Patient- Intervention- Comparison- Outcome-question.

We found two other systematic reviews on AR versus laser surgery[2;13]. Roberts and colleagues (2008) analyzed one randomized controlled trial to compare AR with laser surgery on short-term perinatal outcome in their Cochrane meta-analysis[2]. We also accepted case-series for inclusion to obtain the full range of research to date. Rossi and D'Addario reported on mortality and cerebral anomalies representing the sum of a wide variety of cerebral injuries of varying degrees of severity, regardless of subsequent perinatal deaths or overlap in patients between studies, hence susceptible for bias[13-15]. We selected five studies, directly comparing AR with laser surgery on severe cerebral injury and NDI; three comparative studies plus two follow-up studies from the Eurofetus Trial[15;16]. Although well-designed and highly valuable, the two comparative studies published by Lenclen and colleagues were excluded from analysis, due to a considerable overlap in patients with the two Eurofetus RCT follow-up studies[14;17].

Risk of bias and quality assessment using PRISMA statement

Since no randomization took place in the three comparative studies, performance- or selection bias could not be ruled out[18-20]. However, treatment allocation was unlikely correlated with TTTS severity in these studies since allocation to either AR or laser surgery was based on geographical location and treatment availability; consecutive patients were grouped according to treatment center and year of introduction of laser surgery in a center[18]. The RCT performed prospective power analysis and used computer generated central randomization sequences to maintain adequate allocation concealment[15;16]. Four studies employed a prospective design; of which one staged their AR group retrospectively[15;16;19;20]. All but one were multi-center studies[20]. All studies described techniques for both interventions in detail. Completeness of follow-up ranged from 92 to 100%. Outcome assessment, including timing and frequency of postnatal brain imaging, was fully described in two of five studies and blinded in two[15;16]. Four studies accounted for TTTS stage in their comparison of AR to laser of which one stratified stage on outcome[19]. Two studies reported worse outcomes with increased TTTS stage in both groups; one study found increased TTS stage associated with poorer outcome in the AR group only[19].

Results of individual studies

Summary data for each intervention group are displayed in Table 2. Median gestational age at intervention was 20 to 22 weeks for both intervention groups and comparable in all but one study; 21.6 weeks at first AR versus 20.7 weeks at laser surgery[19]. This study reported an increased incidence of severe cerebral injury following AR which was related to increased TTTS stage. This effect was not observed in their laser group. All but one study staged TTTS, according to Quintero's classification system[1;18]. TTTS stage at intervention was comparable between groups, with limited stage I cases in all studies. Gray and colleagues excluded stage I cases[20]. AR resulted in lower overall survival rates when compared to laser ranging from 39-59% compared to 54-77%, respectively. Treatment with AR resulted in higher neonatal mortality rates ranging from 14-55% compared to 6-15% when treated with laser. Median gestational age at birth was lower with AR, ranging from 28-31 weeks versus 32-34 weeks with laser. Accordingly, birth weight of donor and recipient twins was lower in the AR group ranging from 940-1612 grams versus 1750-2000 grams with laser.

Table 2. Characteristics of the Studies included for Review on Cerebral Injury and Neurodevelopmental Impairment after Amnioreduction versus Laser Surgery.

Reference Design FUP	Selection Allocation Inclusion Data collection	Patient Year Location	Baseline characteristics	Comparison Year Location	Baseline characteristics	Operationalization Outcome measure Blinding	Outcome	Comments
1. Hecher et al 1999[18] Comparative Multicenter Pregnancy outcome	Consecutive, geographical location GA < 25, single MC placenta on ultrasound, oliguria, small-empty bladder, polyuria, distended bladder Prospective FUP	N=86 AR '92-96 Bonn, DE	GA AR 20.4 TTTS < 25 Stage NR IUFD 41% (35/86) NND 14% (7/51) Survival 51% (44/86) GA birth 30.7* BW D 1145* BW R 1560	N=146 FSL '95-97 Hamburg, DE	GA FSL 20.7 TTTS < 25 Stage NR IUFD 35% (51/146) NND 6% (6/95) Survival 61% (89/146) GA birth 33.7* BW D 1750* BW R 2000	IVH III-IV, PVL, parenchyma defects, microcephaly Ultrasound Timing unclear Blinding NR	Cerebral injury AR 18% (8/44) > FSL 6% (5/89)* (ex IUFD, ex NND): **AR** **FSL** + 8 5 - 36 84	Groups different at FUP Survival R > D FSL group* Regimen ultrasound NR Individual observations NNDs cerebral injury NR Adjustment interdependency in overall survival analysis.
2. Quintero et al 2003[19] Comparative Multicenter 18 months	Consecutive, geographical location GA < 27, single placenta, oligohydramnios, polyhydramnios, similar genitalia Retrospective FUP	N=156 AR '90-00 Tampa, FL Perth & Brisbane, AUS	GA AR 21.6* TTTS < 27 Stage I-IV IUFD 17% (26/156) NND 31% (40/130)* Survival 58% (90/156) GA birth 29* BW D 1219* BW R 1612*	N=190 FSL '97-00 Tampa, FL	GA FSL 20.7* TTTS < 27 Stage I-IV IUFD 28% (54/190) NND 10% (14/136)* Survival 64% (122/190) GA birth 32* BW D 1781* BW R 1940*	IVH III-IV, PVL, ventriculomegaly, microcephaly, CP Outcome measure NR Timing NR No blinding	Cerebral injury AR 18% (23/130) > FSL 3% (4/136)* (ex IUFD, inc NND): **AR** **FSL** + 23 4 - 107 132	Groups different at FUP Outcome measure NR Lost to FUP: n=1 AR NNDs with cerebral injury NR Multivariate analyses inc GA intervention, group and TTTS stage.
3. Senat et al 2004[15] RCT Multicenter 6 months	Consecutive 6 countries Computer generated randomization sequence GA 15-26, oliguric oligohydramnios, polyuric polyhydramnios, distended bladder Prospective FUP	N= 140 AR '99-02 17 centers: FR, BE, NL, CH, USA, IT	M GA AR 21 TTTS < 26 Stage I-IV IUFD 16% (29/140) GA birth <24 11% (16/140) NND 43% (41/95)* Survival 39% (54/140)* GA birth 29* BW D & R 1359*	N=144 FSL '99-02 3 centers: NR	M GA FSL 21 TTTS < 26 Stage I-IV IUFD 19% (27/144) GA birth <24 17% (24/144) NND 13% (12/93)* Survival 56% (81/144)* GA birth 33* BW D & R 1757*	IVH III-IV, cystic PVL, blind/deaf, motor disability Ultrasound twice in first 2 weeks, MRI on indication Blinded outcome assessor	IVH AR 8.4% (8/95) = FSL 2.2% (2/93) (ex IUFD, inc NND) PVL AR 15% (14/95) = FSL 9% (8/93) (ex IUFD, inc NND) PVL AR 10% (6/58) = FSL 5% (4/88) (ex IUFD, ex NND) Cerebral injury at 6 mo AR 19% (10/54) > FSL 7% (6/81)* (ex IUFD, ex NND): **AR** **FSL** + 10 6 - 44 75	Groups different at FUP Overrepresentation stage II-III in both groups All children with IVH AR group died One child with IVH FSL survived Adjustment for twin clustering Subgroup analysis IUFD co-twin.

Reference Design FUP	Selection Allocation Inclusion Data collection	Patient Year Location	Baseline characteristics	Comparison Year Location	Baseline characteristics	Operationalization Outcome measure Blinding	Outcome	Comments
4. Gray et al 2006[20] Comparative Single center Perinatal outcome	Consecutive AR <2002> FSL TTTS stage ≥ II, oligohydramnios, polyhydramnios, thin dividing membrane sacs on ultrasound Retrospective AR Prospective FSL	N=54 AR '94-02 Brisbane, AUS	GA AR 20 TTTS < 28 Stage ≥ II IUFD 24% (13/54) NND 22% (9/41)* Survival 59% (32/54)* GA birth 28* BW D 940* BW R 1312	N=62 FSL '02-03 Brisbane, AUS	GA FSL 21 TTTS < 28 Stage ≥ II IUFD 18% (11/62) NND 6% (3/51)* Survival 77% (48/62)* GA birth 34* BW D 1780* BW R 1870	PVH III-IV, cystic PVL, cerebral atrophy, ischemic brain injury Ultrasound first week of life and after on indication Blinding NR	No PVH III-IV PVH AR 7% (3/41) > FSL 0% (0/51* (ex IUFD, inc NND) Cerebral injury FSL 0% (0/51) < AR 12% (5/41)* (ex IUFD, inc NND): **AR** **FSL** + 5 0 - 36 51	Groups different at FUP One center Survival R > D FSL* No stage I TTTS NNDs with cerebral injury NR Two pregnancies FSL group intention-to-treat basis
5. Salomon et al 2010[14] RCT Multicenter 1-6 years	Subgroup Eurofetus RCT delivered in FR Prospective FUP	N=120 AR '99-02 Paris, FR	GA diagnosis 21 TTTS < 26 Stage I-IV IUFD 39% (47/120) NND 55% (26/73)* Survival 39% (47/120)* GA birth NR BW NR	N=136 FSL '99-02 Paris, FR	GA diagnosis 21 TTTS < 26 Stage I-IV IUFD 37% (50/136) NND 15% (13/86)* Survival 54% (73/136)* GA birth NR BW NR	NDI: CP, blind, deaf 1-2 yr: questionnaire neurodevelopment 1-2-4-5 yr: ASQ 5 yr: neurologic exam 6 yr: WISC Blinded outcome assessor	M ASQ 2 yr: AR 192 (±76) = FSL 203 (±80) M ASQ 4 yr: AR 227 (±81) = FSL 241 (±58) M ASQ 5 yr: AR 229 (±80) < FSL 261 (±54)* M WISC 6 yr: AR 91 (±33) = FSL 91 (±20) Developmental delay: AR 14% (4/28) = FSL 5.5% (3/55) CP, blind, deaf 5-6 yr: AR 15% (6/41) = FSL 13% (9/69): **AR** **FSL** + 6 9 - 35 60	Subgroup Eurofetus RCT Groups different at FUP: NND AR > FSL* FUP 5 yr: n=10 exam GP Lost to FUP 6 yr: 8% (10/120) (n=4 FSL, n=6 AR) Large SDs ASQ and WISC AR group NDI as a composite outcome NR Cumulative incidence death or impairment with Kaplan-Meier curves.

FUP = follow-up; N = the number of children; GA = median gestational age in weeks; MC = monochorionic; AR = amnioreduction; DE = Germany; TTTS = twin-twin transfusion syndrome; NR = not reported; IUFD = intrauterine fetal death; NND = neonatal death; BW = median birth weight in grams; D = donor; R = recipient; FSL = fetoscopic laser coagulation; IVH III-IV = intraventricular hemorrhage grade III or IV; PVL = periventricular leukomalacia; ex = excluding; FL = Florida; AUS = Australia; CP = cerebral palsy; inc = including; RCT = randomized controlled trial; FR = France; BE = Belgium; NL = the Netherlands; CH = Switzerland; USA = United States America; IT = Italy; M = mean; MRI = magnetic resonance imaging; PVH = periventricular hemorrhage; NDI = neurodevelopmental impairment; ASQ = Ages and Stages Questionnaire; WISC = Wechsler Intelligence Scale for Children; GP = general practitioner; SDs = standard deviations; values are medians unless stated otherwise; * indicates a significant difference at P<.05.

Synthesis of results

A fixed effect model was used throughout because of the small number of included studies to reliably assess between study variability. To assess the odds of severe cerebral injury in children treated with either AR or laser, data were derived from four studies with 269 children in the AR group versus 357 children in the laser group[15;18-20]. The odds of severe cerebral injury in live-born children treated with AR were seven- to eight-times higher when compared to children treated with laser (OR 7.69, 95% CI 2.78-20.0, *P* = .00; fig. 2). With subsequent neonatal deaths excluded from outcome analysis, the odds were three-times higher in the AR group compared to the laser group (OR 3.23, 95% CI 1.45-7.14, *P* = .00; fig. 2).

To assess the odds of cPVL ≥ II in live-born children, data were derived from two studies, one RCT and one comparative study, with 136 children in the AR and 144 children in the laser group [15;20]. The OR demonstrated no significant difference in cPVL ≥ II in live-born children treated with either AR or laser (OR 2.08, 95% CI .86-5.00, *P* = .10; fig. 3).

Two studies reported on the incidence of IVH ≥ III in live-born children[15;20]. Data were available from 136 children treated with AR versus 144 children treated with laser[15]. The OR demonstrated no significant difference in IVH ≥ III in live-born children treated with either AR or laser (OR 3.56, 95% CI .82-14.29, *P*=.09; fig. 4). Senat and colleagues identified eight (8/95) cases of IVH ≥ III in live-born children in their AR group versus two live-born children (2/93) in their laser group. Of these ten cases, only one child, treated with laser, was alive at six months of age[15]. According to Gray and colleagues, none of the live-born children developed IVH ≥ III in their first week of life[20].

There were insufficient long-term outcome data to assess the odds of NDI as a composite outcome. In their original article, Salomon and colleagues did not report individual observations of cognitive developmental delay as measured with the Ages and Stages Questionnaire (ASQ) and the Wechsler Intelligence Scale (WISC-IV)[16]. Salomon and colleagues did provide individual observations of CP, blindness or deafness. Data were available from 41 children treated with AR and 69 children treated with laser[16]. At 6-year follow-up, four children presented with CP, one child was blind and one child was deaf in the AR group (15%; 6/41) versus six children with CP, two children with blindness, and one child with deafness in the laser group (13%; 9/69). The absence of differences in long-term outcome was probably due to the significant higher neonatal death rate in the AR group that is, 55% (26/73) versus 15% (13/86) in the laser group. Kaplan-Meier curves showed that the probability of survival without major neurological impairment was lower with AR, adjusted for TTS stage (hazard ratio .61, 95% CI .41-.90, *P* = .01)[16]. Individual results of early brain imaging of these children were not reported.

Since the number of included studies was too small for a reliable assessment, construction and analysis of the funnel plot was precluded.

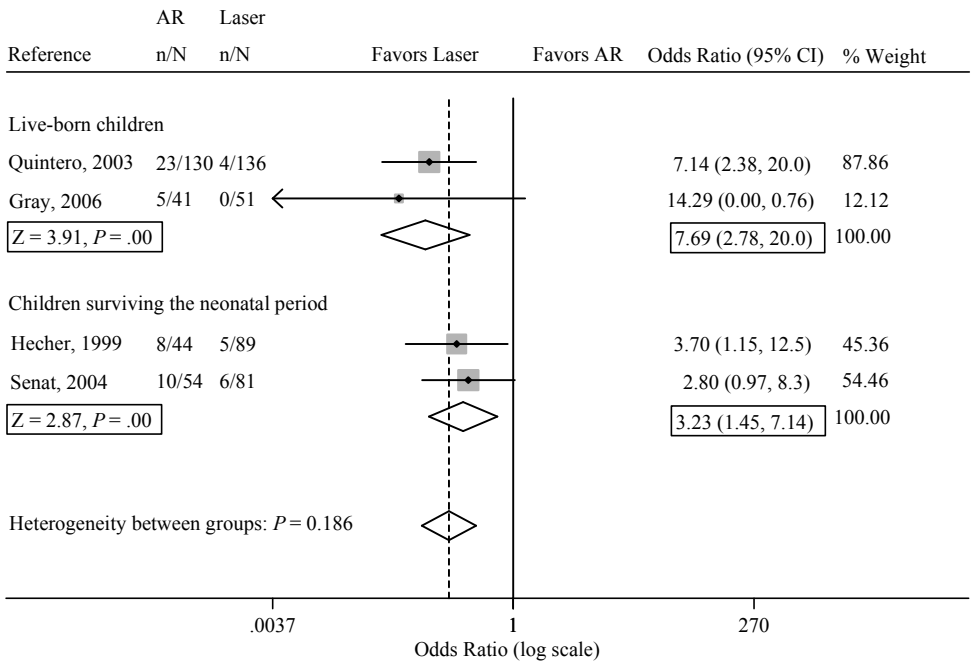

Figure 2. Fixed effect analysis of severe cerebral injury after amnioreduction versus laser surgery.

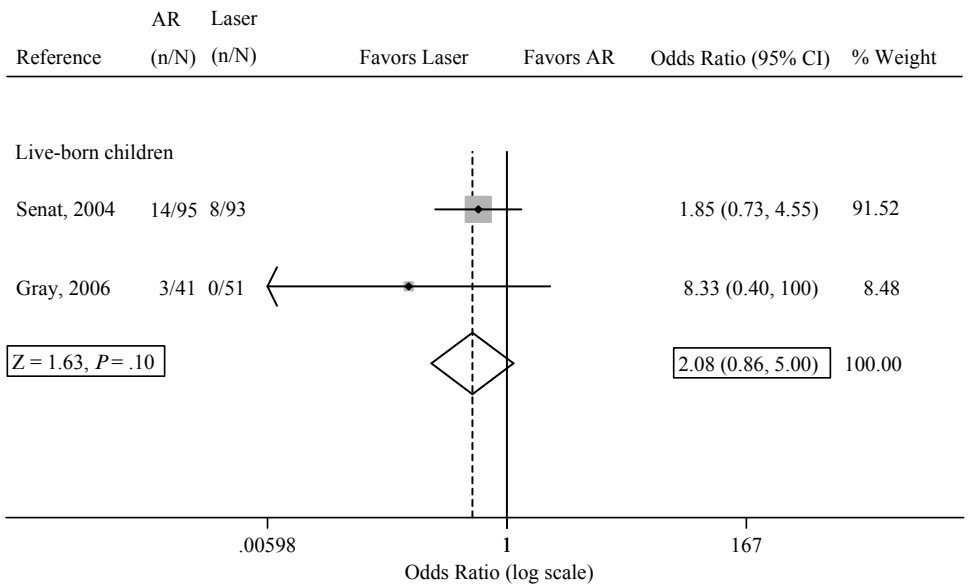

Figure 3. Fixed Effect Analysis of Cystic Periventricular Leukomalacia in Amnioreduction versus Laser Surgery.

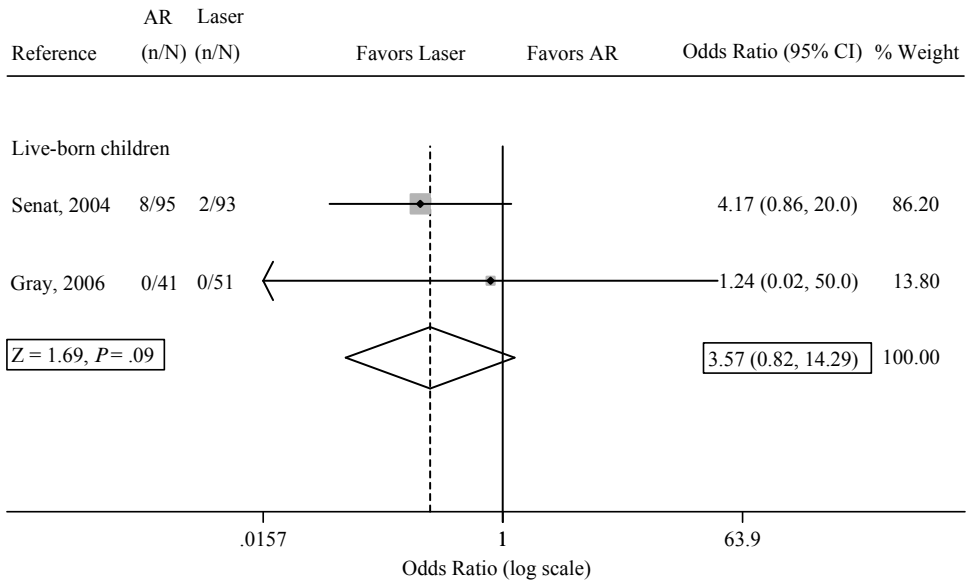

Figure 4. Fixed Effect Analysis of Intraventricular Hemorrhage in Amnioreduction versus Laser Surgery.

Discussion

The objective of our systematic review and meta-analysis was to evaluate severe cerebral injury and long-term impairment in MC twins treated with AR compared to laser for TTTS. We found an ample seven-fold higher risk of severe cerebral injury in live-born children treated with AR compared to laser surgery. In children surviving the neonatal period, the odds were three-times higher following AR versus laser. Detailed analysis per type of severe cerebral injury demonstrated no significant difference between treatments regarding the incidence of cPVL ≥ II and IVH ≥ III. Importantly, there were not enough follow-up data to analyze long-term neurodevelopmental impairment in children treated with AR compared to laser surgery.

Roberts and colleagues showed in their Cochrane review of only one trial that more children were alive without neurological abnormality at six months following laser surgery compared to AR (RR 1.66; 95% CI 1.17 to 2.35 adjusted for clustering, one trial)[2]. They reported no difference in the children alive at six months with neurological abnormality between interventions (RR 0.58; 95% CI 0.18 to 1.86 adjusted for clustering, one trial). The authors suggest that this might be secondary to plasticity of the developing brain or the demise of more severely affected fetuses. No data were available on outcome beyond six months at the time of writing their Cochrane review.

We aimed to present the full range of the research to date and included case-series as well, with a longer follow-up period.

Rossi and D'Addario showed in their meta-analysis of four studies comparing AR to laser that fetuses treated with AR were less likely to survive when compared to laser (overall survival: OR 2.04; 95% CI 1.52-2.76, $P < .0001$; neonatal death: OR .24, 95% CI .15-.40, $P < .001$)[13]. However, among these four studies, two studies have a considerable overlap in patients since both studies included participants of the Eurofetus RCT recruited and delivered in France[15;21]. Furthermore, their analysis of cerebral injury represented a sum of a wide variety of cerebral anomalies regardless of severity of the injury. Also, perinatal deaths were not taken into consideration in their outcome analysis. We excluded studies to avoid overlap between patients, specified cerebral injury according to type and severity and studies were a priori analyzed into two groups i.e., studies including and studies excluding neonatal deaths in outcome analysis.

We speculate that the increased risk of severe cerebral injury following AR is due to the higher rate of prematurity, which is a well-known risk factor for neonatal morbidity and mortality[3]. In addition, since AR is only a symptomatic intervention, fetuses remain exposed to TTTS for a longer period of time when compared to fetuses treated with laser coagulation of the placental anastomoses. The lack of difference in cPVL ≥ II and IVH ≥ III in live-born children between groups could be due to the small sample size since only two studies reported individual observations of these injuries in live-born children. The only RCT follow-up study concluded that there is no difference in neurodevelopmental impairment between interventions. However, these conclusions are likely biased by the significantly higher neonatal death rate in their AR group[2;16].

The main limitation of the current systematic analysis is the small number of studies available for review and small sample size. Studies directly comparing AR to laser on outcome are scarce. The majority of the studies included in this systematic review employed a comparative design which is highly susceptible for bias. Among the studies, there were no stringent criteria regarding what constitutes severe cerebral injury. Although cranial ultrasound is useful for detecting neurologic morbidity, its sensitivity for subsequent neurodevelopmental impairment is not high[3]. In addition, a normal cranial ultrasound scan without cerebral injury does not necessarily equate with normal neurodevelopmental outcome[20]. This can only be ascertained by long-term follow-up to childhood in order to determine outcome in terms of CP, cognitive and socio-emotional development[3;20].

Our study highlights the crucial lack of studies focusing on cerebral injury and long-term neurodevelopmental outcome in TTTS. Although serial AR and laser surgery have been introduced more than 2 decades ago, most studies in TTTS have focused mainly on immediate perinatal outcome[2]. Knowledge on long-term outcome and quality of life

of survivors is indispensable for determining best practice for clinicians as well as for counseling future parents using evidence-based information. This requires cooperation between obstetricians, pediatricians and other experts in the field of child cognitive and social-emotional development in order to look beyond perinatal survival as well as cooperation between international treatment centers to obtain reliable data with large enough case series with sufficient power. We suggest defining what is considered severe cerebral injury and neurodevelopmental impairment consistently, to provide individual information on all cases including early brain imaging in order to reliably estimate the effect on later development. It is important to continuously assess development of the children including formal psychological testing and standardized measures of well documented psychometric quality, with increasing reliability of results with increasing age. Table 3 represents a proposition for future research.

Conclusion

Setting up a new RCT with long-term follow-up after AR versus laser surgery is not ethical, since higher overall survival rates and better perinatal outcomes have already been established with laser surgery. However, long-term follow-up with emphasis on child cognitive, socio-emotional development and quality of life is indispensible for conducting future RCTs in all fields of fetal medicine, in order to implement new techniques.

Table 3. A proposition for future research: Assessment according to age in years.

Development	Neonate	2 years	4 years	7 years	8 years	10 years	12 years	14 years	16 years
Brain:	Imaging of the brain								
Senses:	Hearing	Vision							
Physical:		Neurologic exam, Cerebral Palsy, Gross Motor Function Classification System							
Cognitive:		BSID, WPPSI		WISC					
Psychosocial:		Achenbach System, Quality of Life							
School:			Special education, number of grades below age appropriate educational level						
Neuropsychological:				Learning, language, executive functioning, attention, visual spatial abilities, memory, fine motor development					
Neuropsychiatric:				Attention Deficit Hyperactivity Disorder, Autism Spectrum Disorder					

BSID = Bayley Scales of Infant and Toddler Development for children 1 month to 3 years of age; WPPSI = Wechsler Preschool and Primary Scale of Intelligence for children 2 years and 6 months to 7 years and 11 months of age; WISC = Wechsler Intelligence Scale for Children 6 to 16 years of age.

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Chapter 6

Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery

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Abstract

Objective
To compare the incidence of neurodevelopmental impairment in surviving children from pregnancies with twin-twin transfusion syndrome treated with laser surgery between two time periods.

Study design
We compared the neurodevelopmental outcome between the first consecutive cohort of twin-twin transfusion syndrome pregnancies treated with laser surgery from 2000 to 2005, with a cohort treated between 2008 and 2010. Neurological, cognitive and motor development was evaluated using Bayley scales at 2 years of age corrected for prematurity.

Results
A total of 229 twin pregnancies were treated with laser surgery, 113 in the first cohort and 106 in the recent cohort. Overall survival increased from 70% (158/226) to 80% (170/212) (P = .014). The incidence of neurodevelopmental impairment decreased from 18% (28/152) to 6% (10/155) (P < .01). In multivariate analysis, severe cerebral injury at birth was independently associated with neurodevelopmental impairment (OR 34.86, 95% CI 11.83-102.75, P < .01).

Conclusion
Overall survival in twin-twin transfusion syndrome has improved over time, with a concomitant reduction in the incidence of neurodevelopmental impairment. Research focused on prevention of cerebral injury is needed to further improve outcomes of these complicated twin pregnancies.

Introduction

Twin-twin transfusion syndrome (TTTS) is a major complication of monochorionic twin pregnancies and is the result of inter-twin blood transfusion through placental vascular anastomoses. Fetoscopic laser coagulation of the anastomoses is considered by many specialists the treatment of choice since it was first described two decades ago.[1;2] Despite improved short-term and long-term outcomes with increasing experience and advances in technique, TTTS treated with laser surgery is still associated with severe cerebral injury ranging from 3% to 16%[3-6] and neurodevelopmental impairment ranging from 8% to 18%.[7] Our objective was to compare the incidence of neurodevelopmental impairment in a cohort of TTTS pregnancies recently treated with laser surgery at our center between 2008 and 2010 with a previously published first cohort, treated at our center between 2000 and 2005.[8]

Materials and Methods

The Leiden University Medical Center serves as the national referral center for laser treatment in TTTS pregnancies in The Netherlands since 2000. Surviving children of all TTTS pregnancies treated with fetoscopic laser surgery were routinely assessed in our long-term outcome clinic since the start of our laser program, except, for organizational reasons, in the period between 2006 and 2007. We previously reported on the neurodevelopmental outcome at two years of age of our first cohort, treated between 2000 and 2005. We evaluated neurodevelopmental outcome of all surviving children treated between 2008 and 2010, and compared the two groups.

TTTS was diagnosed by using standard prenatal ultrasound criteria[9] and staged according to standard criteria.[10] All fetoscopic laser procedures were performed by the same group of experienced operators during both study periods. Details on the laser technique used at our center and the short-term outcome results have previously been reported.[11] During the second study period, the majority of TTTS cases were also included in a randomized controlled trial, the Solomon study (NTR1245) comparing laser coagulation of the entire vascular equator, the Solomon technique, with the standard selective laser technique.

The following antenatal and neonatal data were recorded: gestational age at the time of laser treatment, stage of TTTS, occurrence of twin anemia-polycythemia sequence (TAPS) or recurrence of TTTS after laser, fetal demise, gestational age at delivery, birth weight, severe cerebral injury and neonatal death. TAPS was diagnosed according to antenatal and/or postnatal criteria.[12] Severe cerebral injury was defined

as intraventricular hemorrhage (IVH) ≥ grade III,[13] cystic periventricular leukomalacia (cPVL) ≥ grade II,[14] ventricular dilatation ≥ 97th percentile,[15] porencephalic cysts, arterial or venous infarction detected on cerebral imaging. Socio-economic status (SES) of the parents was registered as high, average or low according to the Dutch Sociaal en Cultureel Planbureau.[16] The follow-up visit was assessed at age 2 years (corrected for prematurity) and included a physical and neurologic examination and an assessment of cognitive and motor development using the Dutch version of the Bayley Scales of Infant and Toddler Development second edition (BSID-II) in the old cohort and third edition (BSID-III) in the new cohort by certified examiners.[17;18] Both tests (BSID-II and BSID-III) provide a cognitive development and motor development score that follow a normal distribution with a mean of 100 and a standard deviation (SD) of 15. When each separate score was below 70, which is > 2 SD below the mean, this was indicative of a severe delay in either cognitive or motor development. A score below 85, > 1 SD below the mean, was indicative of at least mild to moderate delay. Cerebral palsy (CP) was defined according to the European CP Network and classified as diplegia, hemiplegia, quadriplegia, dyskinetic, or mixed.[19] A composite outcome, termed neurodevelopmental impairment (NDI), was defined as any of the following: CP, cognitive development score of less than 70, motor development score of less than 70, bilateral blindness, or bilateral deafness requiring amplification.

The primary aim of our study was to compare the incidence of NDI between both cohorts. The secondary aim was to determine risk factors associated with NDI. The institutional review board of the Leiden University Medical Center approved the study and all parents gave written informed consent for their children.

Statistics

Data are reported as means with standard deviation (SD) or as medians with inter quartile range (IQR), as appropriate. Statistical analysis was performed using the t-test and Mann-Whitney test for continuous variables. Chi-square test and Fisher's exact test were used for categorical variables, as appropriate. Analysis for risk factors possibly contributing to the neurodevelopmental impairment was conducted using univariate and multivariate regression methods. The following potential risk factors for neurodevelopmental impairment were studied in a univariate logistic regression model: gestational age at laser surgery, Quintero stage, fetal demise of one twin, gestational age at delivery, birth weight, treatment failure defined as post-laser TAPS or recurrent TTTS, and severe cerebral injury. The multivariate logistic regression model included all variables that showed significant association in the univariate analysis. Results are expressed as odds ratio (OR) with 95% confidence interval (CI). All analyses were conducted using the Generalized Estimated Equation (GEE) module to account for the

effect that observations within twins are not independent. A *P*-value of less than 0.05 was considered significant. Statistical analysis was executed with computer software (SPSS 20.0, SPSS, Inc., Chicago, IL).

Results

During the first study period between 2000 and 2005, 113 TTTS pregnancies were treated with fetoscopic laser surgery. During the second study period, between 2008 and 2010, we treated 106 TTTS pregnancies. The incidence of intrauterine fetal demise was 26% (58/226) and 16% (33/212), respectively ($P = .01$) with significant more double fetal demises in the first cohort (17% versus 7%; $P < .01$). Although in both groups the majority of pregnancies were classified as Quintero stage II or III, the recent cohort comprised significantly more stage III diagnoses at therapy ($P = .02$). Median gestational age at birth and median birth weight in live-born neonates was higher in the first cohort, 34 versus 32 weeks ($P < .01$) and 1982.5 versus 1700 grams ($P < .01$), respectively.

Severe cerebral injury was detected in 10% (16/168) of live-born neonates in the first cohort and 6% (11/179) in the recent cohort ($P = .32$). Neonatal death rate was similar, 6% (10/168) and 5% (9/179), respectively ($P = .90$). Overall survival to 30 days was significantly higher in the recent cohort, 70% (158/226) versus 80% (170/212) respectively ($P = .01$). Baseline characteristics of the entire cohort are presented in Table 1.

Table 1 Baseline characteristics for the entire cohort.

	Cohort 2000-2005 N=113 pregnancies	Cohort 2008-2010 N=106 pregnancies	*P*
SES Low	32/113 (28)	27/106 (25)	.65
SES Intermediate	55/113 (49)	44/106 (41)	.34
SES High	26/113 (23)	35/106 (33)	.31
Gestational age at laser - weeks	20.1 ± 3.1	20.1 ± 3.3	.93
Quintero stage	2 (1)	3 (1)	.35
I – n (%)	11 (10)	14 (13)	.53
II – n (%)	49 (43)	30 (28)	**.02**
III – n (%)	46 (41)	61 (58)	**.02**
IV – n (%)	7 (6)	1 (1)	.07
Fetal demise	58/113 (26)	33/106 (16)	**.01**
Single	20/113 (9)	19/106 (9)	1.00
Double	38/113 (17)	14/106 (7)	**<.01**
TAPS or recurrent TTTS	15/113 (13)	16/106 (15)	.59
TAPS	9/113 (8)	15/106 (14)	**.04**
Recurrent TTTS	6/113 (5)	1/106 (1)	**.02**
Gestational age at birth - weeks	34 (5)	32 (6)	**<.01**
Birth weight - grams	1982.5 (1258)	1700 (870)	**<.01**
Severe cerebral injury	16/168 (10)	11/179 (6)	.32
Neonatal death	10/168 (6)	9/179 (5)	.90
Overall perinatal survival	158/226 (70)	170/212 (80)	**.01**

N, number; *SES*, socio economic status; *TAPS*, twin anemia-polycythemia sequence;
Data are presented as mean ± standard deviation, median (IQR), n (%) or n/N (%).

In both cohorts, follow-up data were obtained from 97% (153/158 and 165/170) of surviving children. Seven children could not be assessed since the parents declined consent. Contact information was lost for two children. One child was excluded from the analysis due to infantile Tay-Sachs disease; the co-twin was a fetal demise.

All children underwent physical and neurological examination but three children did not complete the motor scale of the BSID-III and eight children did not complete the cognitive and motor scale. No significant differences in antenatal and neonatal characteristics and SES were found between the included and lost-to-follow-up group. A flow chart showing the derivation of the study population is shown in Figure 1. Baseline characteristics of the TTTS survivors included for follow-up are presented in Table 2.

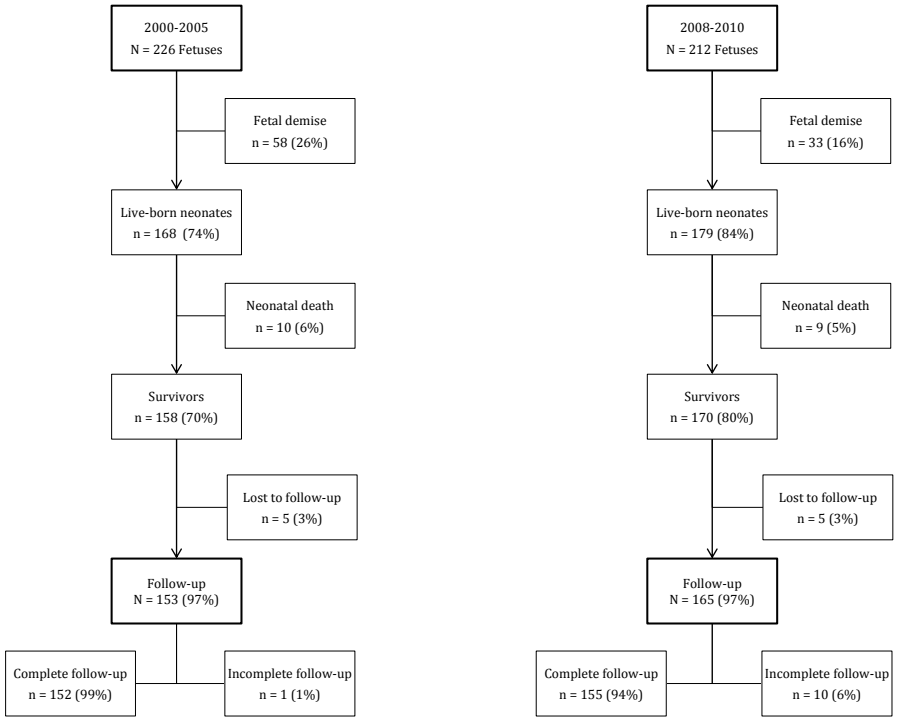

Figure 1 Flow chart showing the derivation of the study population.

Table 2 Baseline characteristics of long-term TTTS survivors for follow-up

	Cohort 2000-2005 N=153 children	**Cohort 2008-2010 N=165 children**	***P***
SES Low	35 (23)	49 (30)	.52
SES Intermediate	76 (50)	76 (46)	.58
SES High	42 (27)	40 (24)	.20
Donor	79 (52)	84 (51)	.88
Gestational age at laser (weeks)	20.4 ± 3.4 (15-28)	20.4 ± 3.4 (14-29)	.86
Quintero stage - median (range)	2 (1)	3 (1)	.07
I – n (%)	19 (12)	22 (13)	.87
II – n (%)	69 (45)	45 (27)	**<.01**
III – n (%)	59 (39)	95 (58)	**<.01**
IV – n (%)	7 (5)	2 (1)	.09
Fetal demise co-twin	19 (12)	17 (10)	.60
Gestational age at birth (weeks)	34 (5)	33 (6)	**<.01**
37-40 weeks	37 (24)	15 (9)	**<.01**
32-36 weeks	70 (46)	82 (50)	.50
24-31 weeks	46 (30)	68 (41)	**.05**
Birth weight (grams)	2000 (1202.5)	1710 (851)	**<.01**
Female	79 (52)	91 (55)	.53

SES, socio-economic status.
Data are presented as mean ± standard deviation (range), median (IQR) or n (%).

The incidence of NDI in children with complete follow-up was 18% (28/152) in the first cohort, versus 6% (10/155) in the recent cohort ($P < .01$). NDI was due to CP in 12 cases in the first compared to 5 cases in the recent cohort ($P = .07$). CP was classified as quadriplegia (n = 5), diplegia (n = 2), and hemiplegia (n = 5) and as quadriplegia (n = 2) and hemiplegia (n = 3) respectively. Severe cognitive developmental delay occurred in 14 cases in the first cohort compared to 5 cases in the recent cohort ($P = .03$). Severe motor development delay was detected in 20 cases in the first cohort compared to 5 cases in the recent cohort ($P < .01$). Details on the combinations of abnormal findings detected in the children with NDI are presented in Table 3. In the first cohort, 36% (10/28) of children with NDI also had severe cerebral injury, versus 60% (6/10) in the recent cohort ($P = .27$). Mean BSID cognitive scores in the first cohort were seven points lower compared to the recent cohort, 94.6 ± 17.0 versus 102.0 ± 12.3, respectively ($P < .01$). Mean BSID motor scores in the first cohort were 12 points lower, 89.7 ± 17.7 versus 101.8 ± 13.8, respectively ($P < .01$).

Table 3 Combination of abnormal findings in children with neurodevelopmental impairment

	2000-2005 n=153	2008-2010 N=165
CP	1 (1)	2 (1)
Cognitive development <2 SD	6 (4)	3 (2)
Motor development <2 SD	6 (4)	1 (1)
CP and cognitive and motor development <2 SD	4 (3)	1 (1)
CP and cognitive development <2 SD	1 (1)	0
CP and motor development <2 SD	5 (3)	2 (1)
CP and bilateral deafness	1 (1)	0
Cognitive and motor development <2 SD	3 (2)	1 (1)
Cognitive and motor development <2 SD and deafness	1 (1)	0
Neurodevelopmental impairment[a]	28 (18)	10 (6)

N, number; CP, cerebral palsy; *SD,* standard deviation.
Data are expressed as n (%).
[a]Neurodevelopmental impairment included any of the following: Cerebral Palsy, cognitive development <2 SD, motor development <2 SD, bilateral deafness or blindness.

Univariate analysis of potential risk factors for NDI was performed for both cohorts (Table 4). Risk factors found to be associated with NDI were advanced gestational age at laser surgery (OR, 1.12 for each week; 95% CI, 1.01-1.25; $P = .03$), lower birth weight (OR, 1.08 for each 100-gr decrease; 95% CI, 1.01-1.15; $P = .02$) and severe cerebral injury at birth (OR, 40.00; 96% CI, 13.39-119.46; $P < .01$). We found a (small) positive correlation between gestational age at laser and Quintero stage (r = .11; $P = .05$) and a strong positive correlation between gestational age at birth and birth weight (r = .85; $P < .01$).

Table 4 Analysis of potential risk factors for neurodevelopmental impairment

Characteristics	NDI (n=38/318)	No NDI (n=280/318)	*P*	Univariate OR (95% CI)	*P*	Multivariate OR (95% CI)
Gestational age at laser -weeks[a]	21.6 ± 3.9	20.2 ± 3.3	**.03**	1.12 (1.01-1.25)	.07	1.10 (.99-1.23)
Birth weight - grams[b]	1505 (937)	1893 (990)	**.02**	1.08 (1.01-1.15)	.47	1.03 (.95-1.10)
Severe cerebral injury, yes	16/21 (76)	5/21 (24)	**<.01**	40.00 (13.39-119.46)	**<.01**	34.86 (11.83-102.75)
Severe cerebral injury, no	22/297 (7)	275/297 (93)	-	-	-	-
SES Low	8/82 (10)	74/82 (90)	.14	2.01 (.80-5.04)		
SES Intermediate	15/152 (10)	137/152 (90)	-	-		
SES High	15/84 (18)	69/84 (82)	-	-		
Quintero stage I	1/41 (2)	40/41 (98)	.06	.09 (.01-1.10)		
Quintero stage II	12/114 (11)	102/114 (89)	-	-		
Quintero stage III	23/154 (15)	131/154 (85)	-	-		
Quintero stage IV	2/9 (22)	7/9 (78)	-	-		
FD of co-twin, yes	3/36	33/36	.48	1.56 (.45-5.35)		
FD of co-twin, no	35/282	247/282				
TAPS or recurrent TTTS, yes	3/47 (6)	44/47 (94)	.21	.46 (.14-1.56)		
TAPS or recurrent TTTS, no	35/271 (13)	236/271 (87)	-	-		
Gestational age at birth - weeks[c]	31 (5)	33 (6)	.12	.93 (.85-1.02)		

NDI, neurodevelopmental impairment; *OR*, odds ratio; *CI*, confidence interval; *SES*, socio-economic status; *FD*, fetal demise; *TAPS*, twin anemia-polycythemia sequence; *TTTS*, twin-twin transfusion syndrome.
Data are presented as mean ± standard deviation, median (range) or n/N (%).
[a]OR calculated per week increment; [b]OR calculated for each 100-gr decrease; [c]OR calculated for each week less.

We found no statistically significant difference in NDI between donors and recipients (P = .86), between males and females (P = .07) and between children with and without treatment failure (P = .21). All (7 of 7) children with recurrent TTTS had normal neurodevelopmental outcome, three (3 of 24) children with TAPS had NDI.
Risk factors associated with NDI by univariate analyses were entered in a multivariate logistic regression model to measure the independent association with NDI (Table 4). We found that only severe neonatal cerebral injury was still significantly associated with NDI (OR, 34.86; 95% CI, 11.83-102.75; P < .01).

Comment

This is the first study evaluating changes in long-term neurodevelopmental outcome in TTTS survivors treated with laser surgery over time. Overall survival rate improved from 70% to 80% with a significant reduction in (double) fetal demise. Increased survival was associated with a concomitant decrease in incidence of long-term neurodevelopmental impairment, from 18% in the first 6 years of our laser program (2000-2005) to 6% in the recent cohort (2008-2010). The incidence of NDI in our recent cohort is also lower compared to the 11% rate of NDI recently reported in a systematic review by Rossi, Vanderbilt & Chmait[20] who used data from pregnancies treated between 1995 and 2006. Our study shows that although TTTS remains one of the most lethal conditions in perinatal medicine, the outcome (in terms of its most important parameter, disease-free survival) has significantly improved in the last decade.
Several factors may explain this improvement in disease-free survival, including improvements in fetal and prenatal care strategies (monitoring and management options), increased awareness and knowledge of potential (treatable) complications in TTTS, learning curve effect associated with a highly technical procedure such as laser surgery and the introduction of a new laser technique (Solomon technique) since 2008.[21] Since many of these changes occurred simultaneously, it is impossible to assess the contribution of each of these factors. Whether improvement in long-term outcome could be related to the Solomon laser technique, is currently being evaluated in a large long-term follow-up program. Long-term outcome results in the Solomon study are awaited in 2015.
One limitation of our study that requires attention is the use of the BSID-III instead of the BSID-II in the analysis of the recent cohort. Several studies report a significant underestimation of developmental delay using the BSID-III compared to BSID-II assessment, with cognitive and motor score differences ranging from three up to 18 points in favor of BSID-III assessment.[22-26] In our study, mean BSID-III cognitive scores were seven points higher than mean BSID-II cognitive scores, in accordance with what the BSID-III manual states.[18] Mean BSID-III motor scores were 12 points higher than

mean BSID-II motor scores. To account for possible underestimation of delay in the new cohort, we first applied a correction of 7 points on both scales. With this correction, the rate of NDI in the recent cohort did not change. Then, following the example of Vohr et al (2012),[26] we applied a correction of 15 points on both scales, raising our threshold for cognitive and motor delay from a score of < 2 SD (test scores < 70) to a score of < 1 SD (test scores < 85). With this correction, the rate of NDI increased from 6% (10/155) to 10% (15/155). Compared to 18% (28/152) of children with NDI in the first cohort using BSID-II assessment, the difference between the two cohorts remains significant (P = .03).

Interestingly, the rate of impairment was lower in the recent cohort, despite the fact that the median gestational age at birth was significantly lower. The most plausible factor causing a decrease in gestational age at birth in the recent cohort is related to a gradual change of obstetrical management in monochorionic twin pregnancies over the last decade. Although the optimal timing of delivery in monochorionic twin pregnancies remains controversial, guidelines in The Netherlands nowadays advise to induce delivery between 36 and 37 weeks. As shown in this study, the percentage of monochorionic twin pregnancies delivering between 37 and 40 weeks was significantly higher in the first cohort.

This also provides several clues to further understand which children may be at increased risk for adverse long-term outcome. In a univariate analysis including both cohorts we found that advanced gestational age at laser surgery, low birth weight and severe neonatal cerebral injury are potential risk factors for NDI. The association between low birth weight (which was strongly related with low gestational age at birth) and neurodevelopmental impairment is not surprising since low birth weight and low gestational age are well-recognized risk factors for long-term impairment.[8;27;28] The association between advanced gestational age at laser and neurodevelopmental outcome is more difficult to explain. A possible explanation could be that the fetal brain may be more susceptible for circulatory imbalance and development of TTTS at a more advanced age. Alternatively, we also found a positive correlation with advanced age at treatment for TTTS and Quintero stage. Previous studies have shown that higher Quintero stage may have an impact on long-term neurodevelopmental impairment.[8] However, we found an increased number of Quintero stage III cases in the recent cohort. We speculate that there has been a gradual change in our interpretation of Doppler waveform analysis, with in more recent years considering milder ductus venosus waveform changes as being abnormal.

Multivariate logistic regression showed that severe neonatal cerebral injury detected at birth is an independent risk factor for long-term neurodevelopmental impairment. Given the increased risk of antenatally acquired cerebral injury in TTTS survivors,

cranial ultrasound scans should be routinely performed at birth.[29] However, although the predictive value of cerebral imaging techniques is increasing, its predictive accuracy remains subject of debate.[30] Presence of long-term impairment can only reliably be ascertained by accurate and standardized long-term follow-up until at least childhood.
One of the limitations of this study is the absence of a non-TTTS control group. Another potential limitation is related to the as yet unclear predictive value of the Bayley scales for future cognitive and motor development. In addition, some developmental problems become more apparent only at a later age, especially at school age. Therefore follow-up until at least school age is highly recommended.
In conclusion, we found a significant increase in survival rate and a concomitant decrease in long-term neurodevelopmental impairment in TTTS after laser surgery from 2000-2005 to 2008-2010, suggesting major improvement in overall outcome in TTTS. We were able to establish several risk factors for neurodevelopmental impairment, including advanced gestational age at laser, low birth weight and severe neonatal cerebral injury. Although the optimal management of TTTS remains a major challenge for obstetricians and neonatologists, our data can be used to counsel parents in a more optimistic way compared to a decade ago.

Clinical Implications

- Overall survival in twin-twin transfusion syndrome has improved over time, with a concomitant reduction in the incidence of neurodevelopmental impairment.
- Although the optimal management of TTTS remains a major challenge for obstetricians and neonatologists, our data can be used to counsel parents in a more optimistic way compared to a decade ago.
- Research focused on prevention of cerebral injury is needed to further improve outcomes of these complicated twin pregnancies.
- Presence of long-term impairment can only reliably be ascertained by accurate and standardized long-term follow-up until at least childhood.

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Chapter 7

Neurodevelopmental outcome at 2 years in twin-twin transfusion syndrome survivors randomized for the Solomon trial

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Abstract

Background

The preferred treatment for twin-twin transfusion syndrome (TTTS) is fetoscopic laser coagulation of inter-twin vascular anastomoses on the monochorionic placenta. Severe postoperative complications can occur when inter-twin vascular anastomoses remain patent including twin-anemia polycythemia sequence (TAPS) or recurrent TTTS. To minimize the occurrence of residual anastomoses, a modified laser surgery technique, the Solomon technique, was developed in which the entire vascular equator is coagulated. In the Solomon randomized controlled trial (NTR1245), the Solomon technique was associated with a significant reduction in TAPS and recurrence of TTTS when compared to the standard laser surgery technique. Although a significant improvement in perinatal outcome was shown after the Solomon technique, the clinical importance should also be ascertained with long-term follow-up of the surviving children.

Objective

To compare the long-term neurodevelopmental outcome in surviving children with TTTS included in the Solomon randomized trial and treated with either the Solomon technique or standard laser surgery technique.

Study design

Routine standardized follow-up in survivors, at least 2 years after the estimated date of delivery, was performed at two of the five centers participating in the Solomon trial, Buzzi Hospital Milan (Italy) and Leiden University Medical Center (The Netherlands). The primary outcome of this follow-up study was survival without long-term neurodevelopmental impairment (NDI) at age 2 years. NDI was defined as: Cerebral Palsy, cognitive and/or motor development score of less than 85, bilateral blindness or deafness. Cognitive and motor development was evaluated using Bayley-III. All analyses per fetus, neonate or child were conducted using the generalized estimated equation module to account for the effect that observations between co-twins are not independent.

Results

The primary outcome, survival without NDI, was detected in 95/141 (67%) in the Solomon group and in 99/146 (68%) in the standard group (P = .92). NDI in long-term survivors included for follow-up was detected in 12/107 (11%) in the Solomon and in 10/109 (9%) in the standard group (P = .61). NDI was due to cerebral palsy in 1 (1%)

case (spastic unilateral) in the Solomon group and in 2 (2%) cases (spastic unilateral and spastic bilateral) in the standard group (P = .58). Cognitive development < 85 was detected in 2/105 (2%) children in the Solomon group and in 6/106 (6%) children in the standard group (P = .23). Motor development < 85 occurred in 8/103 (8%) children in the Solomon group and 3/104 (3%) in the standard group (P = .23).

Conclusion
We found no difference in survival without NDI between the Solomon and standard laser technique. In view of the reduction of short-term complications and absence of increased adverse long-term effects, these data support the use of the Solomon technique in the treatment of TTTS.

Introduction

Twin-twin transfusion syndrome (TTTS) is a major complication of monochorionic twin pregnancies and is the result of unbalanced inter-twin blood flow through placental vascular anastomoses. The preferred treatment for TTTS is fetoscopic laser coagulation of the anastomoses, with an overall survival rate of up to 74%.[1] Although the goal of fetoscopic laser surgery is to coagulate all anastomoses, in up to 33% of pregnancies some vascular connections remain patent.[2;3] These residual anastomoses can cause severe complications such as twin-anemia polycythemia sequence (TAPS) or recurrent TTTS.[1;4] To minimize the occurrence of residual anastomoses and their associated complications, a modified fetoscopic laser surgery technique was developed called the 'Solomon technique', in which a coagulation line is drawn along the entire vascular equator.[1] In the Solomon randomized controlled trial, this technique was associated with a significant reduction in TAPS and recurrence of TTTS when compared to the standard laser surgery technique.[1] Although a significant improvement in perinatal outcome was shown after the Solomon technique, the clinical importance should also be ascertained with long-term follow-up of the surviving children. The aim of this study was to compare the long-term neurodevelopmental outcome in surviving children included in the Solomon randomized trial.

Materials and Methods

The Solomon trial was an open-label, international, multicenter, randomized controlled trial (NTR1245). The background of the trial, methods, baseline characteristics and perinatal outcome have been reported previously.[1;5] The protocol of the trial can be found on http://www.studies-obsgyn.nl/solomon/page.asp?page_id=791. In brief, the trial included 274 women with MC twin pregnancies up to 26 weeks' gestation complicated by TTTS. Women were randomly assigned to the Solomon technique or standard technique. All fetoscopic laser procedures were undertaken by experienced operators, each of whom had done at least 60 previous laser procedures and were competent to undertake the Solomon technique. Routine standardized follow-up in survivors at least 2 years of age was performed at two of the five centers participating in the Solomon trial, Buzzi Hospital Milan (Italy) and Leiden University Medical Center (The Netherlands). Of the 274 patients included in the Solomon trial, 156 (57%) were randomized in one of these two centers. The follow-up study was approved by the Institutional Review Board of both centers. 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, antenatal and/or postnatal TAPS, recurrence of TTTS, gestational age at birth, birth weight, severe neonatal morbidity, cerebral injury and neonatal death (death of a live-born baby within the first four weeks of life). The presence of TAPS was identified according to previously published antenatal and postnatal criteria.[6] In brief, antenatal TAPS was diagnosed when Doppler ultrasound examination revealed an increase in peak systolic velocity in the middle cerebral artery of > 1.5 Multiples of the Median (MoM) in one fetus that coincided with a decreased velocity of < 1.0 MoM in the co-twin, in the absence of twin oligo-polyhydramnios sequence. Postnatal TAPS diagnosis is based on inter-twin hemoglobin (Hb) difference ≥ 8.0 g/dL and at least one of the following criteria: reticulocyte count ratio > 1.7 or small anastomoses (< 1 mm) at the placental surface.[6]

Severe neonatal morbidity was defined as: respiratory distress syndrome, chronic lung disease (defined as oxygen dependency at 36 weeks gestational age), patent ductus arteriosus needing medical therapy or surgical closure, necrotizing enterocolitis ≥ grade 2, retinopathy of prematurity ≥ stage 3, ischemic limb injury, amniotic band syndrome, or severe cerebral injury. Severe cerebral injury includes: intraventricular hemorrhage ≥ grade 3,[7] cystic periventricular leukomalacia ≥ grade 2,[8] ventricular dilatation ≥ 97th percentile,[9] porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome.[10] Neuroimaging was performed using either fetal or neonatal ultrasound. In case of suspected cerebral injury, magnetic

resonance imaging (MRI) was performed. 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).

Follow-up assessments were performed by trained psychologists and pediatricians unaware of the treatment allocations. A follow-up visit was performed at age 2 years corrected for prematurity that is, 2 years after the estimated date of delivery, and included a physical and neurological examination and an assessment of cognitive and motor development using the Bayley scales of Infant and Toddler Development third edition (Bayley-III).[11] The Bayley-III provides cognitive and motor composite scores with a normed mean of 100 and a standard deviation (SD) of 15. Cerebral palsy was defined according to the European CP Network and classified as spastic bilateral, spastic unilateral, dyskinetic (dystonic or choreo-athetotic), ataxic, or mixed.[12] The functional severity was classified according to the Gross Motor Function Classification System (GMFCS) for Cerebral Palsy.[13]

The primary outcome of this follow-up study was survival without neurodevelopmental impairment (NDI). Neurodevelopmental impairment was defined as the presence of at least one of the following: Cerebral Palsy (GMFCS II-V), a cognitive composite score of less than 85 (> -1 SD), a motor composite score of less than 85 (> -1 SD), bilateral blindness, or bilateral deafness requiring hearing aids. Severe NDI was defined as: Cerebral Palsy (GMFCS II-V), a cognitive composite score of less than 70 (> -2 SD), a motor composite score of less than 70 (> -2 SD), bilateral blindness, or bilateral deafness requiring hearing aids.

Statistics

Data are reported as means with standard deviation (SD) or as medians with range, as appropriate. Baseline characteristics were compared using the *t*-test and Mann-Whitney test for continuous variables. Chi-square test and Fisher exact test were used for categorical variables, as appropriate. All analyses per fetus, neonate or child were conducted using the generalized estimated equation module to account for the effect that observations between co-twins are not independent. A two-sided *P*-value of less than 0.05 was considered significant. Statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY).

Results

A total of 76 pregnancies, 152 fetuses, were treated with the Solomon and 77 pregnancies, 154 fetuses, with the standard laser surgery technique for TTTS (Figure 1). Overall survival did not differ significantly between the Solomon (118/152, 78%) and the standard group (117/154, 76%) (P = .77). Cord occlusion of one of the twins was performed in four cases due to severe cerebral injury (Solomon group n=1, standard group n=1) or life-threatening condition of the co-twin (Solomon group n=1, standard group n=1). After treatment with Solomon laser surgery, one pregnancy was terminated following the diagnosis of trisomy 21. In both groups, neonatal death within 28 days of birth occurred in 6/124 (6%) of cases. In the standard group, intensive care treatment was withdrawn in three neonates due to severe cerebral injury.

Five children were excluded from the analysis due to infantile Tay-Sachs disease (n=1; the co-twin was a fetal demise), Neurofibromatosis type I (n=2) and inherited moderate-severe hearing loss (n=2). Long-term follow-up data were obtained from 221/235 (94%) survivors. Fourteen children were lost to follow-up (n=4 declined consent, n=1 mother unable to travel due to pregnancy, n=9 loss of contact information). Perinatal characteristics of the lost-to follow-up group were similar to the children included for follow-up (data not shown and available on request). Table 1 presents the baseline characteristics and Table 2 presents the neonatal characteristics of the children included for follow-up. The Solomon technique was associated with a reduced risk of TAPS or recurrent TTTS compared to the standard group, 4/107 (4%) versus 26/109 (24%), respectively (P = .00). Antenatal treatment in the cases with post-laser TAPS or recurrent TTTS included intrauterine blood transfusion (n=14), cord occlusion (n=2), laser surgery re-intervention (n=6), induction of labor (n=2) or expectant management (n=20). Severe neonatal morbidity including severe cerebral injury did not differ between the two groups.

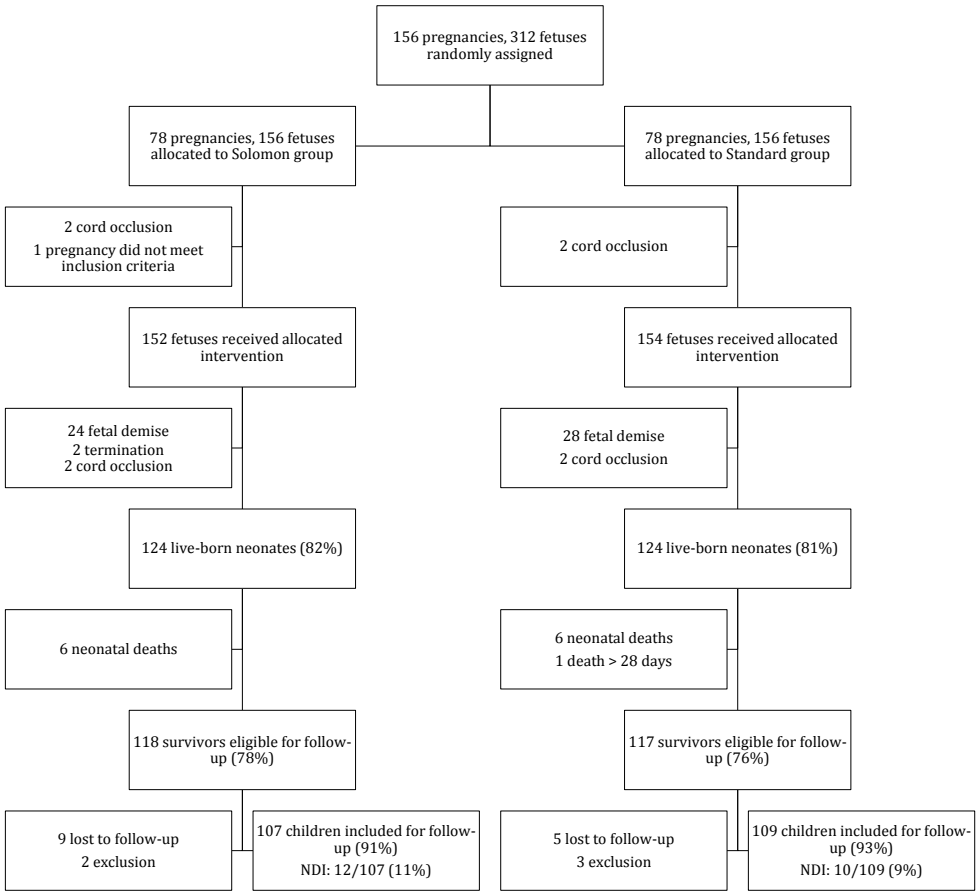

Figure Flow chart showing the derivation of the study population.
NDI, neurodevelopmental impairment.

Table 1 Baseline characteristics of the 216 children included for follow-up.

	Solomon N=107	Standard N=109	*P*
Donor, n (%)	53 (50)	56 (51)	.43
Mean gestational age at laser ± SD - weeks	19.4 ± 2.5	20.2 ± 2.8	.09
Median Quintero stage (range)	3 (1-4)	3 (1-4)	.27
Stage I, n (%)	6 (6)	17 (16)	.10
Stage II, n (%)	42 (39)	34 (31)	.44
Stage III, n (%)	56 (52)	56 (51)	.92
Stage IV, n (%)	3 (3)	2 (2)	.97
Fetal demise co-twin, n (%)	9 (8)	12 (11)	.54
Maternal educational level, n (%)			
Low	15 (14)	14 (13)	.92
Average	48 (45)	45 (41)	.81
High	44 (41)	50 (46)	.76

TTTS, twin-twin transfusion syndrome, *TAPS*, twin anemia-polycythemia sequence

Table 2 Neonatal baseline characteristics of the 216 children included for follow-up.

	Solomon N=107	Standard N=109	*P*
Female, n (%)	62 (58)	52 (48)	.40
Mean gestational age at birth ± SD - weeks	32.8 ± 3.0	32.7 ± 3.1	.99
Mean birth weight ± SD - grams	1864.2 ± 568.2	1898.7 ± 561.2	.72
Severe neonatal morbidity, n (%)	23 (22)	24 (22)	.93
Respiratory distress syndrome	21 (20)	19 (17)	.63
Chronic lung disease	2 (2)	4 (4)	.60
Patent ductus arteriosus	3 (3)	7 (6)	.36
Necrotizing enterocolitis	-	1 (1)	-
Retinopathy of prematurity	-	-	-
Ischemic limb injury	1 (1)	-	-
Amniotic band injury	1 (1)	1 (1)	.99
Severe cerebral injury	4 (4)	5 (5)	.79
Intraventricular hemorrhage ≥ grade 3	2 (2)	3 (3)	.75
Periventricular leukomalacia ≥ grade 2	-	1 (1)	-
Ventricular dilatation ≥ 97th percentile	3 (3)	3 (3)	.97
Porencephalic or parenchymal cysts	-	-	-
Other severe cerebral injury	1 (1)	4 (4)	.21

The primary outcome, survival without NDI, was detected in 95/141 (67%) in the Solomon group and in 99/146 (68%) in the standard group (*P* = .92). NDI in long-term survivors included for follow-up was detected in 12/107 (11%) in the Solomon and in 10/109 (9%) in the standard group (*P* = .61). NDI was due to cerebral palsy in 1 (1%) case (spastic unilateral) in the Solomon group and in 2 (2%) cases (spastic unilateral and spastic bilateral) in the standard group (*P* = .58). Cognitive development < 85 was detected in 2/105 (2%) children in the Solomon group and in 6/106 (6%) children in

the standard group (*P* = .23). Motor development < 85 occurred in 8/103 (8%) children in the Solomon group and 3/104 (3%) in the standard group (*P* = .23). Severe NDI was found in 3/107 (3%) in the Solomon group and in 6/109 (6%) in the standard group (*P* = .47). The long-term outcome of the children included for follow-up is presented in Table 3. Details on the combinations of findings detected in the children with NDI are presented in Table 4a and b.

Table 3 Long-term outcome at 2 years of the 216 children included for follow-up

	Solomon N=107	**Standard N=109**	***P***
Cerebral Palsy, n (%)	3 (3)	3 (3)	.98
GMFCS ≥ grade II	1 (1)	2 (2)	.58
GMFCS < grade II	2 (2)	1 (1)	.56
Bilateral blindness or deafness requiring hearing aids, n (%)	-	-	-
	N=105	**N=106**	
Mean cognitive composite score ± SD	101.6 ± 10.6	100.6 ± 13.9	.61
Cognitive composite score > -1SD, n (%)	2 (2)	6 (6)	.23
Cognitive composite score > -2SD, n (%)	2 (2)	3 (3)	.75
	N=103	**N=104**	
Mean motor composite score ± SD	99.7 ± 12.7	100.9 ± 12.7	.78
Motor composite score > -1SD	8 (8)	3 (3)	.23
Motor composite score > -2SD	2 (2)	3 (3)	.75
Neurodevelopmental impairment[a], n (%)	12 (11)	10 (9)	.61
Severe neurodevelopmental impairment[b], n (%)	3 (3)	6 (6)	.47

GMFCS, Gross Motor Function Classification System; *SD,* standard deviation.
[a]Neurodevelopmental impairment: Cerebral Palsy (GMFCS II-V), cognitive development > -1SD, motor development > -1SD, bilateral blindness or deafness.
[b]Severe neurodevelopmental impairment: Cerebral Palsy (GMFCS II-V), cognitive development > -2SD, motor development > -2SD, bilateral blindness or deafness.

Table 4a Combination of abnormal findings in children with neurodevelopmental impairment in the Solomon group

Case	Twin	GA birth	Birth weight	Neonatal morbidity	Cerebral palsy	Cognitive score	Motor score	Other
1	donor	36	2160	-	-	85	76	
2	recipient	36	2400	-	-	90	82	
3	donor	37	2880	-	-	95	82	
4	recipient	37	2910	-	-	95	76	
5	donor	37	2500	-	-	85	79	TAPS
6	recipient	37	2120	-	-	85	82	TAPS
7	donor	29	1280	RDS	-	95	82	
8	donor	33	1940	-	-	80	97	
9	recipient	32	1649	-	-	95	82	
10	recipient	33	2520	-	-	80	85	
11	recipient	33	1740	Middle cerebral artery infarction	GMFCS IV	95	61	
12	recipient	39	3260	-	-	65	67	Fetal demise co-twin

GA, gestational age; *RDS*, Respiratory Distress Syndrome; *TAPS*, twin anemia-polycythemia sequence; GMFCS*,* Gross Motor Function Classification System

Table 4b Combination of abnormal findings in children with neurodevelopmental impairment in the Standard group

Case	Twin	GA birth	Birth weight	Neonatal morbidity	Cerebral palsy	Cognitive score	Motor score	Other
1	donor	33	2012	RDS	-	80	88	
2	recipient	33	2150	RDS	-	75	76	
3	donor	34	1675	-	-	80	64	
4	recipient	34	2230	-	-	75	67	
5	donor	37	2615	-	-	75	82	
6	recipient	37	2310	-	-	75	82	
7	donor	29	1565	-	-	55	NA	Speech language problems
8	recipient	29	1515	RDS, IVH-3, Ventricular dilatation > 97th	-	55	NA	Speech language problems
9	donor	31	777	IVH-3	GMFCS II	59	64	
10	recipient	32	1635	PVL-3	GMFCS II	100	85	TAPS

GA, gestational age; *RDS*, Respiratory Distress Syndrome; *NA*, not assessed; *IVH*, intraventricular hemorrhage; *PVL,* Periventricular leukomalacia; *GMFCS,* Gross Motor Function Classification System; *TAPS*, twin anemia-polycythemia sequence.

Comment

We found no difference in survival without NDI between the Solomon and standard laser technique. Overall, NDI was detected in 10% (22/206) of survivors included for follow-up that is, 11% in the Solomon group and 9% in the standard group.

Our study shows that, although the Solomon laser technique strongly reduces the risk of short-term complications (TAPS and recurrent TTTS),[1] the effect on the long-term neurodevelopmental outcome appears negligible. Several explanations can be considered to explain the lack of difference between the two treatment groups in this follow-up study. First, the Solomon trial was primarily designed and powered to detect a difference in short-term outcome. Follow-up was only available from two of the five centers participating in the Solomon trial and results cannot be generalized to the total trial population. A second explanation for the lack of difference could be that timely detection and adequate management and treatment (intrauterine transfusion, laser surgery re-intervention) in patients with short-term complications (TAPS or recurrent TTTS) in the standard group reduced the risk for long-term impairment. In addition, the lack of difference in Bayley scores could also be related to early interventions for children with developmental impairment. However, no difference in the rate of early interventions including physical therapy (39% vs 41%, P = .89), speech-language therapy (9% vs 12%, P = .65) and psychological interventions (4% vs 7%, P = .54), was found between the Solomon and standard group, respectively.

The primary outcome of our follow-up study was a composite of survival without NDI to exclude bias related to a positive correlation between high (fetal or neonatal) death rate and selective feticide or withdrawal of intensive care treatment in cases with severe cerebral injury. In the Eurofoetus trial, laser surgery was shown to be superior to amnioreduction in TTTS, but the long-term outcome was similar between the two treatment groups.[14;15] However, the long-term evaluation did not take into account the fact that in a relatively large number of live-born neonates in the amnioreduction group, intensive care treatment was withdrawn due to severe cerebral injury. Had these children survived, the differences in long-term neurodevelopmental outcome between both groups could have been much more evident. In our study, cord occlusion was performed in one case with severe cerebral injury following the Solomon technique (n=1). In the standard group, cord occlusion was performed in one case and intensive care treatment was withdrawn from three neonates due to severe cerebral injury (n=4). Had these neonates with severe cerebral injury survived, long-term impairment would have increased from 11% (12/107) to 12% (13/107) in the Solomon group and from 9% (10/109) to 13% (14/109) in the standard group.

Overall, NDI was detected in 10% (22/206) of children randomized for the Solomon trial. Reported mild to severe impairment rates in the literature vary widely from 0

to 22%.[15-20] Care must be taken when comparing the results of these studies, as this large discrepancy is due to different methodology, differences in (neonatal) death rates, considerable heterogeneity within the small case series and lack of uniform outcome criteria. In this study, NDI was clearly defined as the presence of cerebral palsy (GMFCS II-V), a Bayley-III cognitive and/or motor score below 85, bilateral blindness and/or deafness. We used a cut-off point of 85 (test scores < 1 SD) instead of 70 (test scores < 2 SD) since studies report a significant underestimation of developmental delay using the Bayley-III compared to Bayley-II assessment, with differences up to 18 points in favor of Bayley-III assessment.[21;22] In our study, severe NDI, defined as Bayley-III scores < 70, was detected in 3% in the Solomon group and in 6% in the standard group. We believe that these percentages underestimate the real incidence of impairment in the children treated with fetoscopic laser surgery for TTTS.

Although TTTS remains one of the most lethal conditions in perinatal medicine, the outcome (in terms of its most important parameter, survival without neuro-developmental impairment) has significantly improved over time.[23] In our study, the majority of children (90%) had no neurodevelopmental impairment at 2 years of age. Several factors may explain this improvement, including advancements in fetal and prenatal care strategies (monitoring and management options), increased awareness and knowledge of potential (treatable) complications in TTTS and learning curve effect associated with a highly technical procedure such as fetoscopic laser surgery.[23]

Our findings may be limited by several factors including the relatively small sample size and power, as discussed above. Another potential limitation is related to the limited predictive value of the Bayley scales for future cognitive and motor performance. An average Bayley-III score at age 2 years does not imply within average school performance at age twelve and vice versa. In addition, some developmental problems such as attention deficit- or speech-language problems become more apparent at a later age, especially at school age. Therefore follow-up until at least school age, preferably at age 2, 5 and 8 years, is highly recommended. We intend to perform long-term follow-up in all TTTS survivors at 5 and 8 years.

To date, this is the largest follow-up study of a randomized controlled trial in fetal therapy. We were able to follow-up 94% of survivors with individual and detailed follow-up assessments using standardized tests to evaluate neurological, cognitive and motor development. Follow-up assessments were performed by trained psychologists and pediatricians unaware of the treatment allocations minimizing potential bias.

In conclusion, the results of the Solomon trial imply that the use of the Solomon technique reduces the risk of complications associated with TAPS and recurrent TTTS in monochorionic pregnancies treated with laser surgery for TTTS.[1] The Solomon technique does not increase the risk of long-term impairment. In view of the reduction

of short-term complications and absence of increased adverse long-term effects, we recommend the use of the Solomon technique in the treatment of TTTS.

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Chapter 8

Neurodevelopmental outcome in twin anemia polycythemia sequence after laser surgery for twin-twin transfusion syndrome

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Abstract

Objective

To evaluate the long-term neurodevelopmental outcome in children who developed twin anemia-polycythemia (TAPS) after laser surgery for twin-twin transfusion syndrome (TTTS).

Methods

Neurological, motor and cognitive development was assessed in a consecutive cohort of TTTS survivors treated with laser between 2004 and 2011 and complicated by post-laser TAPS. Primary outcome was neurodevelopmental impairment (NDI), a composite outcome including any of the following: cerebral palsy, bilateral deafness, blindness, severe motor and/or cognitive developmental delay (< -2 SD). A risk analysis on cognitive outcome was performed.

Results

During the study period, 33/306 (11%) monochorionic twin pairs developed TAPS after laser surgery for TTTS. Survival was 53/66 (80%). Long-term outcome was assessed in 47/53 (89%) children. The incidence of NDI was 4/47 (9%), occurring in one donor (1/20, 5%) and three recipients (3/27, 11%) (P = .63). Risk factors for low cognitive scores are low gestational age at birth (P = 0.02) and low birth weight (P < = 0.01). Lowest cognitive scores were detected in the subgroup of TAPS survivors treated with intrauterine transfusion (median score: 82.5).

Conclusions

Neurodevelopmental impairment and cognitive delay was found in almost 1 in 5 children surviving post-laser TAPS. Better treatment and ideally prevention of this complication after laser for TTTS is urgently warranted.

Introduction

Twin anemia-polycythemia sequence (TAPS) is a chronic form of feto-fetal transfusion in monochorionic (MC) twins through small anastomoses at the placental surface.[1] TAPS is characterized by large inter-twin hemoglobin (Hb) difference without signs of twin oligo-polyhydramnios sequence (TOPS). TAPS may occur spontaneous (spontaneous TAPS) or after twin-twin transfusion syndrome (TTTS) treated with laser (post-laser TAPS). The incidence varies between 1-5% in spontaneous TAPS and 1-16% in post-laser TAPS.[2-7] Antenatal diagnosis is based on Doppler ultrasound abnormalities showing an increased peak systolic velocity in the middle cerebral artery in the donor twin, suggestive of fetal anemia, and decreased velocities in the recipient twin, suggestive of polycythemia, without concomitant signs of TOPS. Postnatal diagnosis is based on inter-twin Hb difference ≥ 8.0 g/dL and at least one of the following criteria: reticulocyte count ratio ≥ 1.7 or small anastomoses (< 1 mm) at the placental surface. Perinatal mortality and morbidity rates in TAPS are not well known, and outcome may vary from two healthy neonates to severe neonatal morbidity, including severe cerebral injury, or neonatal death.[2;7;8]

In TTTS treated with laser surgery, the risk of adverse long-term neurodevelopmental outcome is increased, ranging from 6% to 18%.[9-11] Whether TTTS survivors who developed TAPS after laser surgery are also at increased risk of adverse long-term outcome is not known. The aim this study was to evaluate long-term neurodevelopmental outcome in post-laser TAPS survivors and to compare outcome between donors and recipients.

Methods

All consecutive TTTS pregnancies treated with fetoscopic laser surgery at our center between 2004 and 2011 were eligible for this study. The Leiden University Medical Center is the national referral center for fetal therapy in the Netherlands, including laser surgery for TTTS. All TTTS cases complicated with TAPS after laser surgery (post-laser TAPS), were included in this follow-up study. The study was approved by the Institutional review board at the Leiden University Medical Center and all parents gave written informed consent for their children.

TAPS was identified using previously published criteria and staging system.[2] In brief, antenatal TAPS was diagnosed when Doppler ultrasound examination revealed an increase in peak systolic velocity in the middle cerebral artery of > 1.5 Multiples of the Median (MoM) in one fetus that coincided with a decreased velocity of < 1.0 MoM

in the co-twin, in the absence of TOPS. Postnatal TAPS diagnosis is based on inter-twin hemoglobin (Hb) difference ≥ 8.0 g/dL and at least one of the following criteria: reticulocyte count ratio > 1.7 or small anastomoses (< 1 mm) at the placental surface.[2] Antenatal and postnatal TAPS is staged from stage 1 to 5 according to a previously published staging system.[2]

The following antenatal and neonatal data were recorded: gestational age at laser treatment, Quintero stage of TTTS, fetal demise, age at detection of antenatal or postnatal TAPS, antenatal or postnatal TAPS stage, TAPS management in antenatally detected TAPS cases (expectant management, intrauterine transfusion, laser and cord coagulation), gestational age at birth, birth weight, severe neonatal morbidity including severe cerebral injury and neonatal death. Severe neonatal morbidity was defined as the presence of at least one of the following: respiratory distress syndrome (requiring medical ventilation and surfactant), patent ductus arteriosus (requiring medical therapy or surgical closure), necrotizing enterocolitis ≥ grade 2, retinopathy of prematurity ≥ stage III or severe cerebral injury. Severe cerebral injury was defined as at least one of the following: intraventricular hemorrhage (IVH) ≥ grade III,[12] cystic periventricular leukomalacia (cPVL) ≥ grade II,[13] ventricular dilatation ≥ 97th percentile,[14] porencephalic cysts, arterial or venous infarction detected on cerebral imaging.

A follow-up visit was performed at a minimum age of 24 months and included a neurologic examination and an assessment of cognitive and motor development using the Dutch version of the Bayley Scales of Infant and Toddler Development (BSID). Before 2006, the second edition of the BSID was used (BSID-II), while the third edition (BSID-III) was used from 2006 onwards.[15;16] Children at the age ≥ 3 years were tested with the Wechsler Preschool and Primary Scale of Intelligence scale third edition (WPPSI-III).[17] These three tests (BSID-II, BSID-III and WPPSI-III) provide cognitive scores that follow a normal distribution with a mean of 100 and a standard deviation (SD) of 15. BSID-II and BSID-III also provide motor development scores. When each separate score was below 70, > 2 SD below the mean, this was indicative of a severe delay in either cognitive or motor development. Cerebral palsy (CP) was defined according to the European CP Network and classified as diplegia, hemiplegia, quadriplegia, dyskinetic, or mixed.[18]

The primary outcome measure was a composite outcome termed neurodevelopmental impairment (NDI), including at least one of the following: CP, cognitive development score of less than 70 (< - 2 SD), motor development score of less than 70 (< - 2 SD), bilateral blindness, or bilateral deafness requiring amplification. The primary aim of our study was to assess the incidence of NDI in post-laser TAPS cases and to compare outcome between donors and recipients. Secondary outcome was estimation of risk factors associated with lower cognitive scores including gestational age at birth, birth weight, gestational age at TAPS diagnosis, TAPS management in the antenatal detected

TAPS cases and severe neonatal morbidity (including severe cerebral injury).
Data are reported as means with standard deviation (SD) or as medians with range, as appropriate. Statistical analysis was performed using the t-test and Mann-Whitney test for continuous variables. Chi-square test and Fisher's exact test were used for categorical variables, as appropriate. Analysis for risk factors possibly contributing to cognitive outcome was conducted using univariate and multivariate regression methods. The potential risk factors for cognitive outcome were studied in a univariate logistic regression model. The multivariate logistic regression model included all variables that showed significant association in the univariate analysis. Analyses were conducted using the Generalized Estimated Equation (GEE) module to account for the effect that observations within twins are not independent. Results are expressed as *P*-values. A *P*-value of less than 0.05 was considered significant. All statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA).

Results

A total of 306 MC twin pregnancies were treated with fetoscopic laser surgery for TTTS between 2004 and 2011. A total of 33/306 (11%) of the MC twin pairs were diagnosed with TAPS after laser surgery for TTTS. Fetal death occurred in 7/66 (11%) cases, neonatal death in five cases (5/59, 8%) and in one case (1/59, 2%) sudden (unexplained) infant death occurred at the age of two months. Overall survival rate in the post-laser TAPS group was 53/66 (80%). Six children (6/53, 11%) were lost to follow-up due to declined consent or loss of contact information. Follow-up assessments were performed in 47/53 (89%) children. Baseline characteristics of the TAPS survivors included for follow-up are presented in Table 1.

Table 1 Baseline characteristics of post-laser TAPS survivors for follow-up.

	N=47 children
TAPS donor	20 (43)
Gestational age at laser (weeks)	21 (15-27)
Quintero stage	2 (1-4)
Antenatal TAPS stage[a]	2 (1-5)
Stage 1	4 (14)
Stage 2	11 (39)
Stage 3	4(14)
Stage 4	7 (25)
Stage 5	2 (7)
Postnatal TAPS stage[b]	2 (1-4)
Stage 1	8 (42)
Stage 2	9 (47)
Stage 3	0 (0)
Stage 4	2 (11)
Stage 5	0 (0)
Gestational age at birth (weeks)	32 (26-41)
Birth weight (grams)	1635 (750-3667)
Female	22 (47)
Severe cerebral injury[c]	2/46 (4)
Severe neonatal morbidity[d]	18/47 (38)

TAPS, twin-anemia polycythemia sequence; *N,* number. Data are presented as median (range) or n (%).
[a]TAPS stage in the antenatal detected TAPS cases (*n* = 28)
[b]TAPS stage in de postnatal detected TAPS cases (*n = 19)*
[c]Denominator is the number of children who underwent cranial ultrasound.
[d]Severe neonatal morbidity was defined as any of the following characteristics: respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis≥ stage II or severe cerebral injury.

TAPS was detected antenatally in 28/47 (60%) cases and postnatally in the remaining 19/47 (40%) cases. Median gestational age at birth in TAPS cases detected antenatally and postnatally was 32 (26-37) and 32.5 (26-41) completed weeks, respectively (*P* = 0.62). Of the 28 antenatally detected post-laser TAPS cases, 17 were managed expectantly, eight underwent IUT, two were treated with re-laser surgical intervention and in one case cord coagulation of the co-twin was performed. Intrauterine treatment was offered in all cases of TAPS stage 3 and 4. In TAPS stage 1 or 2, intrauterine treatment was offered only in case TAPS was rapidly progressing (within a couple of days), or when the fetus showed other signs of severe anemia not meeting criteria for stage 3, such as increasing heart size or prehydropic signs. In case of treatment, laser surgery was the first choice of treatment if this appeared technically feasible. Laser surgery in TAPS can be more challenging due to the absence of the oligo polyhydramnios sequence. Intrauterine transfusion was chosen in case laser was not perceived feasible. Cord coagulation was performed in one case where we observed severe cerebral injury in

the ex TTTS recipient (new TAPS donor)[19]. Median gestational age at birth of the cases treated intrauterine (IUT, laser or cord coagulation) was 29 (26-33) weeks compared to 33 (27-41) weeks in the cases treated expectantly (P = 0.07).

Of the 47 children neonatal cranial ultrasound was performed in all but one case (46/47, 98%). This child was born at term age in the referral hospital where cranial ultrasound was not part of standard procedure. Two children were diagnosed with severe cerebral injury. In one case, the TAPS donor (former TTTS recipient) was diagnosed with cystic PVL grade III. In the other case, a TAPS recipient (former TTTS recipient), cerebral imaging showed venous infarction and IVH grade II.

Long-term neurodevelopmental outcome in the 47 children was assessed at a median age of 28 (24-96) months. Twenty-nine Children completed were assessed with the BSID tests using either the second edition (n = 9) or third edition (n=20). In three (3/29, 10%) children motor development could not be assessed due to child's refusal. 16 Children completed WPPSI-III. One twin pair was already tested elsewhere, due to behavioral difficulties, with the Snijders Oomen Non-Verbal Intelligence Scale (SON). Previous assessment with the WPPSI failed and the SON was used to obtain a reliable view of their capacities. One twin had mild-to-moderate cognitive delay and the co-twin scored within the normal range of intelligence.

The incidence of NDI in the studied cohort was 4/47 (9%), occurring in one donor (1/20, 5%) and three recipients (3/27, 11%) (P = 0.63). CP was diagnosed in one (1/47, 2%) case. Severe cognitive delay was detected in two (2/47, 4%) children and severe motor delay, in one (1/47, 2%) child. The long-term outcome is reported in Table 2. Patient characteristics of the 4 children with NDI are presented in Table 3.

Table 2 Long-term outcome in the post-laser TAPS group

	Overall N=47	**Donor N=20**	**Recipient N=27**	***P***
Cerebral palsy	1/47 (2)	1/20 (5)	0/27 (0)	0.43
Cognitive score	95.3 ± 12.5	94.5 ± 11.3	95.8 ± 13.4	0.74
Cognitive development < -2 SD	2/47 (4)	0/20 (0)	2/27 (7)	0.50
Cognitive development < -1 SD	8/47 (17)	3/20 (15)	5/27 (19)	1.0
Motor score[a]	93.9 ± 12.4	93.2 ± 7.8	94.4 ± 15.3	0.81
Motor development < -2 SD[a]	1/26 (4)	0/11 (0)	1/15 (7)	1.0
Motor development < -1 SD[a]	5/26 (19)	1/11 (9)	4/15 (27)	0.36
Bilateral blindness/deafness	0/47 (0)	0/20 (0)	0/27 (0)	-
Neurodevelopmental impairment[b]	4/47 (9)	1/20 (5)	3/27 (11)	0.63

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

[a]Total number of children with assessment of motor development with Bayley scales, 11 donors and 15 recipients.

[b]Neurodevelopmental impairment included any of the following: Cerebral Palsy, cognitive development < 2 SD, motor development < 2 SD, bilateral deafness or blindness.

We performed a subgroup analysis on cognitive outcome of the antenatal TAPS cases according to the prenatal management (Table 4). We found that the subgroup of TAPS survivors treated with intrauterine transfusions had the lowest mean cognitive score compared to the other subgroups (Table 4).

We also performed univariate analysis of potential risk factors for cognitive outcome in the whole cohort. Risk factors for low cognitive scores are low gestational age at birth ($P = 0.02$) and low birth weight ($P < 0.01$). Since these two risk factors are highly correlated ($r = 0.87$, $P < 0.01$), no multivariate analysis was performed. In the antenatal TAPS cases ($n = 28$), intrauterine transfusion was a significant risk factor for low cognitive scores ($P = 0.05$).

Table 3 Patient characteristics of the 4 post-laser TAPS survivors with NDI

Case	TAPS donor/ recipient	Highest TAPS stage	Treatment TAPS	GA birth weeks	Birth weight	Neonatal morbidity	Cerebral imaging	Long-term outcome
1	Recipient (former TTTS donor)	4	Expectant management	29	1080	RDS, renal failure (transplant at 3 years)	IVH-I	Cognitive delay < -2SD
2	Recipient (former TTTS donor)	2	IUT	29	1009	RDS	No abnormalities	Cognitive delay < -2SD
3	Recipient (former TTTS donor)	3	Cord coagulation co-twin	28	955	RDS	No abnormalities	Motor delay < -2SD
4	Donor (former TTTS recipient)	2	IUT followed by re-laser surgery intervention	32	1635	No	cPVL-III	CP: quadriplegia

TAPS, twin anemia polycythemia sequence; GA, gestational age; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; SD, standard deviation; IUT, intrauterine transfusion; cPVL, cystic periventricular leukomalacia; CP, cerebral palsy.

Table 4 Cognitive scores in the 28 post-laser TAPS survivors diagnosed antenatally.

Treatment antenatal TAPS	N	Antenatal TAPS stage	Gestational age at birth	Cognitive score
Expectant management	17	2 (1-5)	33 (27-41)	93 (69-109)
Intrauterine transfusion	8	3.5 (2-4)	29 (26-33)	82.5 (67-105)
Laser surgery	2	2 (2-2)	32 (32-32)	112.5 (100-125)
Cord coagulation	1	3 (3-3)	28 (28-28)	99 (99-99)

TAPS, twin anemia polycythemia sequence; N, number
Data are expressed as n (%) or median (range)

Discussion

This is the first study evaluating long-term neurodevelopmental outcome in TTTS survivors who developed TAPS after laser surgery. NDI was detected in 9%, with no difference between donors and recipients. Our results suggest that impairment in post-laser TAPS cases is frequent but is within the range of the incidence of NDI reported in case series of TTTS treated with laser (range 6% to 18%).[9-11] Unfortunately due to logistic reasons we did not have the opportunity to perform follow-up in the years 2006-2007. This is the reason why our cohort could not be compared with the whole cohort of TTTS treated with laser. Larger studies, possibly with a case-control study design, are needed to determine if post-laser TAPS leads to an increased risk of impairment compared to uncomplicated TTTS cases.

The incidence of CP of 2% in our series was similar to previously published TTTS follow-up studies, ranging from 3 to 12%.[9-11] In the general population, CP occurs in approximately 6% at 28 to 31 weeks, 0.7% at 32 to 36 weeks, and 0.1% in term infants.[20] Severe cognitive delay (4%) and severe motor delay (2%) was in the lower range compared to outcomes after TTTS in general (0% to 25%).[11] According to the normal distribution of intelligence, severe cognitive delay occurs at a 2.3% rate in the general population.

Cerebral injury and neurologic impairment in TAPS survivors can theoretically be due to several factors, including among others, hematologic disorders (anemia and polycythemia, leading to impaired cerebral oxygenation), morbidity related to TTTS, preterm delivery, or the type of antenatal TAPS treatment. In a univariate risk factor analysis on cognitive scores, we found that low gestational age and low birth weight were important risk factors for cognitive delay. Low gestational age at birth and low birth weight are known to be independently associated with increased risk for severe cerebral lesions[21] and impaired neurodevelopmental outcome.[22] In a subgroup analysis on antenatal detected/managed TAPS cases, we found that the TAPS subgroup treated with IUT had the lowest median cognitive score (82.5) compared to the other subgroups. A possible explanation for the low cognitive scores could be that these cases were born at a lower gestational age at birth of 29 weeks (IQR 27.5-33) due to induced labor or planned caesarean for severe anemia or polycythemia. IUT may temporarily improve the condition of the donor, allowing prolongation of the pregnancy. However, IUT may also worsen the polycythemia in the recipient twin and lead to possible severe complications such as severe cerebral injury.[8] Additionally IUT is a symptomatic treatment, not a causal treatment for TAPS.

One of the limitations of our study is the use of different developmental tests, that is BSID-II (n = 9), BSID-III (n = 20) and WPPSI-III (n = 16). Previous studies have reported

a significant underestimation of developmental delay using the BSID-III compared to BSID-II assessment.[23;24] Of the 3 children with severe developmental delay, 2 were tested with BSID-III and one with BSID-II. Children at the age ≥ 3 years were tested with WPPSI. With advanced age a more reliable view of capacities can be obtained. Two children were already tested elsewhere with the Snijders Oomen Non-Verbal Intelligence Scale due to failure of previous WPPSI assessment. The most important limitation of this study was the relatively small sample size. Although this is the largest study to date reporting on neurodevelopmental outcome in post-laser TAPS our data should be interpreted with care.

Since post-laser TAPS is caused by small residual anastomoses that might have been missed at initial laser treatment for TTTS, it is of high importance to reduce the amount of these residual anastomoses. A recent published randomized controlled trial showed a significant reduction of the incidence of post-laser TAPS without any identifiable adverse outcomes.[25] To reduce the amount of residual anastomoses and the incidence of TAPS, we advise the use of the Solomon technique, where the whole vascular equator is coagulated, for laser treatment in TTTS.

In conclusion, this is the first study reporting on neurodevelopmental outcome in post-laser TAPS. We report a 9% incidence of NDI and 17% incidence of mild-to-moderate cognitive delay, without difference between donors and recipients. Risk factors for lower cognitive score are lower gestational age at birth and birth weight. Antenatal TAPS management consisting of IUT was a risk factor for lower cognitive scores. Larger studies are needed to reliably investigate long-term neurodevelopmental outcome and evaluate risk factors for adverse outcome. Since TAPS is a rare disease, collaboration between international fetal therapy centers is of utmost importance to increase sample size.

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PART IV

SPECIFIC COMPLICATIONS IN MONOCHORIONIC PREGNANCIES

Chapter 9

Cerebral injury in monochorionic twins with selective intrauterine growth restriction: A systematic review

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Abstract

Objective
To estimate incidence and risk factors of severe cerebral injury in survivors from monochorionic pregnancies with selective intrauterine growth restriction (sIUGR) and/or birth weight discordance (BWD).

Methods
Electronic databases were searched for studies describing perinatal and neurologic outcome in monochorionic twins with sIUGR and/or BWD. Exclusion criteria were twin–twin transfusion syndrome, twin anemia–polycythemia sequence, selective feticide or laser treatment.

Results
Eleven articles were included in the systematic review. Analysis was hampered by different methodology and definitions of cerebral injury. The incidence of severe cerebral injury varied from 0% to 33% (average 8%, 52/661), and was higher in studies including single intrauterine demise [odds ratio (OR) 2.92; 95% confidence interval (CI) 0.89- 9.56] and studies with a median gestational age at birth of ≤32 weeks (OR 1.56; 95% CI 1.06–2.27). The risk of severe cerebral injury was higher in pregnancies with abnormal umbilical artery Doppler (13.5% vs 2.5%; OR 7.69; 95% CI 2.56–25.00) and in larger twins (9% vs 5%; OR 1.93; 95% CI 0.95–3.92).

Conclusion
The incidence of severe cerebral injury in monochorionic twins with sIUGR and/or BWD is approximately 8% and is associated with abnormal umbilical artery Doppler, larger twins, intrauterine fetal demise and low gestational age at birth.

Introduction

Monochorionic (MC) twins are at increased risk of several complications including twin–twin transfusion syndrome (TTTS), twin anemia–polycythemia sequence (TAPS), selective intrauterine growth restriction (sIUGR) and/or birthweight discordance (BWD). Although most studies in the last decades focused primarily on TTTS, the attention is now gradually shifting to other complications, in particular MC twins with sIUGR and BWD. The incidence of sIUGR is reported to be higher than of TTTS and may occur in up to 25% of MC pregnancies.[1–3] In contrast with TTTS, the optimal management in MC twins with sIUGR is not clear, and international consensus on the best treatment strategy is lacking. Treatment options include expectant management, elective preterm birth, fetoscopic laser coagulation of the vascular anastomoses and selective feticide.[2;3] The main cause of sIUGR and BWD is unequal placenta sharing. The growth restricted fetus often has a small placental share and a velamentous cord insertion. The clinical outcome however also depends on the presence of vascular anastomoses in the MC placenta.[4–7] Superficial arterio-arterial or veno-venous anastomoses are known to allow bidirectional blood-flow with low resistance related to their large diameter.8 Because of these anastomoses, intrauterine fetal death (IUFD) of the growth-restricted twin may cause concomitant death or neurological damage in the normal co-twin because of acute exsanguination. In addition, even when both twins survive, the risk of adverse outcome appears to be increased.[3;4;9] However, the short-term and long-term outcomes in MC twins with sIUGR remain unclear, and conflicting results on the risk of cerebral injury have been reported.[1–3;6;9] We performed a systematic review, focusing on the neurological outcome in MC pregnancies with sIUGR and/or BWD following a natural history, to estimate the incidence and risk factors of severe cerebral injury in surviving co-twins.

Methods

Sources

To retrieve studies and articles for this review, online electronic databases were searched from inception until July 2012, with aid of a librarian from the Walaeus Medical Library of the Leiden University Medical Center. The databases included PubMed, Embase, Web of Science, COCHRANE, CINAHL, Academic Search Premier, ScienceDirect and MEDLINE. The following MESH terms or keywords were used with various spelling options: MC, sIUGR, BWD, fetal weight discordance and fetal growth retardation, intrauterine growth retardation, and IUGR in combination with selective. A manual search of the reference

lists of the primary articles was carried out to identify relevant articles not captured by the electronic searches. The search was limited to English language articles.

Study selection

The following inclusion criteria were applied: MC twins with sIUGR and/or BWD and assessment of perinatal outcome, including the presence of severe cerebral injury in live born infants on the basis of postnatal cranial ultrasound. sIUGR was defined as an estimated fetal weight (EFW) in one fetus <10th percentile.[1;2;6;10–12] BWD was described as a difference in birth weight varying from ≥18% to ≥25%. The difference in birth weight was calculated as the difference between the fetal weight of the larger and the smaller twin, divided by the fetal weight of the larger twin ((in formula: body weight of larger twin - body weight of smaller twin)/body weight of larger twin × 100%).[3;13–17] Severe cerebral injury was defined as the presence of intraventricular hemorrhage (IVH) grades III and IV, periventricular leucomalacia (PVL) grade II or more, porencephalic cysts and/or ventricular dilatation (width of one or both lateral ventricles exceeded the 97th percentile).[1;3;7;10;18]

We excluded MC twin pregnancies with TTTS or TAPS and MC twin pregnancies treated with selective feticide or laser treatment. Further exclusion criteria for this systematic review were as follows: case reports, reviews, conference abstracts, book chapters and guidelines. When multiple articles described (partly) the same cases, the articles with the largest study population were included. In case of lack of detailed information, no attempt was made to contact principal investigators to request details regarding their raw data.

The primary outcome was the incidence of severe cerebral injury. When the severity of the cerebral injury could not be determined in all cases (as a result of lack of detailed information), the lowest and highest limits of the incidence of severe cerebral injury are reported. When gestational age (GA) at birth and birth weight were reported separately for different subgroups, a weighted mean was calculated.

The following potential predictors for severe cerebral injury were studied: GA at birth (per study cohort), study cohorts with single IUFD (compared with cohorts without IUFD), abnormal umbilical artery (UA) Doppler measurements (compared with normal Doppler) and larger twin (compared with smaller twin). For the risk analysis, we calculated a mean incidence instead of a range. Type I sIUGR was classified as UA Doppler with positive end-diastolic flow, type II was defined as persistent absent or reversed end-diastolic flow (AREDF), and type III as intermittent AREDF. For this study, we divided the three types in two groups: normal UA Doppler (type I) and abnormal UA Doppler (types II and III). The results of the mixed effects logistic regression models were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). A p value .05

was considered to indicate statistical significance. Analysis was performed using SPSS 20 (SPSS Inc., Chicago, IL).
For this literature review, no ethical committee approval was sought.

Results

We obtained 242 articles with the online electronic database search of which 13 articles met the inclusion criteria. As a result of overlapping study populations, three articles had to be excluded.[1;5;13] Manual search of the reference lists provided one more relevant article, resulting in a total of 11 included articles. A flowchart of the derivation of the included studies is shown in Figure 1, and characteristics of the study designs are reported in Table 1. The number of infants included in the studies ranged from 24 to 108. Inclusion criteria and definitions of sIUGR and/or BWD and cerebral injury varied considerably between the studies. The protocol for timing and frequency of cranial ultrasounds in surviving neonates was different for most studies, and not always described.

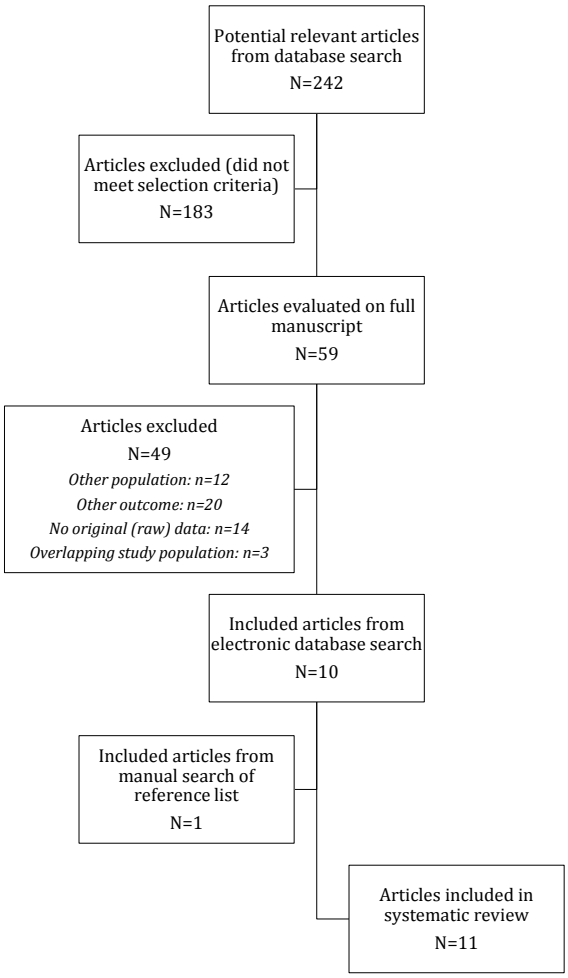

Figure 1 Flowchart showing the derivation of the included studies.

Detailed information on the incidence and type of severe cerebral injury in the 11 studies is shown in Table 2. The incidence of severe cerebral injury varied from 0% up to 33% between the studies. The lowest and highest limits of the mean incidence of severe cerebral injury were 7% (45/661) and 9% (57/661), respectively, depending on the interpretation of the severity of the cerebral injury.

The first study, by Quintero et al.[2;3] reported the outcome in 17 MC twin pregnancies with sIUGR (EFW <10th percentile in one twin), including eight pregnancies with IUFD (six single and two double IUFD). The incidence of severe cerebral injury in live-born infants was 4–13% (1–3/24). Two of the three cases with cerebral injury could not be classified as mild or severe based on the reported information.

Table 1: Information on the study designs and criteria in the 11 included studies included in the systematic review

First author, year	Population	Patients	Definition sIUGR/BWD	Exclusion	Brain imaging
1. Quintero, 2001	USA, 1997-2000	24 MC infants with sIUGR	EFW <10th percentile in one twin	TTTS	Unknown
2. Gratacós, 2004	Spain/Belgium, 2 years	75 MC infants with sIUGR; 40 normal & 35 abnormal UA Doppler	BWD of ≥25% & EFW <5th percentile in one twin	TTTS, cord occlusion	≤day 4 and at 28±7 days
3. Adegbite, 2005	UK, 1991-1998	30 MC infants with BWD and GA at birth ≤34 weeks	BWD of ≥20% & abdominal circumference ≤5th percentile with an abnormal UA Doppler in the smaller twin	Fetal aneuploidy, major congenital malformations, IUFD of both twins, triplets, feticide, embryo reduction	Shortly after birth, at 1 week and if necessary repeated fortnightly
4. Leduc, 2005	Canada, 1994-2002	50 MC infants with BWD	BWD of ≥25%	TTTS, chromosomal/structural anomalies, mono-amnionicity, IUFD	Unknown
5. Cordero, 2005	USA, 1990-2004	48 MC infants with BWD	BWD of ≥20%	None	Unknown
6. Alam Machado, 2009	Brazil, 1998-2004	24 MC infants with BWD	BWD of ≥20%	TTTS, fetal abnormalities, IUFD, delivery <26 weeks gestation	Unknown
7. Chang, 2009	Taiwan, 2006-2008	54 MC infants with sIUGR; 28 normal & 26 abnormal UA Doppler	EFW <10th percentile in one twin	TTTS, anomalies, IUFD or death <24 hrs.	≤day 4 and if necessary serial scans
8. Ishii, 2009	Japan, 2001-2008	108 MC infants with sIUGR; 44 type I, 40 type II, 24 type III	EFW <10th percentile in one twin	TTTS, fetal malformation	Ultrasound with confirmation by MRI
9. Weisz, 2011	Israel, 2004-2008	74 MC infants with sIUGR; 38 normal & 36 abnormal UA Doppler	EFW <10th percentile & co-twin with EFW >10th percentile	TTTS, major fetal anomalies	Unknown
10. Breathnach, 2011	Ireland, 2007-2009	82 MC infants with BWD	BWD of ≥18%	TTTS, major fetal structural abnormalities	Ultrasound with confirmation by MRI
11. Lopriore, 2012	Netherlands, 2002-2011	94 MC infants with BWD	BWD of ≥25%	TTTS, anomalies, IUFD, mono-amnionicity, triplets	Day 1, 3 and 7, followed by 1 scan weekly until discharge

Table 2: Incidence and type of severe cerebral injury in the 11 studies included in the systematic review

First author, year	Incidence[a]		Severe cerebral injury	Comment
	Lowest	Highest		
1. Quintero, 2001	4% (1/24)	13% (3/24)	1 porencephalic cysts, 1 IVH and 1 ventriculomegaly	IVH and ventriculomegaly were not specified
2. Gratacós, 2004	12% (9/75)	17% (13/75)	8 PVL ≥II, 1 occipital cavitated infarction and 4 IVH	IVH grade unknown; cases with IVH and parenchymal brain damage were added up to calculate overall incidence.
3. Adegbite, 2005	27% (8/30)	33% (10/30)	2 cerebral atrophy with PVL, 6 SEH with ventricular dilatation and cystic lesions and 2 IVH grade I-III	IVH grade unknown
4. Leduc, 2005	4% (2/50)		2 IVH grade III-IV	
5. Cordero, 2005	2% (1/48)	4% (2/48)	1 IVH grade III-IV and 1 PVL	PVL grade unknown
6. Alam Machado, 2009	8% (2/24)		2 multicystic leuko-encephalomalacia	
7. Chang, 2009	2% (1/54)		1 cystic PVL with ventriculomegaly	
8. Ishii, 2009	15% (16/108)		16 neurologic morbidity	Neurologic morbidity (defined as IVH gr III-IV, cPVL, blindness or deafness) was not individually specified.
9. Weisz, 2011	5% (4/74)		3 PVL and 1 porencephalic cysts	
10. Breathnach, 2011	0% (0/82)	4% (3/82)	3 IVH	IVH grade unknown
11. Lopriore, 2012	1% (1/92)		1 PVL grade III	
Overall	7% (45/661)	9% (57/661)		

[a]When the severity of the cerebral injury could not be determined in all cases, the lowest and highest limits of the incidence of severe cerebral injury are reported.

In 2004, Gratacos et al.[9] published a prospective study of 84 MC twin pregnancies with IUGR in one fetus (EFW <5th percentile) together with a BWD of ≥25%. Six pregnancies with IUFD (three single and three double IUFDs) were included in the study, all with intermittent A/REDV. The median GA at birth in this group was low, 30.7 weeks. The incidence of severe cerebral injury was high, ranging from 12-17% (9-13/75). Four cases with IVH were not graded and could not be classified as mild or severe injury. To calculate this incidence, we added up the cases with IVH with the cases with parenchymal brain damage. No overlap between the cases was reported. The incidence of severe brain damage was particularly increased in infants with intermittent A/REDV and in larger twins.

Adegbite et al.[19] considered twins discordant when (1) interpair difference in birth weight was ≥20% with normal amniotic fluid in the larger twin and (2) an abdominal circumference of ≤5th percentile with an abnormal UA Doppler in the smaller twin. They included a small population of 30 infants without IUFD that were prematurely delivered between 24 and 34 weeks' gestation. The incidence of severe cerebral injury in this group was high, 27–33% (8–10/30). In two cases with IVH, it was not possible to determine whether the injury was mild or severe.

In 2005, Leduc et al.[16] reported on 25 MC pregnancies complicated with a BWD of ≥25% in a prospective study. Pregnancies with IUFD were excluded. The incidence of IVH grade III or IV was 4% (2/50). No information was reported on the difference between normal versus abnormal UA Doppler and larger versus smaller twins.

Cordero et al.[15] described a group of 54 MC infants with BWD of ≥20%. Cerebral ultrasounds were performed in 48/54 (89%) infants, and the incidence of severe cerebral injury was reported to range from 2% to 4% (1-2/48). PVL was not graded; therefore, it was not possible to classify one case as mild or severe. No information about the incidence related to UA Doppler and larger versus smaller twin was reported. There were no pregnancies with IUFD in this group. Alam Machado et al.[17] reported the neurologic complications (defined asmulticystic leuko-encephalomalacia) in 12 MC twin pregnancies with a BWD of ≥20%. Pregnancies with IUFD were excluded. The incidence of neurologic complications was 8% (2/24). The difference between normal versus abnormal UA Doppler and larger versus smaller twins was not reported.

In a prospective study, Chang et al.[20] reported in 27 MC pregnancies with sIUGR (defined as EFW <10th percentile in one twin) resulting in two live born infants on the occurrence of severe cerebral injury. Of these 54 infants, only one (2%) child was diagnosed with severe cerebral injury. This was an appropriate for gestational age twin delivered at 27^{+5} weeks with cystic PVL and ventriculomegaly, showing signs of cerebral palsy and developmental delay at follow up.

Ishii et al.[6] described the outcome in a large group of 63 MC twin pregnancies with sIUGR (defined as EFW <10th percentile in one twin). There were 18 cases of IUFD, which left 108 surviving children. Besides IVH grades III and IV and cystic PVL, the definition of neurologic morbidity also included blindness and/or deafness and was recorded in an observational period up to six months of age. The overall incidence of neurological morbidity was 15% (16/108). This occurred mainly in the group with abnormal UA Doppler findings. The incidence was similar between larger and smaller twins.
In a prospective study, Weisz et al.[7] reported the outcome in 37 MC twin pregnancies with sIUGR (defined as an EFW <10th percentile in one twin and a co-twin with an EFW>10th percentile). In 74 infants with sIUGR, the incidence of severe cerebral injury was 5% (4/74). All cases with severe cerebral injury occurred in pregnancies with an abnormal UA Doppler. The incidence of severe cerebral injury was higher in larger twins compared with smaller twins. There were no pregnancies with IUFD.
Breathnach et al.[14] established a threshold for intertwin BWD of ≥18% in a prospective study. They included pregnancies with two live fetuses and intact membranes at 24 weeks of gestation. In 82 discordant MC infants, the mean GA at birth was relatively high with almost 35 weeks. The range of severe cerebral injury was 0-4% (0-3/82). It was not possible to classify the injury as mild or severe because grades of IVH and PVL were not reported. The incidence of cerebral injury was similar between larger and smaller twins.
Recently, our group reported the outcome in 47 MC twin pregnancies with a BWD≥25%.[3] The majority of these pregnancies (94%, 44/47) were also classified as sIUGR (EFW<10th percentile) and staged on the basis of UA Doppler. Only pregnancies resulting in two live born twins were included. Cranial ultrasound scans were performed in 92/94 (98%). Cranial ultrasounds were not performed in two cases because of quick discharge from the hospital in good condition. The overall incidence of severe cerebral injury was 1% (1/92) and was similar between larger and smaller twins. The case with severe cerebral injury was the larger twin from an MC pregnancy with sIUGR type III born at a GA of 28^{+6} weeks and diagnosed with PVL grade III at 2 weeks of age.

Risk analysis

Table 3 shows detailed information on the risk factors in the included studies. The median GA at birth in the reported cohorts ranged from 30.6 to 34.9 weeks, indicating that the timing of delivery varied substantially between the studies. Some studies included the surviving twin from sIUGR pregnancies with single IUFD, whereas other studies had no cases with IUFD or explicitly excluded these cases. The results of the risk analysis for potential risk factors (IUFD, abnormal UA Doppler, larger twin and low GA at birth) for severe cerebral injury are shown in Table 4.

Table 3 Perinatal risk factors for severe cerebral injury in the 11 included studies included in the systematic review.

First author, year	Survivors (n)	GA at birth (weeks, median)	BW AGA twin (g, median)	BW IUGR twin (g, median)	IUFD included	Incidence of severe cerebral injury			
						Normal Doppler	Abnormal Doppler	Larger Twin	Smaller Twin
1. Quintero, 2001	24	30.6	-	-	Yes	-	-	7-14% (1-2/14)[a]	0-10% (0-1/10)[a]
2. Gratacós, 2004	75	30.7	1507	972	Yes	5-10% (2-4/40)[a]	20-26% (7-9/35)[a]	21-26% (8-10/39)[a]	3-8% (1-3/36)[a]
3. Adegbite, 2005	30	32	1650	1070	No	-	-	-	-
4. Leduc, 2005	50	33.2	-	-	No	-	-	-	-
5. Cordero, 2005	48	32.3	1742	1286	No	-	-	-	-
6. Alam Machado, 2009	24	34.9	-	-	No	-	-	-	-
7. Chang, 2009	54	33.4	1870	1265	No	0% (0/28)	4% (1/26)	4% (1/27)	0% (0/27)
8. Ishii, 2009	108	32	-	-	Yes	2% (1/44)	23% (15/64)	14% (8/56)	15% (8/52)
9. Weisz, 2011	74	34	1858	1416	No	0% (0/38)	11% (4/36)	8% (3/37)	3% (1/37)
10. Breathnach, 2011	82	34.7	-	-	No	-	-	0-5% (0-2/41)[a]	0-2% (0-1/41)[a]
11. Lopriore, 2012	94	33.2	2118	1411	No	0% (0/28)	2% (1/60)	2% (1/46)	0% (0/46)
Total (range)	24-108	30.6-34.9	1507-2118	972-1416		2-3% (3-5/178)[a]	13-14% (28-30/211)[a]	8-10% (22-27/260)[a]	4-6% (10-14/249)[a]

[a]When the severity of cerebral injury could not be determined in all cases, a range (n/N) is reported.

Table 4: Univariate analysis of potential risk factors for severe cerebral injury in MC twins with sIUGR and/or BWD

	OR (95% CI)	P-value
Studies with IUFD ***versus*** studies without IUFD	2.92 (0.89-9.56)	.076
Studies with median GA at birth of ≤32 wks ***versus*** >32 wks	1.56 (1.06-2.27)	.022
Abnormal ***versus*** normal UA Dopplers	7.69 (2.56-25.00)	<.001
Larger ***versus*** smaller twins	1.93 (0.95-3.92)	.070

OD, odds ratio; CI, confidence interval; IUFD, intrauterine fetal death; GA, gestational age; UA, umbilical artery.

Intrauterine fetal demise: Of the 11 studies included in our systematic review, three also included MC pregnancies with IUFD. The incidence of severe cerebral injury in these three studies was higher compared to studies without IUFD (OR 2.92; 95% CI 0.89–9.56).

Abnormal UA Doppler measurements: In 5 of the 11 studies, UA Doppler measurements were reported in relation to the risk of cerebral injury. Risk analysis showed a significantly increased incidence of cerebral injury in the group with abnormal UA Doppler compared with the group with normal UA Doppler measurements (OR 7.69; 95% CI 2.56–25.00).

Larger versus smaller twin: In 7 of the 11 studies, the risk of cerebral injury was reported in relation to the larger or the smaller twin. Risk analysis showed a slightly increased incidence of cerebral injury in larger twins compared with smaller twins (OR 1.93; 95% CI 0.95–3.92).

Low GA at birth: We studied the association between the median GA at birth reported for each cohort and the incidence of severe cerebral injury. In Table 4, this relation is shown for studies with a median GA at birth of ≤32 weeks versus >32weeks of gestation. The risk of cerebral injury was significantly increased in studies with a lower GA at birth (OR 1.56, 95% CI 1.06–2.27). Furthermore, we performed a logistic regression analysis where we included random effects to account for heterogeneity between studies. We found that the log odds for cerebral injury decreased by 0.44 (P = 0.02) for each additional week GA at birth (Figure 2).

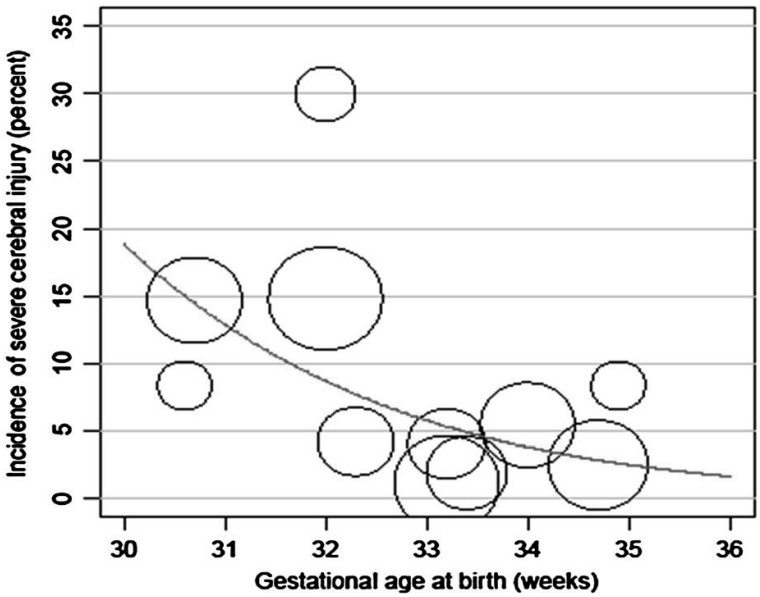

Figure 2 Incidence of severe cerebral injury in relation with median gestational age at birth in 11 studies of MC twins with sIUGR and/or BWD.
Logistic regression of severe cerebral injury and gestational age at birth ($P = 0.02$).
The circles reflect study size.

Discussion

This systematic review shows that the incidence of severe cerebral injury in MC twins with sIUGR and/or BWD varies greatly between the studies, from 0% up to 33%, with an average of approximately 7-9% (45-57/661). The incidence appears to be similar compared with the risk of severe cerebral injury reported in dichorionic twins (8%) and lower compared with MC twins having TTTS (range: 9% to 38%).[21–23]

In addition, we found that the highest incidence of severe cerebral injury was reported in cohorts with lower median GA at birth and in studies including cases with IUFD. Furthermore, the risk for cerebral injury appears to be higher in pregnancies with abnormal UA Doppler findings. These three risk factors are likely to be interdependent. Which of these factors independently contributes the most to the increased risk of cerebral injury could not be determined in this study because we could not perform an individual patient meta-analysis.

Interestingly, the risk of cerebral injury is approximately doubled in the larger twin compared to the smaller twin. Two different theories could explain this finding. According to Gratacos et al., the group with intermittent A/REDF was identified as

having the most elevated risk of IUFD of the smaller twin and neurological damage in the larger twin.

Theoretically, this group may be more prone to in utero fetofetal blood transfusions through the large arterio-arterial anastomoses resulting in hypoxic injury, particularly in the larger twin.[4;11] The increased risk of cerebral injury in the group with abnormal Doppler found in this study may be in agreement with this theory. An alternative theory, recently postulated by our group, suggests that cerebral injury in the larger baby does not primarily develop in utero, but rather after birth as a result of (iatrogenic) premature delivery and subsequent brain immaturity. Detailed analysis of the timing of detection of severe cerebral injury is required to determine whether the injury is mainly due to antenatal factors (such as abnormal UA Doppler and impaired cerebral perfusion of the fetal brain) or postnatal factors due to (extreme) prematurity. Detection of cerebral injury early after birth (within 1-2 weeks) suggests an antenatal etiology, whereas late detection (≥ 2 weeks after delivery) suggests a postnatal etiology.

Low GA at birth, particularly extreme prematurity, is a well-defined risk factor for cerebral injury. Because preterm delivery occurs more frequently in MC twins with sIUGR and/or BWD compared with the general population, the development of cerebral injury is bound to be correlated with this factor. As shown in Figure 2, the highest incidence of cerebral injury was reported in studies with the lowest GA at birth. The cause of the wide variation in GA at birth between the studies is not clear. The optimal timing of delivery in MC twins with sIUGR and/or BWD is not known and may vary between centers. Some centers may be more prone to induce delivery or perform a cesarean delivery at an earlier age to decrease the risk of fetal demise. In case of IUFD of one twin, concomitant death or neurological damage in the co-twin may occur as a result of acute exsanguination through the vascular anastomoses. As emphasized by previous studies, the risk of abnormal postnatal cranial imaging in the surviving MC cotwin may be as high as 34%.[24] In agreement with these findings, the data in our meta-analysis confirm that the risk of cerebral injury in three studies including cases with IUFD is increased (Table 4).

However, the strategy of 'early delivery' in MC twin pregnancies with sIUGR and/or BWD may theoretically not only decrease the risk of fetal demise but also increase the risk of cerebral injury related to prematurity. How to balance the benefit of prolonging the pregnancy in preventing prematurity-related cerebral injury against the harm of risking single IUFD and concomitant damage of the co-twin is a true clinical challenge and urgently warrants more study.

Besides expectant management and elective preterm birth, other management options in MC twins with sIUGR include fetoscopic laser coagulation of the vascular anastomoses and selective feticide. Both methods aim to lead to complete separation of

both fetal circulations and therefore prevent acute exsanguination from one fetus into the other. However, the invasiveness of both methods is associated with complications such as premature rupture of membranes, chorioamnionitis and preterm delivery.[25] In addition, fetoscopic laser coagulation of the vascular anastomoses is technically more challenging in sIUGR pregnancies compared with TTTS pregnancies, because of the absence of a polyhydramnios.[5]

Our results should be interpreted with care due to the uncertainty related to a high degree of heterogeneity between the studies as well as their retrospective nature and inclusion of relative small cohorts. All studies applied different inclusion criteria and various definitions of sIUGR and BWD and different outcome measures. A distinction between types of sIUGR was often not made. Furthermore, detailed information on the timing of fetal demise and cerebral imaging was rarely reported. Because of a lack of detailed information, we could not determine the severity of all cases with cerebral injury. Comparison between the selected studies was therefore difficult to perform, and knowledge on the incidence and risk factors of severe cerebral injury in this group remains limited Another potential limitation of our study is the inclusion of only studies reported in English. Imposing language restrictions may result in bias.

Identification of the true risk factors for cerebral injury in MC twins with sIUGR and/or BWD can only be assessed through meta-analysis of published cohorts using an individual patient analysis or large prospective studies. Whether fetal surgery or obstetrical interventions may improve the outcome in this risk group remains to be elucidated and outcome studies assessing the natural history are urgently required. Finally, although the predictive value of cerebral imaging techniques is increasing, its predictive accuracy remains subject of debate.[23] Presence of clinically relevant, long-term impairment can only reliably be ascertained by accurate and standardized long-term follow-up until at least childhood.

Authors' contributions

All authors contributed to the study concept, design and implementation, and to the content and development of this manuscript.

What's already known about this topic?

- sIUGR may occur in up to 25% of monochorionic twin pregnancies.
- The main cause of sIUGR is related to unequal placenta sharing, in which the growth restricted fetus often has a small placental share and a velamentous cord insertion.
- The optimal management in monochorionic twins with sIUGR is not clear and international consensus on the best treatment strategy is lacking.

What does this study add?

- The average incidence of severe cerebral injury in monochorionic twins with sIUGR is approximately 8%, but varies greatly between the studies from 0% up to 33%.
- Severe cerebral injury in monochorionic twins with sIUGR is associated with abnormal umbilical artery Doppler, intrauterine fetal demise and low gestational age at birth.

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Chapter 10

Single fetal demise in monochorionic pregnancies: Incidence and patterns of cerebral injury

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Abstract

Objective
To evaluate the incidence, type and severity of cerebral injury in the surviving monochorionic (MC) co-twin after single fetal demise.

Methods
All MC pregnancies with single fetal demise that were evaluated at the Leiden University Medical Center between 2002 and 2013 were included. Perinatal characteristics, neonatal outcome and the presence of cerebral injury, observed on neuroimaging, were recorded for all co-twin survivors.

Results
A total of 49 MC pregnancies with single fetal demise, including one MC pair from a dichorionic triplet, were included in the study ($n = 50$ co-twins). The median gestational age at the occurrence of single fetal demise was 25 weeks and the median interval between single fetal demise and live birth was 61 days, with a median gestational age at birth of 36 weeks. Severe cerebral injury was diagnosed in 13 (26%) of the 50 co-twins and was detected antenatally in 4/50 (8%) and postnatally in 9/50 (18%) cases. Cerebral injury was mostly due to hypoxic-ischemic injury resulting in cystic periventricular leukomalacia, middle cerebral artery infarction or injury to basal ganglia, thalamus and/or cortex. Risk factors associated with severe cerebral injury were advanced gestational age at the occurrence of single fetal demise (odds ratio (OR), 1.14 for each week of gestation; 95% CI, 1.01-1.29; $P = 0.03$), twin–twin transfusion syndrome, developing prior to single fetal demise, (OR, 5.0; 95% CI, 1.30-19.13; $P = 0.02$) and a lower gestational age at birth (OR, 0.83 for each week of gestation; 95% CI, 0.69-0.99; $P = 0.04$).

Conclusions
Single fetal demise in MC pregnancies is associated with severe cerebral injury occurring in 1 in 4 surviving co-twins. Routine antenatal and postnatal neuroimaging, followed by standardized long-term follow-up, is mandatory.

Introduction

Monochorionic (MC) pregnancies are at an increased risk for complications, including twin–twin transfusion syndrome (TTTS), twin anemia–polycythemia sequence and selective intrauterine growth restriction (sIUGR). In the case of single fetal demise in a MC pregnancy, severe complications may arise. Several reports have shown that the co-twin is at an increased risk of fetal demise or severe morbidity due to injury to the brain, gastrointestinal tract or kidneys.[1] These complications are thought to be caused by acute fetal exsanguination into the low-pressure circulation of the demised fetus through the placental vascular anastomoses. Acute hypovolemia, hypotension and anemia may subsequently result in hypoxic-ischemic multi-organ damage, particularly to the brain, and even double fetal death.[2] In a meta-analysis, Hillman *et al.* reported a rate of abnormal postnatal cranial imaging and neurodevelopmental impairment after single fetal demise of 34% and 26%, respectively.[3] However, this meta-analysis was based on a few small series and case reports, which may have introduced selection or publication bias, hampering accurate estimation of the incidence. In addition, little is known on the type, severity and risk factors of cerebral injury after single fetal demise. The aim of this study was to determine the incidence and characteristics of severe cerebral lesions in the surviving co-twin of a large case series of MC pregnancies after single fetal demise.

Methods

In this retrospective analysis of collected data, we included all consecutive MC pregnancies with single fetal demise diagnosed at or referred to the Leiden University Medical Center (LUMC), between June 2002 and November 2013. The LUMC is the national referral center in The Netherlands for fetal therapy. Ethical approval from the parents is not required for this type of retrospective study with anonymized data in The Netherlands. We excluded MC pregnancies with fetal demise occurring after laser treatment for TTTS, selective feticide, cases with double fetal demise occurring on the same day, and cases with single fetal demise occurring during the first trimester. Dichorionic (DC) triplets were included if the fetal demise occurred in one of the MC twins. In such cases, the outcomes of the MC co-twin were analyzed only.

We recorded the presence and characteristics of fetal and/or neonatal cerebral injury detected on antenatal and/or postnatal neuroimaging examination. Neuroimaging was performed using either fetal or neonatal ultrasound or magnetic resonance imaging (MRI). The fetal MRI was performed using a 1.5-T MRI system (Philips Medical Systems,

Best, The Netherlands) and included T_2 turbo spin echo sequences in three directions and T_2* fast field echo. The neonatal MRI was performed using a 3-T MRI system (Achieva 3T; Philips Medical Systems, Best, The Netherlands) and included a 3D T_1 turbo field-echo, T_2 turbo spin echo, T_2* fast field echo, and diffusion weighted sequences. We recorded the neuroimaging data obtained within the first year of life. Cerebral injury was categorized as follows: intraventricular hemorrhage (IVH), parenchymal hemorrhage, cystic periventricular leukomalacia (cPVL), porencephalic cyst, ventricular dilation, arterial or venous infarction, and hypoxic-ischemic injury of basal ganglia, thalamus and/or cortex. Severe cerebral injury was defined as at least one of the following: IVH ≥ Grade III[4], cPVL ≥ Grade II,[5] ventricular dilatation ≥ 97th percentile,[6] porencephalic cyst, arterial or venous infarction, basal ganglia, thalamic and/or cortical injury, or other severe cerebral lesions associated with an adverse neurological outcome.[7]

The following obstetric parameters were recorded: amnionicity, sIUGR (defined as an estimated fetal weight of the growth-restricted fetus < 10th centile), TTTS (including Quintero stage and management),[8] congenital anomalies, gestational age at detection of fetal demise, cause of fetal demise, fetal middle cerebral arterial (MCA) peak systolic velocity (PSV) Doppler measurement, presence of fetal cerebral injury and gestational age at detection, time between fetal demise and delivery, and mode of delivery. In the surviving co-twins that were treated with intrauterine blood transfusion (IUT) for anemia, we recorded the hemoglobin level prior to the transfusion.

The following neonatal parameters were recorded: gestational age at birth, birth weight, gender, occurrence of perinatal asphyxia, neonatal death, presence of respiratory distress syndrome, patent ductus arteriosus, hemoglobin level at birth and need for a blood transfusion, and need for inotropic support. Perinatal asphyxia was defined as the presence of three or more of the following five criteria: non-reassuring cardiotocogram patterns, umbilical cord arterial pH < 7.10 and base excess ≥ 16mmol/L or lactate > 10mmol/L, an Apgar score < 5 at 5 minutes after birth, failure of spontaneous breathing at 5 minutes after birth, and onset of multiple organ failure.

Statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA) and reported as *n* (%), mean ± SD or median (interquartile range (IQR)). Statistical analysis was performed using the *t*-test and Mann–Whitney *U*-test for continuous variables. The chi-squared test and Fisher's exact test were used for categorical variables. Risk factors possibly contributing to severe cerebral injury were studied in a univariable regression model. The multivariable model included all variables that showed a significant association in the univariable analysis. Results are expressed as odds ratio (OR) with 95% confidence interval (CI). A *P*-value < 0.05 was considered as statistically significant.

Results

A total of 49 MC pregnancies, including one MC triplet and two DC pregnancies with a MC component, fulfilled our inclusion criteria and were included in the study ($n = 50$ fetuses). The median gestational age at the diagnosis of single fetal demise was 25 (20–29.3) weeks' gestation. In 35/49 (71%) pregnancies, a likely cause of single fetal demise could be identified: monoamnionicity (5/49, 10%), sIUGR (13/49, 27%) or TTTS (17/49, 35%). In all monoamniotic twin pregnancies, umbilical cord entanglement was present. In one monoamniotic pregnancy, fetal co-twin demise was detected at 42 days after single fetal demise. In the sIUGR pregnancies, single fetal demise occurred most often in the growth-restricted fetus (11/13, 85%). In the TTTS pregnancies, single fetal demise occurred mostly in the donors (14/17, 82%). Two pregnancies with sIUGR were also complicated by congenital anomalies in the demised twin (Potter syndrome, transposition of the great arteries and esophageal atresia). MCA-PSV Doppler measurements were performed in 47/50 (94%) cases after single fetal demise. In three cases, MCA-PSV measurements were not performed due to imminent delivery of the surviving co-twin. Treatment with IUT due to signs of severe acute anemia shortly after the demise of the co-twin was performed in 6/50 (12%) cases. The median interval between fetal demise and delivery was 58 (9.5–106) days. Figure 1 presents a flow chart showing the derivation of the study population. Details of the antenatal characteristics of the study group are reported in Table 1.

Table 1 Antenatal characteristics of the 49 monochorionic (MC) pregnancies after single fetal demise

Characteristic	MC pregnancies
Female fetus	25 (50)
Twin–twin transfusion syndrome	17 (35)
Amnioreduction	4/17 (24)
Selective IUGR	13 (27)
Monoamniotic twin	5 (10)
GA at single fetal demise (weeks)	25 (20–29.3)
Interval between fetal demise and delivery (days)	58 (9.5–106)
IUT after single fetal demise	6 (12)
Hb level at transfusion (g/dL)	5 (3–5.9)
Cerebral injury	4 (8)
GA at detection (weeks)	26.5 (22.3–30.8)

Data are given as *n* (%), *n*/*N* (%) or median (interquartile range).
GA, gestational age; Hb, hemoglobin; IUGR, intrauterine growth restriction; IUT, intrauterine transfusion.

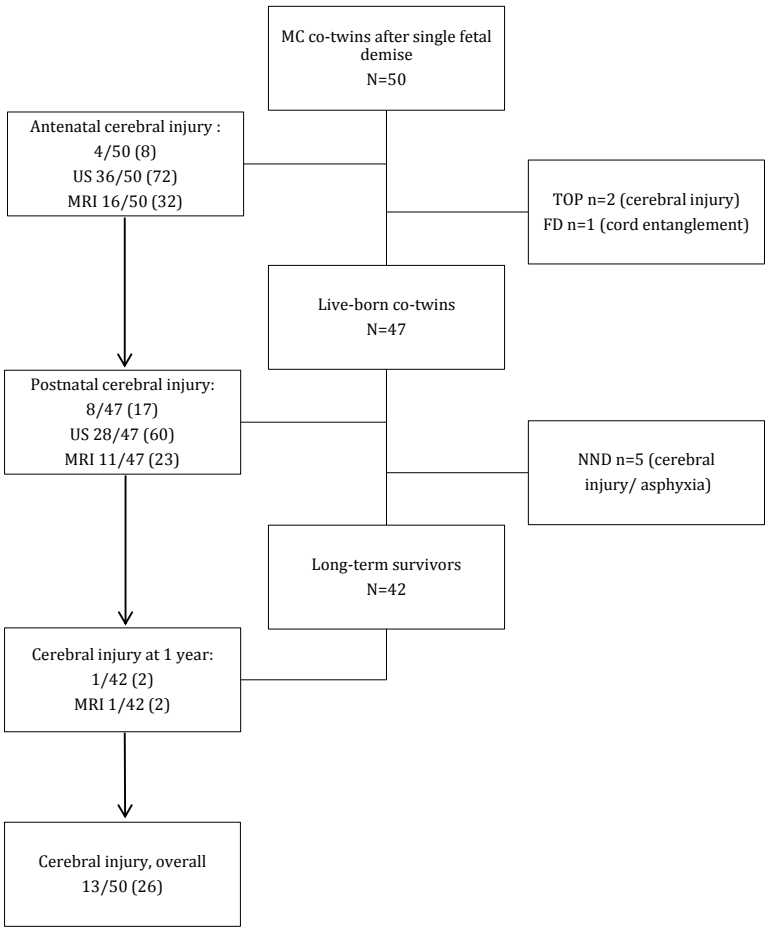

Figure 1 Derivation of the study population.
MC, monochorionic; *US,* ultrasound; *MRI*, magnetic resonance imaging; *TOP*, termination of pregnancy; *FD,* fetal demise; *NND*, neonatal death.

Serial fetal cranial ultrasound after single fetal demise was performed in 36/50 (72%) fetuses, of which, 16/36 (44%) also underwent fetal MRI. In 4/36 (11%) fetuses, severe cerebral injury was detected at a median gestational age of 26.5 (22.3–30.8) weeks. Among these were two pregnancies complicated with TTTS, of which, single fetal demise of the donor twin occurred at 19 and 23 weeks' gestation. Fetal MRI at 21 and 26 weeks, respectively, revealed infarction of the MCA in the recipient co-twin. In both cases, the parents opted for termination of the pregnancy after extensive counseling. In the third case of severe cerebral injury, occurring in a DC triplet pregnancy, single fetal demise of the growth-restricted MC co-twin was detected at 25 weeks' gestation. Fetal MRI performed at 27 weeks displayed multicystic encephalopathy in the surviving MC co-twin. In the fourth case, in a TTTS-complicated pregnancy, single fetal demise

of the donor twin was detected at 28 weeks' gestation and fetal MRI at 32 weeks showed cerebral atrophy, diffuse white matter loss and abnormalities of the thalamus and internal capsula in the recipient co-twin. Postnatal MRI confirmed the cerebral abnormalities detected with a fetal MRI in both the surviving co-twins.

The median interval between the occurrence of single fetal demise and delivery of the 47 live-born co-twins was 61 (9–114) days, with a median gestational age at live-birth of 36 (33–38) weeks. Perinatal asphyxia was detected in 6/47 (13%) neonates and three (3/47; 6%) neonates were diagnosed with persistent pulmonary hypertension, of which, one required extracorporeal membrane oxygenation. This child was diagnosed with severe renal failure and died at 2 years of age after a renal transplant[9]. Eight (8/47; 17%) neonates were treated with blood transfusion, of which five also required inotropic medication at birth. These children were born shortly after the demise of their co-twin (range, 0–2 days). One (2%) child presented with an unexpected limb reduction at birth. Neonatal death occurred in 5/47 (11%) co-twins, due to asphyxia and/or severe cerebral injury. Detailed information on neonatal outcome is presented in Table 2.

Table 2 Neonatal outcome of live-born co-twins after single fetal demise

Outcome	Co-twins (*n* = 47)
GA at birth (weeks)	36 (33–38)
Birth weight (g)	2622.5 (1816.3–2985)
Perinatal asphyxia	6 (13)
Respiratory distress syndrome	10 (21)
Patent ductus arteriosus	4 (9)
Renal failure	1 (2)
Hb level at birth (g/dL)*	15 (9.4–18.1)
Blood transfusion at birth	8 (17)
Neonatal death	5 (11)
Age at death (days)	6 (3.5–19)

Data are given as *n* (%) or median (interquartile range). *$n = 23$. GA, gestational age; Hb, hemoglobin.

Postnatal ultrasound was performed, within the first days of life, in 28/47 (60%) neonates, of which, 11/28 (39%) also underwent MRI. Severe cerebral injury was detected on postnatal imaging in 8/28 (29%). Overall, antenatal or postnatal neuroimaging at birth was performed in all but one case (49/50, 98%). This child was born at 37 weeks gestational age in another hospital where cranial ultrasound was not part of standard procedure. However, the child presented with severe developmental delay at almost 1 year of age and subsequent MRI showed severe cerebral injury. In total, severe cerebral injury was detected in 13/50 (26%) co-twins after single fetal

demise. Of these 13 co-twins, eight (62%) were diagnosed with TTTS prior to single fetal demise. The median gestational age at single fetal demise was 27 (24–33) weeks, compared to 23.5 (19–28.75) weeks in co-twins without severe cerebral injury ($P = 0.03$). The median gestational age at live birth was 35 (28–37) and 37 (34–38) weeks, respectively ($P = 0.04$). Details on characteristics and outcome of the 13 co-twins with severe cerebral injury after single fetal demise are presented in Table 3. Figure 2 shows T2-weighted MRI scans of four children with different types of severe cerebral injury.

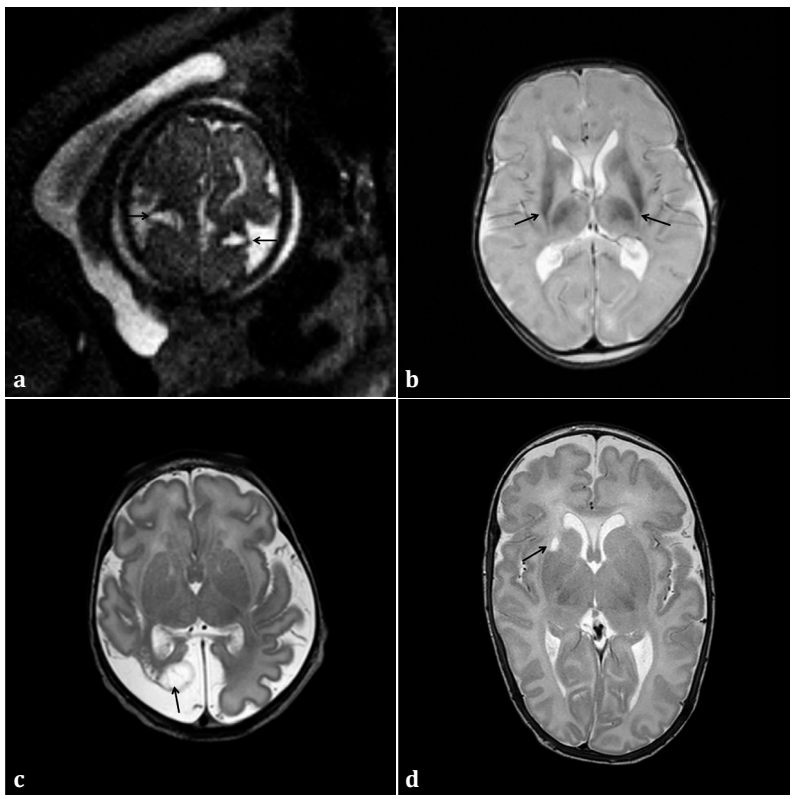

Figure 2 (a) T2-weighted fetal magnetic resonance image (MRI) obtained at 26 weeks' gestation, 3 weeks after single fetal demise in a monochorionic pregnancy, showing bilateral middle cerebral artery infarction (arrows). (b) T2-weighted MRI in a 3-day-old neonate who was born asphyxiated at 36 weeks' gestation, one day after single fetal demise, showing diffuse cortical necrosis, white matter injury and severe basal ganglia/thalamic injury (arrows). (c) T2-weighted postnatal MRI in a 2-day-old neonate who was born at 37 weeks' gestation, 63 days after single fetal demise, showing bilateral severe cerebral atrophy and cystic parenchymal destruction of right posterior cerebral hemisphere (arrow). (d) T2-weighted MRI in a 12-week-old neonate who was born at 28 weeks' gestation, 10 days after single fetal demise, in whom neonatal cranial ultrasound showed Grade II unilateral intraventricular hemorrhage and infarction in the right caudate nucleus (not shown). A cystic lesion in the right caudate nucleus (arrow) can be seen, confirming the ultrasound diagnosis.

Table 3 Characteristics of the 13 co-twins with severe cerebral injury after single fetal demise

Case	Cause of single fetal demise	GA at fetal demise (wks)	GA at birth (wks)	Outcome	Type and age at neuroimaging (wks)	Cerebral injury
1	TTTS donor	19	23	TOP	aMRI (21)	Right MCA infarction
2	TTTS donor	23	34	TOP	aMRI (26)	Bilateral MCAinfarction
3	TTTS and sIUGR small twin	25	37	survival	aMRI (27)	Multicystic encephalopathy
4	TTTS donor	28	37	survival	aMRI (32)	Severe cerebral atrophy, diffuse white matter loss, abnormal thalamus and capsula interna
5	TTTS donor	22	37	survival	pMRI at 1 year	cPVL Grade 3
6	TTTS recipient	26	27	NND	pUS (28)	Bilateral IVH Grade 3 with PVHI, cPVL Grade 3
7	TTTS recipient	27	28	survival	pMRI (40)	Unilateral IVH Grade 2, infarction right nucleus caudatus
8	TTTS donor	29	29	NND	pMRI (30)	Multicystic encephalopathy
9	sIUGR large twin	35	35	survival	pMRI (35)	Diffuse cortical necrosis
10	sIUGR large twin	27	27	NND	pUS (27)	Bilateral IVH Grade 3
11	Unknown	31	31	survival	pMRI (32)	Multicystic encephalopathy
12	Unknown	36	36	NND	pMRI (36)	Diffuse cortical necrosis, white matter injury and severe basal ganglia/thalamic injury
13	Unknown	36	36	NND	pMRI (36)	Cortical necrosis, white matter injury and severe basal ganglia/thalamic injury

aMRI, antenatal magnetic resonance imaging; cPVL, cystic periventricular leukomalacia; GA, gestational age; IVH, intraventricular hemorrhage; MCA, middle cerebral artery; NND, neonatal death; pMRI, postnatal magnetic resonance imaging; pUS, postnatal ultrasound; PVHI, periventricular hemorrhagic infarction; sIUGR, selective intrauterine growth restriction; TOP, termination of pregnancy; TTTS, twin–twin transfusion syndrome; wks, weeks.

Univariable and multivariable analysis of the potential risk factors contributing to severe cerebral injury was performed (Table 4). The associated risk factors were advanced gestational age at occurrence of single fetal demise (OR, 1.14 for each week; 95% CI, 1.01-1.29; $P = 0.03$), diagnosis of TTTS prior to single fetal demise (OR, 5.0; 95% CI, 1.30-19.13; $P = 0.02$) and gestational age at live birth (OR, 0.83 for each week; 95% CI, 0.69-0.99; $P = 0.04$).

Table 4 Analysis of potential risk factors for severe cerebral injury in the surviving co-twin after single fetal demise ($n = 50$)

Characteristics	Cerebral injury ($n = 13$)	No cerebral injury ($n = 37$)	*P*	Univariable OR (95% CI)	*P*	Multivariable OR (95% CI)
GA at single fetal demise (weeks)	27 (24–33)	23.5 (19–28.8)	0.03	1.14 (1.01-1.29)	0.01	1.34 (1.06-1.69)
TTTS	8/13 (62)	9/37 (24)	0.02	5.0 (1.30-19.13)	0.02	19.2 (1.65-223.69)
sIUGR	2/13 (15)	11/37 (30)	0.32	0.43 (0.08-2.27)		
Monoamniotic pregnancy	1/13 (8)	4/37 (11)	0.75	0.69 (0.07-6.78)		
GA at birth (weeks)	35 (28–37)	37 (34–38)	0.04	0.83 (0.69-0.99)	0.05	0.77 (0.59-1.00)

Data are presented as median (interquartile range) or n/N (%).
OR, odds ratio; *CI*, confidence interval; *GA*, gestational age; *TTTS*, twin-twin transfusion syndrome; *sIUGR*, selective intrauterine growth restriction.

Discussion

This study shows that the incidence of severe cerebral injury in MC pregnancies after single fetal demise is high (13/50, 26%) and is mostly due to hypoxic-ischemic injury resulting in cystic PVL, MCA infarction, or injury to basal ganglia, thalamus and/or cortex. The exact mechanisms leading to these different types of cerebral injury are still unclear. In the past, co-twin morbidity after single fetal demise was thought to result from transfusion of thromboembolic material from the demised fetus into the circulation of its co-twin. At present, most experts think that co-twin morbidity results from acute exsanguination from the surviving co-twin into the low-pressure circulation of the demised co-twin. Acute exsanguination results in acute hemodynamic changes (hypovolemia and anemia) leading to hypoxic-ischemic injury and multi-organ failure.[2] In a previous study, we showed that perinatal asphyxia in MC twins, in contrast to DC twins, is strongly associated with acute exsanguination and anemia at birth.[10] However, alternative explanations can be considered to explain the observed cerebral injury. In this study, we found that severe cerebral injury in the surviving co-twin was associated

with the presence of TTTS (diagnosed prior to single fetal demise), higher gestational age at the time of single fetal demise and lower gestational age at birth. In two pregnancies complicated by TTTS, MCA infarction in the recipient twin was detected antenatally. Whether the cerebral lesions in the TTTS cases occurred before or after single fetal demise is not clear. As shown in previous studies, TTTS is known to be associated with the development of antenatal cerebral injury, including arterial infarctions in recipient twins.[11] The combination of TTTS and single fetal demise may increase the risk of cerebral injury. Similarly, a lower gestational age at birth is a well-known risk factor for cerebral injury; prematurity may have accounted for the cases with severe IVH in our series. The explanation for the association between a higher gestational age at single fetal demise and the presence of cerebral injury is not clear. Since the size of placental vascular anastomoses increases with gestational age, we hypothesize that the severity of acute exsanguination may also increase with gestational age, with reduced vascular resistance through the larger vascular anastomoses. However, care should be taken when interpreting these results and speculating on possible explanations due to the relatively small sample size. In order to reliably investigate risk factors contributing to the development of severe cerebral injury in co-twins following single fetal demise, larger studies are needed.

The incidence of severe cerebral injury reported in this study (26%) is slightly lower than the 34% (95% CI, 28.8–46.1%) risk reported in the recent meta-analysis of Hillman *et al*.[3] Methodological differences and heterogeneity between the studies included in this meta-analysis could have led to an overestimation of the true risk. Our study, the largest single-center series to date, with antenatal and postnatal imaging in the majority of cases, still has limited numbers.

In our study, all but one fetus underwent routine antenatal or postnatal cerebral imaging after single fetal demise. In two cases, severe cerebral injury was detected antenatally and had important clinical implications which led to termination of the pregnancy in both cases. In the majority of cases, severe cerebral injury was detected postnatally, and led to either neonatal death or subsequent neurological symptoms related to perinatal asphyxia. In one case, cerebral imaging was not performed at birth but neurological symptoms became apparent several months later. Given the important clinical and prognostic implications, we recommend routine ante- and postnatal imaging in all MC co-twins after single fetal demise, to enable appropriate ante- and postnatal intervention and timely counseling.

The intriguing question remains as to why the majority (37/50; 74%) of surviving co-twins do not suffer from cerebral injury. We speculate that the type and size of the anastomoses may play an important role in the pathogenesis of cerebral injury. Hypothetically, the presence of large arterioarterial or venovenous anastomoses may

allow a more rapid and massive exsanguination due to the low resistance of these anastomoses. However, this relation is not easy to establish, since the interval between fetal demise and birth is often more than 1 week, after which, placental anastomoses cannot be evaluated due to maceration of the placental share of the demised co-twin.

Severe injury on cerebral imaging does not equal long-term developmental delay. Outcome can vary from healthy development to mild impairment on multiple domains to severe developmental delay. The presence of clinically relevant, long-term impairment can only reliably be ascertained by standardized long-term follow-up of these children until at least school age.[11;12] Hillman *et al.* found a rate of long-term neurodevelopmental impairment after single fetal demise in MC co-twins of 26%.[3] Neurodevelopmental impairment was only defined broadly as cerebral palsy or 'minor delay' in motor development and standardized developmental tests were not employed. In addition, the timing of follow-up was often unclear. As such, the true rate of long-term impairment in MC co-twins after single fetal demise is still unknown. We intend to perform long-term follow-up in the surviving MC co-twins after single fetal demise using standardized developmental tests.

Our findings should be interpreted with care due to the retrospective nature of this study and the relatively small number of cases. In addition, the combination of either pre- and/or postnatal imaging is an important limitation. We recommend stringent and precise ante- and postnatal imaging protocols to evaluate accurately the incidence of antenatal cerebral injury and investigate the correlation between antenatal and postnatal imaging findings. Given the extreme rarity of these events, more accurate estimations of the incidence of severe cerebral injury, and other complications such as gastrointestinal or renal lesions, can only be obtained through an international multicenter registry of MC pregnancies with single fetal demise. Nevertheless, this is the largest cohort thus far, showing clearly that single fetal demise in MC pregnancies is associated with an increased risk of severe cerebral injury for the surviving co-twin. We would strongly advocate careful follow-up with antenatal and postnatal neuroimaging investigations as well as long-term neurodevelopmental follow-up in all MC pregnancies with single fetal demise.

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Chapter 11

Perinatal outcome after selective feticide in monochorionic twin pregnancies

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Abstract

Objective
To evaluate the incidence and risk factors of adverse perinatal outcome in complicated monochorionic twin pregnancies treated with selective feticide.

Methods
This was a retrospective analysis of prospectively collected data from a consecutive, national cohort. All monochorionic twin pregnancies treated with selective feticide at Leiden University Medical Center between June 2000 and November 2011 were included. Obstetric and neonatal data were recorded. The primary outcome measure was adverse perinatal outcome, including fetal or neonatal demise or severe neonatal morbidity.

Results
Data on perinatal outcome were obtained in all cases (n=131).Overall perinatal survival rate was 67.2% (88/131). Median gestational age at delivery was 34 (interquartile range, 23–38) weeks. Neonatal mortality and morbidity rate in liveborn children was 4.3% (4/92) and 12.0 % (11/92), respectively. Severe cerebral injury was detected in three children. The overall incidence of adverse perinatal outcome was 41.2% (54/131). Median gestational age at occurrence of preterm prelabor rupture of membranes (PPROM) was 19.0 weeks and 32.0 weeks in cases with and without adverse perinatal outcome, respectively (P = 0.017). Liveborn children with adverse perinatal outcome were born at a lower median gestational age (29.0 weeks) than were children without adverse perinatal outcome (38.0 weeks) (P < 0.001).

Conclusions
The risk of adverse perinatal outcome after selective feticide is high and associated with low gestational age at occurrence of PPROM and low gestational age at delivery. Long-term follow-up to assess neurodevelopmental outcome in survivors is required.

Introduction

The incidence of complications and perinatal mortality is higher in monochorionic (MC) twin pregnancies than in dichorionic (DC) twin pregnancies, owing to the presence of placental vascular anastomoses. These vascular connections can lead to specific complications including twin–twin transfusion syndrome (TTTS),[1] twin anemia–polycythemia sequence,[2] and twin reversed arterial perfusion (TRAP).[3;4] In addition, selective intrauterine growth restriction (sIUGR) occurs more frequently in MC pregnancies and is associated with an increased risk of morbidity and mortality. When intrauterine death of one fetus occurs, the risk of death or cerebral damage in the cotwin is increased, owing to acute exsanguination through the placental anastomoses.[5–7]

Several indications have been described for selective feticide in MC twin pregnancies, including TTTS, TRAP, sIUGR, severe congenital anomalies and higher-order multiple pregnancies.[5;8;9] Reported methods for selective feticide include fetoscopic laser coagulation, bipolar cord coagulation, radiofrequency ablation (RFA), cord occlusion by ligation or photocoagulation and interstitial laser coagulation.[5;6;10;11] Associated complications are premature rupture of membranes in up to 30% of cases and preterm delivery.[5;12] Perinatal survival rates vary between 65 and 92%, depending on technique and indication.[5] However, data on the risk of neonatal complications and long-term neurodevelopmental outcome in surviving twins are limited.[6;13]

The primary objective of this study was to evaluate the incidence of perinatal mortality and neonatal morbidity in a series of MC twin pregnancies treated with selective feticide in a large national cohort. Our secondary objective was to assess possible risk factors for adverse outcome in order to find ways to improve care.

Methods

All MC pregnancies treated with selective feticide at our center between June 2000 and November 2011 were included in this consecutive cohort study. The Leiden University Medical Center is a tertiary medical center and is the national referral center for fetal therapy (including selective feticide) in The Netherlands.[1] MC triplets (or higher order pregnancies) were excluded from the study.

The following obstetric data were recorded: indication for selective feticide and technique used, gestational age at intervention, preterm prelabor rupture of membranes (PPROM) before 37 weeks, gestational age at delivery and mode of delivery. The techniques used comprised fetoscopic laser coagulation, bipolar cord coagulation, interstitial laser coagulation and RFA. For fetoscopic cord coagulation performed before

18 weeks, an embryoscope was used with a diameter of 1.0 mm. For cord coagulation after 18 weeks, a 1.3-mm fetoscope was used through an 8-F introduction sheath. Criteria for intervention in TRAP were signs of cardiac compromise in the pump twin based on abnormal ductus venosus flow.

The following neonatal data were recorded: gender, birth weight, presence of respiratory distress syndrome (RDS), chronic lung disease, patent ductus arteriosus, necrotizing enterocolitis (NEC) ≥ Stage II,[14] neonatal sepsis (defined as a clinically ill neonate with positive bacterial culture), renal failure and severe cerebral injury on cranial ultrasound examination. Severe cerebral injury was defined as the presence of at least one of the following findings on ultrasound scan: Intraventricular hemorrhage (IVH) ≥ Grade III,[15] periventricular leukomalacia ≥ Grade II,[16] ventricular dilatation, arterial or venous infarct or other cerebral anomalies associated with adverse neurological outcome.

The primary outcome measure was a composite outcome termed 'adverse perinatal outcome', which included intrauterine fetal death (IUFD), neonatal death (NND), termination of pregnancy (TOP) or severe neonatal morbidity. NND was defined as the death of a liveborn child delivered after 23 weeks' gestation. Severe neonatal morbidity was defined as the presence of any of the following: RDS, NEC, neonatal sepsis, renal failure or severe cerebral injury. The incidence of adverse perinatal outcome was described by technique employed. Following analyses in recent studies,[5] we evaluated the outcome in the groups treated with selective feticide at ≤ 18 weeks' gestation and > 18 weeks' gestation.

Categorical variables were compared using Fisher's exact test or the chi-square test, as appropriate. Continuous variables were compared using unpaired Student's *t*-test, median test or the Mann-Whitney U-test. Statistical analysis was performed with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 131 MC twin pregnancies were treated with selective feticide during the study period. In cases of DC triplets with an MC component (n=7), only the MC cotwin pair was analyzed. Figure 1 shows mortality within the study population. Information on indication and technique for selective feticide is given in Table 1.

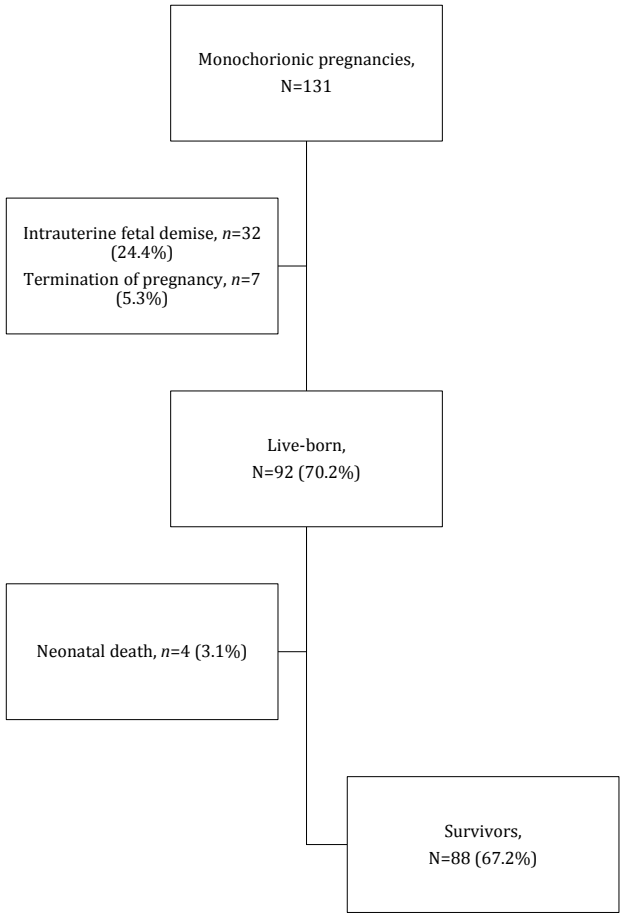

Figure 1 Flowchart showing mortality in study population of 131 monochorionic twin pregnancies that underwent selective feticide.

Table 1 Baseline characteristics in 131 monochorionic pregnancies undergoing selective feticide.

Characteristic	N (%)
Indication	
Twin-twin transfusion syndrome	40 (30.5)
Twin reversed arterial perfusion	39 (29.8)
Congenital malformation,	38 (29.0)
Selective intrauterine growth restriction	11 (8.4)
Other*	3 (2.3)
Technique	
Fetoscopic laser coagulation	69 (52.7)
Bipolar cord coagulation	36 (27.5)
Interstitial laser coagulation	15 (11.5)
Radiofrequency ablation	11 (8.4)
Cesarian delivery	24 (18.3)

* History of preterm delivery and uterus didelphus (n=1), history of preterm delivery and conization of the cervix (n=1), psycho-emotional reasons and amniotic band syndrome (n=1).

IUFD occurred in 32 cases, and in seven cases the pregnancy was terminated after selective feticide. In the 32 IUFD cases, fetal death occurred on average 1.67 (range, 0.0–9.0) weeks after the operation (median gestational age at therapy 16.0 weeks, median gestational age at delivery 17.5 weeks). Reasons for TOP were persistent severe fetal hydrops (*n*=1), chromosomal abnormality (*n*=2) and PPROM (*n*=4). Four liveborn neonates died during the neonatal period because of complications related to prematurity, Potter sequence or severe brain injury. Overall perinatal survival rate was 67.2% (88/131). Perinatal survival rate was higher in the group treated after 18 weeks' gestation than in the group treated before 18 weeks, at 80% (64/80) and 47.1% (24/51), respectively ($P < 0.001$).

Perinatal outcome by technique is given in Table 2. Feticide was performed successfully in all but one pregnancy. In this case, fetoscopic laser coagulation was discontinued because of complex entanglement of the umbilical cords in a monoamniotic twin, with too high a risk of damaging the cord of the healthy twin. This pregnancy was subsequently completely terminated because of mosaicism for trisomy 18.

The reasons for selective feticide in the TTTS group included technical issues (*n*=9), anterior placenta location (*n*=5), severe fetal malformation (*n*=11), severe sIUGR (*n*=6), severe cerebral injury (*n*=3), reversal of TTTS (*n*=2) and parental choice (*n*=4). Quintero stage at time of intervention was Stage 1 (*n*=5), Stage 2 (*n*=6), Stage 3 (*n*=21) and Stage 4 (*n*=8). Higher Quintero stage at intervention was not associated with increased risk of adverse perinatal outcome ($P = 0.29$).

PPROM occurred in 26 (19.8%) cases at a median gestational age of 25.5 weeks, of which 15 (57.7%) occurred before 28 weeks, three (11.5%) between 28 and 32 weeks and eight (30.8%) between 32 and 37 weeks. Four of these pregnancies were terminated because of anhydramnios, in two cases IUFD occurred and one child died in the neonatal period. Median gestational age at therapy was comparable between cases with and without PPROM, at 17.0 and 17.5 weeks, respectively ($P = 0.490$). Median gestational age at delivery in the subgroups with and without PPROM was 30.0 weeks and 35.0 weeks, respectively ($P = 0.047$).

Median gestational age at delivery in the 92 liveborn children was 37 (range, 33-39) weeks. Twenty-five neonates (27.2%) were born between 32 and 37 weeks' gestation, 15 (16.3%) between 28 and 32 weeks and five (5.4%) were born extremely prematurely (before 28 weeks). Detailed information on neonatal morbidity in one case was not available because the mother moved to another country shortly after the operation. She delivered in a foreign hospital at 26 weeks' gestation and the premature neonate died subsequently. Gender and birth weight are unknown.

Severe neonatal morbidity occurred in 15 neonates (16.3%). A total of 27 (29.3%) neonates required admission to the neonatal intensive care unit (NICU) and four of them died in the neonatal period (Figure 1). Detailed information on the cases with adverse perinatal outcome is given in Table 3.

Table 2. Perinatal outcome according to technique used for selective feticide in 131 monochorionic twin pregnancies.

	Fetoscopic laser coagulation (n=69)	Bipolar cord coagulation (n=36)	Interstitial laser coagulation (n=15)	Radiofrequency ablation (n=11)	All cases (n=131)
GA at therapy (weeks)	16.0 (15.0-19.0)	20.5 (18.0-22.0)	16.0 (15.0-18.0)	15.0 (14.0-18.0)	17.0 (15.0-21.0)
GA at delivery (weeks)*	33.0 (20.5-38.0)	35.5 (29.0-38.8)	26.0 (18.0-35.0)	34.0 (20.0-39.0)	34.0 (23.0-38.0)
PPROM	18 (26.1)	5 (13.9) (1 mist)	2 (13.3)	1 (9.1)	26 (19.8)
IUFD	15 (21.7)	5 (13.9)	8 (53.3)	4 (36.4)	32 (21.4)
TOP	7 (10.1)	0	0	0	7 (5.3)
NND	1 (1.4)	3 (8.3)	0	0	4 (3.1)
Survival per indication:					
TTS	15/22	12/16	1/1	0/1	28/40 (70.0%)
TRAP	16/23	-	4/11	4/5	24/39 (61.5%)
Congenital malformation	13/20	11/14	1/2	1/2	26/38 (68.4%)
sIUGR	2/3	4/5	1/1	1/2	8/11 (72.7%)
Other	0/1	1/1	-	1/1	2/3 (66.6%)
Survival rate	46 (66.7)	28 (77.8)	7 (46.7)	7 (63.6)	88/131 (67.2)
Birth weight (g)	2614 ± 1005	2839 ± 1582	2339 ± 1066	3101 ± 1162	2706 ± 1238
Adverse perinatal outcome	27 (39.1)	11 (30.6)	9 (60.0)	7 (63.6)	54/131 (41.2)

Data shown as median (interquartile range), *n* (%), *n*/*n* or mean }SD. GA, gestational age; IUFD, intrauterine fetal death; NND, neonatal death; PPROM, preterm premature rupture of membranes; sIUGR, selective intrauterine growth restriction; TOP, termination of pregnancy; TRAP, twin reversed arterial perfusion; TTTS, twin–twin transfusion syndrome.

Table 3 Cases of selective feticide complicated by neonatal morbidity and/or perinatal mortality in monochorionic twin pregnancies.

Case	Indication for selective feticide	GA at therapy (weeks)	Technique	PPROM (weeks)	GA at birth (weeks)	Birth weight (g)	Neonatal outcome
Neonatal death							
1	Congenital malformation	21	BCC	No	30	1560	NND within a few hours after birth; lung hypoplasia due to congenital renal failure (Potter sequence), neonatal sepsis
2	Congenital malformation	22	BCC	No	26	900	NND on day 10; asphyxia, RDS, IVH Grade IV, renal failure, heart failure
3	Congenital malformation	17	BCC	NA	26	NA	NND; neonatal morbidity unknown
4	TTTS	18	FLC	Yes (19)	30	800	NND after 7 weeks; RDS, PDA, vein of Galen malformation, heart failure, neonatal sepsis
Neonatal morbidity							
5	Congenital malformation	22	BCC	No	25	845	RDS, neonatal sepsis
6	TTTS	14	FLC	Yes (16)	30	2000	RDS, severe neuropathy of auditory nerve (CMV infection)
7	sIUGR	17	ILC	Yes (25)	26	1345	RDS
8	TRAP	18	FLC	Yes (29)	29	1675	RDS
9	TTTS	20	FLC	Yes (25)	28	955	RDS, neonatal sepsis
10	sIUGR	19	FLC	No	26	875	RDS
11	sIUGR	20	RFA	No	28	1150	Neonatal sepsis
12	Congenital malformation	23	BCC	No	29	1512	RDS
13	Other	12	RFA	Yes (34)	34	2165	IVH Grade IV
14	TTTS	21	BCC	No	29	1060	Neonatal sepsis
15	TTTS	21	FLC	Yes (22)	29	1486	RDS

BCC, bipolar cord coagulation; CMV, cytomegalovirus; FLC, fetoscopic laser coagulation; GA, gestational age; ILC, interstitial laser coagulation; IVH, intraventricular hemorrhage; NA, not available; NND, neonatal death; PDA, patent ductus arteriosus; PPROM, preterm premature rupture of membranes; RDS, respiratory distress syndrome; RFA, radiofrequency ablation; sIUGR, selective intrauterine growth restriction; TRAP, twin reversed arterial perfusion; TTTS, twin–twin transfusion syndrome.

Overall, the rate of adverse perinatal outcome (including perinatal mortality and severe neonatal morbidity) was 41.2% (54/131). The rates of PPROM in cases with and without adverse perinatal outcome were 24.1% (13/54) and 16.9% (13/77), respectively (P = 0.275). However, the median gestational age at occurrence of PPROM was significantly lower in cases with than in those without adverse perinatal outcome, at 19.0 weeks and 32.0 weeks, respectively (P = 0.017). In addition, liveborn children with adverse perinatal outcome were born at a lower median gestational age (29.0 weeks) than were children without adverse perinatal outcome (38.0 weeks; $P < 0.001$).

Detailed information on brain imaging was obtained in 28/92 neonates (30.4%). Severe cerebral injury was detected on ultrasound scan in three neonates, including IVH Grade IV (n=2) and vein of Galen malformation (n=1). The case of the child with vein of Galen malformation has been reported previously.[17] Two children with severe cerebral injury died in the neonatal period. In the majority of neonates (64/92), routine ultrasound scans were not performed. Most of these children were in good clinical condition and were discharged from the hospital shortly after birth.

Other neonatal complications in the group of liveborn infants were nine cases of RDS (9.8%), two of chronic lung disease (2.2%), six of patent ductus arteriosus (6.5%), four of neonatal sepsis (4.3%) and one of NEC (1.1%).

Discussion

In this study we evaluated the perinatal outcome after selective feticide in a large cohort (n=131) of MC twin pregnancies and report an overall perinatal survival rate of 67.2%. Survival rates of 65–92% have been reported in the literature.[5] Rossi and D'Addario[5] reported an overall survival rate of 79% in a systematic review. However, care should be taken when comparing results of different studies, as overall perinatal outcome depends on various factors including indication for, and technique and timing of, the selective feticide procedure. Analysis of our results after stratification by type of surgical technique shows that interstitial laser coagulation and RFA are associated with the lowest survival rates, at 46.7% and 63.6%, respectively. Rossi and D'Addario[5] in contrast found the highest survival in the RFA group (86%). Nevertheless, important methodological issues (primarily related to the small number of patients included) prevent accurate comparison between the various reports. Larger studies are urgently needed to reach reliable conclusions on outcome after RFA and interstitial laser coagulation.

Our study also shows that perinatal survival is higher (86.3%) when intervention is performed after 18 weeks' gestation than it is when performed before 18 weeks

(47.1%). Our findings are in accordance with those of Rossi and D'Addario.[5] who also found a higher survival rate (89%) when the procedure was performed after 18 weeks than when it was performed earlier (69%), irrespective of the indication for selective feticide. In support of the hypothesis that gestational age at therapy is associated with adverse perinatal outcome, Lanna et al.[18] found an incidence of miscarriage of 3% when bipolar cord coagulation was performed after 19 weeks' gestation, compared with 45% when performed before 19 weeks. Overall survival rate in this large study (n=118) was 71%, which is comparable with our results. The findings in our study and other reports suggest that intervention should be postponed until after 18weeks' gestation, when possible.[5;18]

The reported rate of neonatal morbidity and mortality (16.3%; 15/92) in our study was higher than the reported rate of 7.0% (19/273) in the systematic review of Rossi and D'Addario.[5] Discrepancies in the results may be due to methodological differences such as under-reporting of neonatal morbidity and/or the use of different definitions of morbidity in the papers included in the review. In addition, most studies included in the systematic review of Rossi and D'Addario had a relatively high rate of loss to- follow-up, whereas we were able to report the perinatal and neonatal outcomes in all cases.

Our study confirms PPROM as one of the major risk factors for adverse perinatal outcome after invasive fetal interventions. In our cohort, in all but one case, neonatal mortality and morbidity occurred in neonates delivered at a gestational age of ≤30 weeks. Our data are in agreement with a recent study by Bebbington et al.[19] of 146 cases treated with selective feticide. The authors report a similar increased rate of PPROM and premature delivery and a clear association with adverse outcome. However, neonatal morbidity was not evaluated or reported.

In two small studies with six and 13 survivors, no neonatal morbidity was detected,[6;20] whereas in another small study, by Tsao et al.[21], one out of 13 children died from complications of prematurity. Lewi et al.[13] found a survival rate of 83% in a group of 80 pregnancies treated with fetoscopic laser and/or bipolar cord coagulation. These authors only described neonatal morbidity (asphyxia and NEC) in the subgroup with neonatal death and cerebral injury only in children who had developmental delay in long-term follow up. Robyr et al.[22] reported 8.7% prematurity-related deaths (4/46) after bipolar cord coagulation, without further describing neonatal morbidity in survivors. Paramasivam et al.[23] reported brain abnormalities in two cases in a group of 32 survivors after treatment with RFA, however other neonatal problems were not discussed. Ilagan et al.[24] reported that 48% of neonates required admission to the NICU after bipolar cord coagulation because of various neonatal morbidities. Perinatal mortality was 3/27 (11.1%). Their conclusions were unfortunately marred owing to a large loss-to-follow-up rate, as only 55% of the patients gave consent for follow-up.

Care should be taken when interpreting our results because of the limitations associated with the retrospective nature of this study. In our cohort, different techniques were used depending on various clinical factors and the operator's preference, and the indications for selective feticide varied. The population was therefore inhomogeneous and difficult to compare with those of other studies. A risk-factor analysis comparing different techniques or indications was envisaged but considered inappropriate owing to small sample size per technique.

Although only a few cases with severe cerebral injury were found, it is possible that some were missed since cranial ultrasound scans were not routinely performed. More research is needed to determine the value of routine ultrasound scans in all liveborn survivors after selective feticide, or in fact after all fetal interventions. Also, because it is difficult to predict long-term outcome based on findings on cranial ultrasound scan, long-term follow-up should be performed to determine quality of life and neurodevelopmental outcome of survivors.

Few studies describe neurodevelopmental outcome in children treated with selective feticide. Moise et al.[6] reported that all of the six survivors in a study of RFA in nine patients were doing well based on telephone follow-up at a mean neonatal age of 4 months. Lewi et al.[13] described long-term follow up in 67 survivors, of whom five had some form of developmental delay. Robyr et al.[22] found one child with developmental delay at age 18 months, although without any abnormalities on brain imaging. Large long-term follow-up studies in survivors after selective feticide are urgently required in order to acquire the knowledge necessary to counsel parents reliably.

In conclusion, selective feticide is associated with a high risk of adverse perinatal outcome. Further research is warranted to find ways to minimize the risks of selective feticide, by optimizing indications, timing and methods used. Outcome studies should include long-term follow-up to assess neurodevelopmental outcome in survivors.

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Chapter 12

Long-term neurodevelopmental outcome after selective feticide in monochorionic pregnancies

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Abstract

Objective
To assess the incidence and risk factors for adverse long-term neurodevelopmental outcome in complicated monochorionic pregnancies treated with selective feticide at our center between 2000 and 2011.

Design
Observational cohort study.

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

Population
Neurodevelopmental outcome was assessed in 74 long-term survivors.

Methods
Children, at least two years of age, underwent an assessment of neurologic, motor and cognitive development using standardized psychometric tests and the parents completed a behavioral questionnaire.

Main outcome measures
A composite outcome termed neurodevelopmental impairment including cerebral palsy (GMFCS II-V), cognitive and/or motor test score of less than 70, bilateral blindness, or bilateral deafness requiring amplification.

Results
A total of 131 monochorionic pregnancies were treated with selective feticide at the Leiden University Medical Center. Overall survival rate was 88/131 (67%). Long-term outcome was assessed in 74/88 (84%). Neurodevelopmental impairment was detected in 5/74 (6.8%, 95%CI 1.1 to 12.5) of survivors. Overall adverse outcome, including perinatal mortality or neurodevelopmental impairment was 48/131 (36.6%). In multivariate analysis, parental educational level was associated with cognitive test scores (regression coefficient B 3.9, 95% confidence interval 1.8 to 6.0). Behavioral problems were reported in 10/69 (14.5%).

Conclusions
Adverse long-term outcome in survivor twins of complicated monochorionic pregnancies treated with selective feticide appears to be more prevalent than in the general population. Cognitive test scores were associated with parental educational level.

Introduction

Monochorionic (MC) pregnancies are associated with increased risks of complications and perinatal mortality. Due to the presence of placental vascular anastomoses, specific complications can arise including twin-twin transfusion syndrome (TTTS), twin anemia polycythemia sequence (TAPS), twin reversed arterial perfusion (TRAP), and selective intrauterine growth restriction (sIUGR). In addition, severe discordant congenital anomalies occur more frequently in MC pregnancies and are associated with increased risk of morbidity and mortality.[1]

In case of spontaneous intrauterine demise of one fetus, its co-twin may subsequently die (15%) or suffer from severe cerebral injury (34%) due to acute exsanguination into the circulation of the demised twin through the placental vascular anastomoses.[2;3] Selective feticide may be an option for the management of abovementioned severe complications, with the aim to optimize the chances for healthy survival of the co-twin.[4] Reported methods for selective feticide include fetoscopic laser coagulation, bipolar cord coagulation, radiofrequency ablation, cord occlusion by ligation or photocoagulation and interstitial laser coagulation.[5-10] Perinatal survival rates following selective feticide vary between 65% and 92%, depending on indication and technique.[6]

Reliable data on the long-term neurodevelopmental outcome in surviving twins is scarce. Developmental delay is reported in 0-12%.[7;10-13] However, in these studies standardized developmental tests and detailed specification of delays were often lacking. In addition, apart from evident impairments such as cerebral palsy or severe cognitive delay, more subtle impairments including behavioral problems require evaluation in order to obtain an integrated picture of the long-term outcome in surviving twins.

The primary objective of this study was to assess the incidence and risk factors for adverse long-term neurodevelopmental outcome and behavioral problems in a consecutive series of MC pregnancies treated with selective feticide in a large national cohort.

Methods

The Leiden University Medical Center (LUMC) is the national referral center for fetal therapy in the Netherlands. We previously reported on the perinatal outcome of 131 MC twin pregnancies treated with selective feticide at our center between June 2000 and November 2011.[14] All surviving co-twins were invited to participate in this long-term follow-up study. The institutional review board of the LUMC approved the study and all parents gave written informed consent for their children.

The following obstetrical data were recorded: indication and technique for selective feticide, gestational age at intervention, fetal demise, termination of pregnancy (TOP) and preterm prelabor rupture of membranes. Following the procedure, patients were discharged after 24 hours with detailed ultrasound of the survivor twin. At 4 to 6 weeks after the procedure, either at our center or at the referring hospital, an ultrasound was performed and in case of suspected cerebral injury, a fetal MRI was performed. Expectant management with routine obstetric care were part of prenatal follow-up until delivery. The following neonatal data were recorded: gender, gestational age at birth in completed weeks, birth weight, neonatal death and severe neonatal morbidity (defined as any of the following: respiratory distress syndrome, necrotizing enterocolitis ≥ stage II,[15] neonatal sepsis, renal failure or severe cerebral injury). Severe cerebral injury was defined as the presence of at least one of the following findings on cranial imaging: intraventricular hemorrhage ≥ grade III,[16] periventricular leukomalacia ≥ grade II,[17] (progressive and non-progressive) ventricular dilatation ≥ 97th percentile,[18] arterial or venous infarct or other cerebral anomalies associated with adverse neurological outcome. Socio-economic status (SES) of the parents was registered as high, average or low according to the Dutch Sociaal en Cultureel Planbureau.[19] Parental education was determined by the level of education of each parent individually. A score of one was given when the parent's education was low (primary school), a score of two for an intermediate educational level (secondary school and intermediate vocational school), and a score of three for higher levels of education (higher vocational school and university). Scores of both parents were then added (range: 2-6).

A follow-up visit was performed at a minimum age of 24 months corrected for prematurity and included a neurologic examination and an assessment of cognitive and motor development using the Bayley Scales of Infant and Toddler Development third edition (Bayley-III) in children at 2 years of age.[20] Bayley-III scores provide cognitive and motor composite scores. Cognitive development of children between 3 and 7 years of age was tested with the Wechsler Preschool Primary Scale of Intelligence third edition (WPPSI-III).[21;22] Children at the age ≥ 7 years were tested with the Wechsler Intelligence Scale for Children third edition (WISC-III).[23] Both the WPPSI and WISC provide a Total IQ (TIQ) score including a Verbal IQ (VIQ) and a Performance IQ (PIQ). Bayley-III, WPPSI and WISC scores follow a normal distribution curve with a mean of 100 and a standard deviation (SD) of 15. A cognitive test score that is, a Bayley-III cognitive composite score, WPPSI TIQ- or WISC TIQ score, below 70 (< -2 SD) indicates severe cognitive delay and scores below 85 (< -1 SD) indicate mild-to-moderate cognitive delay . Cerebral palsy was defined according to the European CP Network and classified as spastic bilateral, spastic unilateral, dyskinetic (dystonic or choreo-athetotic), ataxic , or mixed.[24] Severity was classified according to the Gross Motor Function Classification System (GMFCS)

for Cerebral Palsy.[25] Behavioral problems were assessed using the Achenbach's Child Behavior Checklist (CBCL) versions 1½-5 and 6-18 years.[26;27] These questionnaires measure the occurrence of problem-behavior as reported by parents. For the purpose of this study the Total problem scale, and the two broadband syndrome scales Internalizing (withdrawn, somatic complaints, anxious/depressed) and Externalizing (delinquent or rule-breaking, aggressive) behavior problems were used. T scores were computed from raw scores with higher scores on the syndrome scales indicating greater severity of problems. T scores of the normative sample have a mean of 50 ± SD 10. A clinical score in 10% of the children for the Total, Internalizing and Externalizing behavior problem scales (T score ≥ 64) served as cut-off points for comparison with Dutch normative data.[27]

The primary outcome measure was a composite outcome termed neurodevelopmental impairment (NDI) including cerebral palsy (GMFCS II-V), cognitive and/or motor test score of less than 70, bilateral blindness, or bilateral deafness requiring amplification. In addition, an 'overall adverse outcome' was calculated including perinatal mortality or neurodevelopmental impairment. Secondary outcome was an estimation of risk factors associated with cognitive test scores including indication and technique for selective feticide, gestational age at intervention, preterm prelabor rupture of membranes, gestational age at birth, birth weight, severe neonatal morbidity, age of the children and parental educational level. Third, we assessed behavioral problem scores.

Statistical analysis

All statistical analyses were conducted using SPSS version 20.0 (IBM, Armonk, NY, USA). Data are presented as number and percentage (%), medians with range or as means with SD, as appropriate. Statistical analysis was performed using *t*-test and Mann-Whitney test for continuous variables. Chi-square test and Fisher's exact test were used for categorical variables, as appropriate. The percentages of children with a clinical CBCL score (T score ≥ 64) were compared with normative percentages (10% with clinical score) using a binomial test. Analysis for risk factors contributing to cognitive test scores was conducted using univariate and multivariate regression methods. The potential risk factors for cognitive outcome were studied in a univariate linear regression model. The multivariate linear regression model included all variables that showed significant association in the univariate analysis. Results are expressed as regression coefficients (B) with 95% confidence intervals (95%CI). The level of statistical significance for all analyses was set at $P < 0.05$.

Results

A total of 131 MC twin pregnancies were treated with selective feticide during the study period. Figure 1 represents the derivation of the study population. No differences in perinatal and neonatal characteristics and SES were found between the included and lost-to-follow-up group. Baseline characteristics of the children included for follow-up are presented in Table 1.

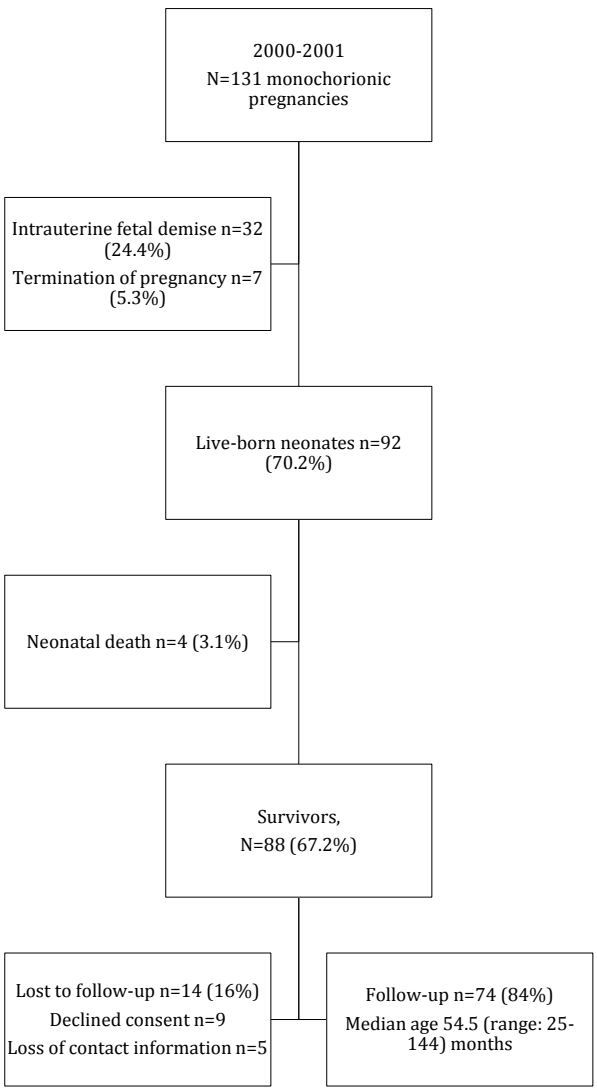

Figure 1 Derivation of the study population

Table 1 Baseline characteristics of 74 monochorionic co-twins included for follow-up

Characteristics	Children *n* = 74
Indication for selective feticide	
TTTS, n (%)	25 (34)
Congenital anomaly, n (%)	21 (28)
TRAP, n (%)	19 (26)
sIUGR, n (%)	8 (11)
Other*, n (%)	1 (1)
Selective feticide technique used	
Fetoscopic laser cord coagulation, n (%)	39 (52.7)
Bipolar cord coagulation, n (%)	25 (33.7)
Interstitial laser coagulation, n (%)	5 (6.8)
Radiofrequency ablation, n (%)	5 (6.8)
Gestational age at intervention, weeks	18 (13-30)
PPROM, n (%)	15 (20)
Gestational age at birth, weeks	37 (25-41)
Birth weight, grams	2675 (800-4740)
Female, n (%)	38 (51)
Neonatal morbidity**, n (%)	8 (11)
Maternal education (*n* = 70)	
Low, n (%)	15 (21)
Intermediate, n (%)	23 (33)
High	32 (46)
Paternal education (*n* = 67)	
Low, n (%)	16 (24)
Intermediate, n (%)	17 (25)
High	34 (51)
Socio-economic status	
Low, n (%)	20 (27)
Intermediate, n (%)	32 (43)
High, n (%)	22 (30)

Data are presented as median (range).
*High risk pregnancy due to history of preterm delivery and conisation of the cervix.
**Neonatal morbidity is defined as any of the following: respiratory stress syndrome, necrotizing enterocolitis, neonatal sepsis, renal failure or severe cerebral injury.
TTTS, Twin-Twin Transfusion Syndrome; TRAP, Twin Reversed Arterial Perfusion; SIUGR, Selective Intrauterine Growth Restriction; PPROM, Preterm Prelabor Rupture of Membranes.

The incidence of neurodevelopmental impairment in survivor twins of monochorionic pregnancies treated with selective feticide was 5/74 (6.8%, 95%CI 1.1 to 12.5) Overall adverse outcome, including perinatal mortality or long-term neurodevelopmental impairment, was 48/131 (36.6%). All 74 long-term survivors underwent physical examination, five of them did not participate in psychometric testing since parents declined consent. Median cognitive score of the children was 100 (range: 95-125), 105 (range: 62-129) and 104 (range: 62-131) according to the Bayley-III (*n* = 8), WPPSI-

III (n = 43) and WISC-III (n = 17), respectively. Overall, median cognitive score was 104 (range: 62-131). The long-term outcome of the 74 children included for follow-up is reported in Table 2. Details on prenatal and neonatal characteristics and the combination of impairments in the five children with neurodevelopmental impairment are presented in Table 3. In one child with deafness and severe cognitive delay, congenital cytomegalovirus (CMV) infection was diagnosed.

Table 2 Long-term neurodevelopmental outcome in the 74 children included for follow-up

Characteristics	**Children** ***n = 74***
Age at follow-up, months	54.5 (25-144)
Neurodevelopmental impairment*, n (%)	5 (6.8)
Cerebral Palsy, n (%)	2 (2.7)
Cognitive development <-2 SD, n (%)	4 (5.4)
Motor development <-2 SD, n (%)	1 (1.4)
Bilateral deafness, n (%)	1 (1.4)
Cognitive score	104 (62-131)
Bayley-III (n = 8)	
Cognitive development score	100 (95-125)
Motor development score	103 (59-121)
WPPSI-III (n = 43)	
Total IQ	105 (62-129)
Verbal IQ	104 (63-129)
Performance IQ	107 (72-126)
WISC-III (n = 17)	
Total IQ	104 (62-131)
Verbal IQ	103 (71-135)
Performance IQ	102 (59-139)
CBCL (n = 69)	
Total behavioral problems	10 (14.5)
Internalizing problems	10 (14.5)
Externalizing problems	3 (4.3)

Data are presented as median (range)
*Neurodevelopmental impairment is defined as any of the following: Cerebral Palsy (GMFCS II-V), cognitive development <2 SD, motor development <2 SD, bilateral blindness or deafness.
Bayley-III, Bayley Scales of Infant and Toddler Development third edition; WPPSI-III, Wechsler Preschool Primary Scale of Intelligence third edition; WISC-III, Wechsler Intelligence Scale for Children third edition; IQ, intelligence quotient; CBCL, Child Behavior Checklist.

Table 3 Prenatal and neonatal characteristics of the five children diagnosed with long-term neurodevelopmental impairment

Case	Gender	Indication	Gestational age at intervention	Technique	Gestational age at birth	Neonatal morbidity	Long-term outcome
1	Female	Recurrent TTTS, post-laser TAPS	19 weeks	Fetoscopic laser coagulation new donor	28 weeks	RDS, sepsis	Motor development < 70 Behavior problems
2	Female	TTTS	20 weeks	Fetoscopic laser coagulation recipient	34 weeks	No (no cerebral imaging performed)	Cognitive development < 70 Behavior problems
4	Female	Severe congenital anomaly, holoprosencephaly	23 weeks	Bipolar cord coagulation	25 weeks	RDS, CLD, PDA, sepsis	Cerebral palsy (spastic bilateral) GMFCS V Cognitive development < 70 Behavior problems
3	Male	TRAP	17 weeks	Interstitial laser coagulation	41 weeks	No (no cerebral imaging performed)	Cerebral palsy (spastic bilateral) GMFCS II Cognitive development < 70
5	Male	Severe congenital anomaly, hydrothorax	14 weeks	Fetoscopic laser coagulation	30 weeks	RDS, CLD, CMV infection	Cognitive development < 70 Deafness Behavior problems

TTTS, Twin-Twin Transfusion Syndrome; TAPS, Twin Anemia Polycythemia Sequence; RDS, Respiratory Distress Syndrome; CLD, Chronic Lung Disease; PDA, Patent Ductus Arteriosus; GMFCS, Gross Motor Functioning Classification System for Cerebral Palsy; TRAP, Twin Reversed Arterial Perfusion; CMV, Congenital Cytomegalovirus.

Univariate analysis of potential risk factors associated with cognitive test scores was performed (Table S1). Gestational age at birth (B 1.0, 95%CI 0.2 to 1.9), birth weight (B 0.4, 95%CI 0.1 to 0.8), neonatal morbidity (B -15.8, 95%CI -27.2 to -4.4) and parental education (B 4.4, 95%CI 2.2 to 6.5) were significantly associated with cognitive test scores. Since gestational age at birth was strongly correlated with birth weight (Pearson correlation 0.9; $P < .01$) multivariate analysis was performed including gestational age at birth, neonatal morbidity and parental education (Table S1). We found that only parental education was still significantly associated with cognitive test scores (B 3.9, 95%CI 1.8 to 6.0).

Complete behavioral questionnaires were obtained from the parents of 69/74 (93%) children. According to the CBCL 1½-5, mean total problem score was 44.2 ± 11.0. Mean internalizing and externalizing problem scores were 44.5 ± 11.0 and 45.1 ± 11.4, respectively. According to the CBCL 6-18, mean scores were 51.1 ± 13.0, 52.6 ± 12.8 and 48.2 ± 10.4, respectively. T scores of the normative sample have a mean of 50 ± 10. Overall, behavioral problems within the clinical range were reported in 10/69 (14.5%) cases, with internalizing problems in 10/69 (14.5%) and externalizing problems in 3/69 (4.3%) cases (Table 2). Compared with normative percentages (10% with clinical score), significantly more total and internalizing problems ($P < .01$) and less externalizing problems were reported ($P < .01$).

Table S1 Analysis of potential risk factors associated with cognitive test scores (Bayley-III cognitive composite score, WPPSI- or WISC TIQ score)

Characteristics	Univariate analysis B (95% CI)	SE	*P*	Multivariate analysis* B (95% CI)	SE	*P*
Indication						
TTTS	-2.2 (-9.9 - 5.5)	3.9	.57			
Congenital anomaly	1.2 (-7.0 - 9.3)	4.2	.78			
TRAP	-0.1 (-8.4 - 8.2)	4.2	.98			
sIUGR	1.3 (-10.1 - 12.6)	5.8	.83			
Technique						
Fetoscopic laser cord coagulation	-1.9 (-9.2 - 5.3)	3.7	.60			
Bipolar cord coagulation	2.9 (-5.0 - 10.7)	4.0	.48			
Interstitial laser coagulation	-11.7 (-25.4 - 2.1)	7.0	.10			
Radiofrequency ablation	10.2 (-5.1 - 25.6)	7.8	.19			
Gestational age at intervention, weeks	-0.3 (-1.4 - 0.7)	0.5	.52			
Preterm prelabour rupture of membranes	-3.5 (-12.3 - 5.2)	4.5	.43			
Gestational age at birth, weeks	1.0 (0.2 - 1.9)	0.4	**.02**	0.3 (-0.7 - 1.3)	0.5	.55
Birth weight, grams	0.4 (0.1 - 0.8)	0.2	**.02**			
Neonatal morbidity	-15.8 (-27.2 - -4.4)	5.8	**.01**	-9.4 (-23.2 - -4.5)	7.1	.18
Age of the children at follow-up	-0.1 (-0.1 - 0.1)	0.1	.85			
Parental education	4.4 (2.2 - 6.5)	1.1	**<.01**	3.9 (1.8 – 6.0)	1.1	**<.01**

Values are regression coefficient B (95%CI), standard error (SE) and *P*.
*multivariate analysis was performed including gestational age at birth, neonatal morbidity and parental education, since gestational age at birth was strongly correlated with birth weight (Pearson correlation 0.9; $P < .01$).
TTTS, Twin-Twin Transfusion Syndrome; TRAP, Twin Reversed Arterial Perfusion; SIUGR, Selective Intrauterine Growth Restriction.

Discussion

Main findings

In the present study, we evaluated the long-term neurodevelopmental outcome in survivors following selective feticide in MC pregnancies. Neurodevelopmental impairment was detected in 6.8% (95%CI 1.1 to 12.5). Overall adverse outcome, including perinatal mortality or long-term impairment was 48/131 (36.6%). One of the children included for long-term follow-up was diagnosed with congenital CMV infection. At five years of age this child had bilateral deafness, severe cognitive delay and behavioral problems. Neurodevelopmental impairment in this case was therefore likely due to the CMV infection. Excluding this case, the rate of impairment was 5.5%. Importantly, one pregnancy was terminated following selective feticide due to severe cerebral injury in the fetus. In addition, intensive care treatment was withdrawn from two neonates due to severe cerebral injury. Had these three children survived, the rate of impairment could have been higher (8/77; 10.4%).

Strengths and limitations

One of the limitations of this study is the lack of a control group. Another important limitation of long-term follow-up studies including ours is the inability to obtain 100% inclusion. We were not able to track 6% of families. Another 10% percent of parents declined consent largely due to emotional reasons. It is possible that parents who have not come to terms with their highly stressful medical history may tend to avoid situations where these difficulties are highlighted.[28] Although no differences in perinatal characteristics and socio-economic status were found between the included and lost-to-follow-up group, losses in follow-up studies are mostly unrelated to prenatal, perinatal, or neonatal medical conditions.[29] However, parents with low educational levels and those with children with severe developmental delay are most likely to drop out of follow-up programs.[29;30] Parental educational level could not be assessed in the lost-to follow-up group. In addition, a high percentage of parents were highly educated in our study. Therefore, the rate of long-term impairment could have been underestimated compared to more general populations. While it is not possible to compel families to participate in follow-up studies, it is possible to hypothesize the impact that might be seen if the outcome of interest occurred in none or all of those who were unwilling to participate (n=9). In our study, the range of impairment in those who survived would be 6.0% (5/83) up to 16.9% (14/83). Including the 3 cases with cerebral injury that did not survive, the range would be 9.3% (8/86) up to 19.8% (17/86).

Care should be taken when interpreting our results due to these limitations. In our cohort, different techniques were used depending on various clinical factors and the

indications for selective feticide varied. The population was therefore inhomogeneous and difficult to compare to other studies.[14] Also, different tests assessing cognitive and motor development were used due to a relative large age range (2-12 years). Ideally, standardized follow-up after fetal therapy should be performed in all children at 2 years, 5 and 8 years to obtain a reliable view of long-term child development. In addition we suggest that routine cerebral imaging should be performed in all survivors after selective feticide to rule out severe cerebral injury and determine the etiology and timing of injury. Unfortunately, in our cohort, routine cerebral imaging was performed only a minority of cases.

Interpretation

The rate of cerebral palsy (2/74; 2.7%) in our study group was higher compared to the general population (0.2%).[24,;31] However, twins in general are estimated to have a 5- to 10-fold increased risk of cerebral palsy because of the higher prevalence of preterm birth and low birth weight.[32] In MC twin pregnancies, the risk of cerebral palsy may be further increased by potential antenatal cerebral injury as a result of transfusion imbalances across placental vascular anastomoses.[32] Although mean cognitive scores were within the normal range, the incidence of severe cognitive delay was 5%. According to the normal distribution of intelligence, severe cognitive delay occurs at a 2.3% rate in the general population. Compared with normative percentages (10%), significantly more behavioral problems (14.5%) were reported.[27;33] It is possible that, besides the obvious impairments such as cerebral palsy, subtle difficulties including behavioral problems are even more frequent. A large proportion of survivor twins (33/74; 45%) was born below 37 weeks. Children born late preterm are reported to be at increased risk of behavioral problems.[34] In addition to fetal- disease and therapy, premature birth and related complications could increase the risk of adverse long-term outcome. At present, there are no published studies on behavioral functioning in survivor twins after selective feticide of the MC co-twin.

Our rate of long-term impairment is in line with the rate reported in literature ranging from 0-12%.[7;10-13] In a series of 36 children, Robyr et al reported one case with 'some form of developmental delay' at 18 months.[11] Follow-up was obtained from a clinical evaluation by the referring pediatrician, a specification of delay was not described and developmental tests were lacking. We employed standardized tests in all children performed by certified child psychologists. Lewi et al reported some form of developmental delay in 5/67 (7.5%) survivors after selective feticide of the co-twin.[12] Four children were diagnosed with minor cognitive or motor delay with Bayley scales (2nd edition) and one child had severe cognitive delay according to a pediatrician. In a series of 6 cases, Moise et al reported all children were doing well at a mean age of

four months.[7] Follow-up was based on a telephone interview with parents. In a large single-center study from Lanna et al, severe neurologic morbidity was detected in 2/84 (2.4%) children with follow-up ranging from 1 to 9 years.[10] Delebaere et al reported developmental delay in 3/26 (12%) children at a mean age of five years according to their medical records or phone calls with the parents.[13] A specification of delay as well as standardized tests were lacking.

In univariate analysis we found that gestational age at birth, birth weight, neonatal morbidity and parental educational level were risk factors for long-term cognitive outcome. In multivariate analysis we found that only parental educational was associated with cognitive test scores. Low gestational age at birth, low birth weight, and often related severe neonatal morbidity, are known risk factors for long-term impairment. In addition, parental educational level is a well-documented and strong determinant of child cognitive development.[35] Due to the small number of cases, we could not determine whether the clinical situation leading to the decision to perform feticide as well as the type of fetal intervention may have had an impact on the long-term outcome. Furthermore, it is not clear whether fetoscopic coagulation of placental vascular anastomoses in addition to cord coagulation may improve outcome by further reducing the risk of intertwin blood flow. At our center we do not perform this procedure after cord coagulation. We assume that cord coagulation guarantees complete cessation of blood flow, also through still patent placental vascular anastomoses.

Conclusion

The present study represents the first study evaluating cognitive, motor and behavioral development using standardized tests in the largest consecutive cohort so far. We found that long-term neurodevelopmental impairment in survivors of MC pregnancies treated with selective feticide appears slightly more prevalent compared to general populations. Long-term cognitive test scores were associated with the level of education of the parents. Large multi-center long-term follow-up studies in survivors after selective feticide are required to counsel future parents reliably. We recommend standardized pre- and postnatal cerebral imaging in all survivor twins after selective feticide along with standardized neurodevelopmental testing to obtain a reliable view of long-term child development.

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PART V

GENERAL DISCUSSION
FUTURE PERSPECTIVES
SUMMARY/SAMENVATTING

General Discussion

Intrauterine transfusion in fetal anemia

Alloimmune hemolytic disease

Despite the use of intravascular IUTs for fetal anemia for over three decades, knowledge on the long-term neurodevelopmental outcome of children treated with IUT, especially in certain subgroups, is limited. The long-term outcome in children treated with IUT for alloimmune anemia is now considered favorable with long-term impairment ranging from 4.8% in recent studies to 12% in older ones.[1] In a large follow-up study performed at our center, including 291 children, we found a 4.8% of long-term neurodevelopmental impairment (NDI) after IUT for alloimmune hemolytic disease (Chapter 2). Significant risk factors for long-term impairment were severe fetal hydrops, the number of IUTs performed, severe neonatal morbidity including severe cerebral injury as well as the educational level of the parents. Nevertheless, even in children without obvious impairments such as cerebral palsy, subtle problems may occur including health-related quality of life (HRQOL) issues or increased behavioral difficulties.[2] In our study on HRQOL and behavioral functioning after IUT for alloimmune anemia we found that, according to parents, the children had more difficulties with cognitive functioning compared to healthy Dutch references (Chapter 2). In addition, behavioral problems were more prevalent and were associated with the level of education of the mother. These results suggest that, besides assessment of neurological disorders, other important and perhaps more subtle aspects of children's well-being require detailed evaluation.

In our follow-up study of a randomized controlled trial (RCT)[3], we found that the neurodevelopmental outcome in children treated postnatally with intravenous immunoglobulins (IVIg) for hemolytic disease of the fetus/newborn was not different from children treated with placebo (Chapter 3). After stratification for treatment with or without IUT, similar results were obtained. According to our RCT analyses, prophylactic IVIg does not reduce the need for exchange transfusion nor the rate of other adverse neonatal outcomes. In addition, IVIg does not appear to have a beneficial effect on the long-term outcome. Standardized follow-up studies with large enough cases series and sufficient power are needed to replicate these findings.

Other indications for intrauterine transfusions

The long-term outcome of survivors of IUT for other indications such as congenital Parvovirus B19 infection and fetomaternal hemorrhage (FMT) is not well known. Up till now, only three relative small studies on the long-term outcome of children treated

with IUT for Parvovirus B19 infection have been performed with severe long-term impairment in up to 12.5% of children.[4-6] The underlying cause for a potential increased rate of adverse neurodevelopmental outcome in parvovirus B19 infection is not yet fully understood.[7] Adverse outcome may be directly related to cerebral injury caused by the viral infection itself or the compromised condition of the fetus with severe anemia and hydrops. Our knowledge on the long-term outcome after FMH is confined to case reports and small series of cases without intrauterine treatment.[7] Since the occurrence of Parvovirus B19- and FMH induced fetal anemia is rare, international multicenter studies are required to determine long-term outcome in these subgroups.

Fetoscopic laser surgery in monochorionic twin pregnancies

Twin-twin transfusion syndrome

The preferred treatment for TTTS is fetoscopic laser coagulation of the placental vascular anastomoses. Before the introduction of this technique, TTTS was managed with serial amnioreduction of excessive amniotic fluid in the amniotic sac of the recipient twin. It is now general knowledge that twins treated with amnioreduction are less likely to survive compared to twins treated with laser surgery.[8;9] In our meta-analysis we found a 7-fold higher risk of severe cerebral injury in twins treated with amnioreduction compared to twins treated with laser surgery (Chapter 5). Since amnioreduction is only a symptomatic intervention, twins remain exposed to TTTS for a longer period of time compared to twins treated with laser surgery. In addition, twins treated with amnioreduction are born on average at 29 weeks' gestation. In comparison, twins treated with laser surgery are born on average almost one month later at 32-33 weeks. Prematurity is a well-known risk factor for neonatal morbidity and adverse long-term neurodevelopmental outcome.[10]

Since the introduction of fetoscopic laser surgery in the Netherlands, overall survival rate has improved from 70% to 80% with a significant reduction in (double) fetal demise (Chapter 6). This significant increase in survival is associated with a concomitant decrease in the incidence of long-term neurodevelopmental impairment (NDI) from 18% in the first years of the laser surgery program to 6% in more recent years (> 2008) (Chapter 6). In 2008 the Solomon trial was initiated, introducing a modified laser technique, the Solomon technique, in the treatment of TTTS.[11] The Solomon technique, in which the whole vascular equator is coagulated, is associated with a significant reduction in short-term complications that is, TAPS and recurrent TTTS, when compared to the standard laser surgery technique. We compared the neurodevelopmental outcome in surviving children included in the trial and treated with either the Solomon technique or standard laser surgery technique (Chapter 7).

We found no difference in our primary outcome that is, survival without NDI, between the two groups. Overall, NDI was detected in 11% of survivors in the Solomon and 9% in the standard group. The lack of difference between the two treatment groups could be that timely detection and adequate management and treatment (IUT, laser surgery re-intervention) in pregnancies with short-term complications in the standard group reduced the risk for long-term impairment. In addition, the Solomon trial was designed and powered to detect a difference in short-term perinatal outcome instead of long-term outcome. In view of the reduction of short-term complications and the absence of increased adverse long-term effects, we recommend the use of the Solomon technique in the treatment of TTTS.

Our follow-up studies in TTTS survivors identified several risk factors for adverse long-term outcome including advanced gestational age at laser surgery (and related Quintero stage), low gestational age at birth (and related low birth weight), severe neonatal morbidity including severe cerebral injury as well as the educational level of the parent(s).

Although TTTS remains one of the most lethal conditions in perinatal medicine, the outcome in terms of its most important parameter that is, survival without NDI, has significantly improved in the last decade.

Twin anemia-polycythemia sequence

Twin anemia-polycythemia sequence (TAPS) may occur spontaneously in up to 5% of MC pregnancies or after TTTS treated with laser surgery (post-laser TAPS) in up to 16%.[12] Data on the neurodevelopmental outcome in TTTS survivors who developed TAPS after laser surgery is confined to one recent study in 47 long-term survivors (Chapter 8). NDI was detected in 9%, with no difference between TAPS-donors and -recipients. Mild-to-moderate cognitive delay was detected in 17%. Low gestational age at birth and related low birth weight were significant risk factors for cognitive delay. These results are within the range of the incidence of NDI in TTTS case series treated with laser (range: 6% to 18%). However, larger case-controlled studies are needed to determine if post-laser TAPS leads to an increased risk of impairment compared with uncomplicated TTTS cases. Knowledge on neonatal morbidity in spontaneous TAPS is scarce and long-term outcome studies in survivors have not been published yet. A TAPS registry website has been set up, an international collaboration of maternal and fetal medicine centers to gain knowledge on TAPS in order to improve treatment for this relatively new MC twin pregnancy condition (https://www.tapsregistry.org/).

Specific complications in monochorionic pregnancies

Selective intrauterine growth restriction in monochorionic twin pregnancies

Selective intrauterine growth restriction (sIUGR) or selective fetal growth restriction may occur in up to 25% of MC twin pregnancies and is related to unequal placental sharing in which the growth restricted fetus has a small placental share and a velamentous cord insertion.[15;16] Our systematic review shows that the incidence of severe cerebral injury in MC twins with sIUGR varies greatly from 0% to 33%, with an estimated average of 8% (Chapter 10). The highest incidence of cerebral injury was reported in studies including pregnancies with single fetal demise, in pregnancies with abnormal umbilical artery Doppler findings and in cohorts with lower gestational age at birth. Results should however be interpreted with care due to the large heterogeneity between studies as well as their retrospective nature and the inclusion of relative small cohorts. Studies applied different inclusion criteria, various definitions of sIUGR and different outcome measures. The optimal management in MC twins with sIUGR is not clear and international consensus on the best treatment strategy is lacking. Whether fetal surgery (fetoscopic laser coagulation of vascular anastomoses or selective feticide) or obstetrical interventions (elective preterm birth) may improve the (long-term) outcome remains to be determined. How to balance the benefit from prolonging pregnancy in preventing prematurity-related cerebral injury against the harm or risking single fetal demise and concomitant damage to the co-twin is a clinical challenge and warrants further study (Chapter 10). On the other hand, the invasiveness of fetal therapy is associated with complications such as premature rupture of membranes (PPROM) and preterm delivery. Outcome studies assessing the natural history of MC pregnancies with sIUGR are therefore urgently required.

Single fetal demise in monochorionic pregnancies

In case of single fetal demise in a MC pregnancy, severe complications may arise resulting from acute exsanguination from the surviving co-twin into the low-pressure circulation of the demised co-twin. Acute hypovolemia, hypotension and anemia may result in hypoxic-ischemic multi-organ damage, particularly to the developing brain, and even double fetal demise.[13] Abnormal postnatal cranial imaging and NDI after single fetal demise are reported in 34% and 26% respectively.[14] In our cohort of 49 MC pregnancies, severe cerebral injury was detected in 26% and was mostly due to hypoxic-ischemic injury resulting in cystic periventricular leukomalacia, middle cerebral artery infarction or injury tot basal ganglia, thalamus and/or cortex (Chapter 8). Risk factors associated with cerebral injury included advanced gestational age at fetal demise, TTTS developed prior to single fetal demise and gestational age at birth.

Nonetheless, abnormal postnatal cranial imaging does not necessarily imply long-term NDI. Outcome can vary from healthy development to mild impairments in multiple domains to severe cognitive delay or cerebral palsy. Stringent antenatal and postnatal neuroimaging protocols to accurately evaluate the incidence of antenatal cerebral injury and to investigate the correlation between antenatal and postnatal imaging findings are strongly recommended. The clinical relevance of these findings should subsequently be determined using long-term neurodevelopmental outcome data of all MC pregnancies with single fetal demise.

Selective feticide in complicated monochorionic pregnancies

In specific complicated MC pregnancies, selective feticide can be offered as an alternative management option with the aim to optimize the chances of healthy survival of the co-twin. Indications include, among others, twin reversed arterial perfusion (TRAP) sequence, sIUGR, monoamniotic twin pregnancies or severe discordant congenital anomalies. We evaluated the perinatal and long-term neurodevelopmental outcome in a large consecutive cohort of 131 MC pregnancies treated between 2000 and 2011 at our center (Chapters 11 and 12). We found an overall perinatal survival rate of 67%, with higher survival (86%) when intervention was performed after 18 weeks' gestation compared to interventions before 18 weeks (47%) (Chapter 11). Adverse perinatal outcome, including mortality or severe neonatal morbidity, was reported in 41% and was associated with low gestational age at PPROM and low gestational age at birth. Long-term NDI was detected in 7% of survivors (Chapter 12). Our rate of long-term impairment is in line with the reported rate in literature ranging from 0% to 12%.[17-21] However, in these studies standardized developmental tests, a clear definition and/or a detailed specification of developmental delay were lacking. In our study, overall adverse outcome, including perinatal mortality and long-term impairment, was 37%. Compared with normative percentages (10%), significant more behavioral problems were reported by parents. Gestational age at birth, birth weight, neonatal morbidity including severe cerebral injury and parental educational level were significantly associated with cognitive test scores. Due to the relative small number of cases, we could not determine whether the clinical situation leading to the decision to perform feticide as well as the type of fetal intervention may have had an impact on the long-term outcome. Again, multi-center efforts are of paramount importance to entangle those factors leading to adverse perinatal and long-term outcome.

Future research perspectives

Although an increasing number of children are being born alive after fetal therapy, knowledge on long-term child development is still limited, especially on potential risk factors for adverse outcome. The majority of follow-up studies available are observational, cross-sectional in design and do not allow for examination of child development over time. The studies are generally limited by small sample size and power and do not include matched controls. In addition, standardized neurodevelopmental testing and clearly specified criteria for impairment are lacking.

The importance of long-term follow-up studies lies in both the necessity of evaluating fetal therapy as well as in evidence-based counselling of future parents. In addition, when a center decides to treat fetuses in utero, with the knowledge that a proportion will develop long-term morbidity, this center also has the responsibility to ensure that survivors will eventually receive the care they need. Long-term follow-up should be an integrated component of fetal therapy. Unfortunately, long-term neurodevelopmental studies are costly and difficult to perform and, consequently, hard to realize. Challenges include, among others, tracking families, motivate families to participate (in order to maintain a lost-to follow-up rate <10-20%), organizing follow-up assessments with trained pediatricians and child psychologists using standardized measures of well documented psychometric quality, as well as complete data acquisition and analysis. In countries where parents have to travel long distances to the follow-up clinic, assessments using (web-based) questionnaires such as the Ages and Stages Questionnaire (ASQ) could be more feasible. Structured long-term follow-up programs of children treated with fetal therapy require a dedicated follow-up team including fetal medicine specialists, neonatologists, child psychologists and research nurses.

This chapter focuses on future perspectives and proposals for future research on the long-term neurodevelopmental outcome in all children treated with fetal therapy.

Intrauterine transfusion in fetal anemia

Alloimmune hemolytic disease

The majority of children treated with IUT for alloimmune hemolytic disease have a normal neurodevelopmental outcome, but some children are at increased risk for adverse long-term outcome. Important risk factors include fetal hydrops, severe neonatal morbidity and low parental education. We suggest routine standardized evaluations at age 2 using questionnaires such as the ASQ. Additionally, questionnaires on school performance, HRQOL and behavioral functioning at age 5 and 8 should be integrated in the follow-up program of IUT to obtain a complete picture of the long-term

outcome since these more subtle impairments may already have a significant impact on care requirements.

Other indications for intrauterine transfusions

Since the occurrence of Parvovirus B19- and FMH induced fetal anemia is rare, international collaboration of fetal medicine centers regarding long-term follow-up is mandatory. In Parvovirus B19, adverse outcome may be directly related to cerebral injury caused by the viral infection itself or the compromised condition of the fetus with severe anemia and hydrops. Standardized ante- and postnatal neuroimaging protocols to accurately evaluate the timing and nature of cerebral injury and to investigate the correlation between imaging findings and long-term outcome is strongly recommended. Whether the outcome of cases with FMH- induced anemia is improved with IUT needs further study. An international registration of cases with FMH, treated with or without IUT, is required to study the natural history, perinatal and neonatal outcome as well as the potential risk factors for adverse long-term outcome.

Fetoscopic laser surgery in monochorionic twin pregnancies

Twin-twin transfusion syndrome

To date, three randomized controlled trials have been performed to determine optimal management and treatment of TTTS.[11;22;23] Another trial, the TTTS stage I trail, is ongoing. These trials were however designed and powered to detect a difference in short-term outcome. Future trials should also be powered to detect a difference in long-term outcome that is, survival without NDI. The sample size in studies including long-term outcome is larger, therefore international multicenter collaboration is required. Long-term follow-up in TTTS survivors, at least at age 2, 5 and 8, should be an integrated component of the fetoscopic laser surgery program in each fetal therapy center. A proposition for long-term assessment according to age in years is presented in Table 1.

Table 1 Follow-up assessment according to age in years.

Fetus	Neonate	2	5	8	12	16 years
Brain development: cerebral imaging						
		Senses: hearing test, vision test				
		Cognitive: Bayley scales/ Ages and Stages Questionnaire, Wechsler scales				
			Physical: Neurological Examination, Cerebral Palsy (GMFCS)			
			Academic: special education, number of grades below age-appropriate level			
				Neuropsychological: learning, language, executive functioning, attention, visual spatial abilities, memory, fine motor development		
				Psychosocial and behavior: internalizing and externalizing behavior, Quality of Life, Achenbach System, Vineland Adaptive Behavioral Scales		
				Developmental problems: attention deficit, hyperactivity, autism spectrum.		

Post-laser TAPS and spontaneous TAPS

TAPS is a newly described disease and long-term neurodevelopmental outcome has not yet been described, except for a small subgroup of post-laser TAPS children. Adequately powered, prospective studies, ideally using a randomized controlled design including long-term follow-up, are urgently needed to determine best management in MC pregnancies complicated by TAPS. The web-based TAPS registry (www.tapsregistry.org) now enables a prospective evaluation of the management of TAPS in all participating fetal medicine centers to further improve our knowledge on this relative new complication. At present, we are setting up a prospective, case-controlled follow-up study to investigate if post-laser TAPS leads to an increased risk of impairment compared with uncomplicated TTTS cases. In addition, we will assess the short- and long-term outcome of all pregnancies complicated by spontaneous TAPS and managed at our center.

Specific complications in monochorionic pregnancies

Selective intrauterine growth restriction

The optimal management in MC twins with sIUGR is not clear and international consensus on the best treatment strategy is lacking. Studies on the natural history are required to improve our knowledge in order to design a multi-center RCT and determine whether fetal surgery (fetoscopic laser surgery of placental vascular anastomoses) or obstetrical interventions improves (long-term) outcome. We intend to assess the long-

term neurodevelopmental outcome in all survivors from sIUGR pregnancies diagnosed at or referred to our center and managed either expectantly or with fetal therapy between 2002 and 2013. The objective is to explore the natural history of MC twin pregnancies diagnosed with sIUGR and to determine potential risk factors for adverse long-term outcome.

Single fetal demise in monochorionic pregnancies

Single fetal demise in MC pregnancies with patent vascular anastomoses poses a high risk for severe cerebral injury in the surviving co-twin. Information on long-term outcome in this subgroup of survivors is missing. A (inter)national database should be developed to register all cases with single fetal demise in order to study the natural history and possible risk factors for adverse long-term outcome. Stringent neuroimaging protocols to accurately evaluate the incidence and nature of antenatal cerebral injury and to investigate the correlation between antenatal and postnatal imaging findings are strongly recommended. The clinical relevance of these findings can be determined using long-term neurodevelopmental outcome data of all MC pregnancies with single fetal demise.

Selective feticide in complicated monochorionic pregnancies

Routine cerebral imaging should be performed in all survivors of selective feticide to rule out severe cerebral injury and determine etiology and timing of possible injury. Following selective feticide, the majority of MC pregnancies are however discharged to the referring hospital. Information at discharge regarding the management of these pregnancies should therefore include ante- and postnatal cerebral imaging in the surviving co-twin. Currently a multi-center RCT (TRAP Intervention Study, TRAPIST) is being set up comparing early (12-14 weeks) versus late intervention (16-18 weeks) in TRAP sequence. Primary outcome is pump-twin neonatal survival at or after 32 weeks. Secondary outcome includes 2-year neurodevelopmental outcome using ASQ in all survivors in the participating centers.

Monoamniotic twins

Since monoamniotic twinning occurs in approximately 1 in 10.000 pregnancies,[24] a multicenter study including Utrecht University Medical Center, Zwolle Isala clinics and LUMC has been set up to gain knowledge on short and long-term outcome of these pregnancies in the Netherlands. The aim of this study is to gain more insight in risk factors for adverse long-term outcome in surviving twins.

Other indications for fetal therapy

Other indications of fetal therapy that require further study include lower urinary tract obstruction (LUTO) and congenital lung lesions including congenital cystic adenomatoid malformation (CCAM) and bronchopulmonary sequestration (BPS). Fetal interventions include (vesicoamniotic or thoracoamniotic) shunting, laser treatment and/or needle drainage. Research suggest that increased perinatal survival with the use of fetal interventions is associated with a significant risk of neonatal morbidity.[25] We are currently investigating the neonatal and long-term outcome of all survivors referred to and treated for abovementioned complications at our center. Potential risk factors for adverse outcome should be studied combining neonatal and long-term data from other fetal therapy centers.

In conclusion, multicenter efforts are of utmost importance to study the natural history in complicated pregnancies with fetal disorders treated with or without fetal therapy, to determine optimal management (timing and type of intervention). Ideal study designs to evaluate new interventions in fetal therapy should be RCTs using 'survival without NDI' as primary outcome. Long-term follow-up, at a minimum of 2 years of age, should be an integrated component of fetal therapy in all fetal medicine centers around the world. In addition, worldwide registries (in analogy with www.tapsregistry.org) to record and evaluate the outcome in large groups of children (treated with and without fetal therapy) are of paramount importance to increase current knowledge in specific subgroups. It is crucial to continuously assess child development including formal psychological testing and standardized measures of well documented psychometric quality, with increasing reliability of results with increasing age of surviving children following fetal therapy.

Summary

An increasing number of fetal diseases are being detected prior to birth due to major improvements in prenatal ultrasound examinations and the wide implementation of screening programs.[26] For various diseases, fetal therapy is a life-saving option or an alternative to postnatal treatment, to prevent permanent organ damage.
A major breakthrough in fetal therapy was the introduction of intrauterine transfusion (IUT) for severe fetal anemia in the early 1960s. Since then, fetal therapy has gradually evolved resulting in a dramatic increase in overall survival in several fetal diseases. In the Netherlands, fetal surgical interventions are concentrated in one center, the Leiden University Medical Center (LUMC), a tertiary medical center which serves as the national referral center for fetal therapy.
Although an increasing number of children are being born alive after fetal therapy, reliable data on the long-term neurodevelopmental outcome remain scarce. Long-term follow-up studies are of paramount importance to increase our knowledge on the quality of survival and to identify potential risk factors for adverse long-term outcome. Detailed and adequate information on long-term outcome is required to improve both fetal management and the quality of antenatal parental counseling. In addition, long-term follow-up studies are essential for conducting future randomized controlled trials (RCT) in all fields of fetal therapy, in order to implement new or modified techniques.
In this thesis, several studies on the long-term neurodevelopmental outcome after fetal therapy for various fetal diseases are presented, including IUT for fetal anemia, fetoscopic laser surgery for twin-twin transfusion syndrome (TTTS) and selective feticide in complicated monochorionic (MC) pregnancies.

Intrauterine transfusion in fetal anemia
In *Chapter 1* an overview of the literature on the long-term neurodevelopmental outcome after IUT for fetal anemia is presented. The review discusses the latest findings on the long-term outcome, possible risk factors for long-term impairment and provides recommendations for future research.
In *Chapter 2* we assessed health-related quality of life (HRQOL) and behavioral functioning in 285 children and adolescents treated before birth with IUT for alloimmune anemia. Significantly lower HRQOL-scores were reported by parents of children 6-11 years compared with Dutch norms on 3 scales: cognitive-, social functioning and positive emotions ($P < .00$, $P = .02$, $P = .04$). In children aged 8-11 the cognitive functioning scale score was significantly lower compared with Dutch norms ($P = .01$). The children aged 12-15 reported higher scores on the negative emotions scale ($P = .02$). When corrected for multiple testing, only the parent-rated cognitive functioning

scale remained significant ($P < .001$). Regarding the HRQOL scores of adolescents ≥ 16 years, no differences were detected. Overall, behavioral difficulties were reported in 37/246 (15%) children 3-16 years, and were associated with maternal educational levels ($P < .001$). Our findings suggest that, for the majority of survivors after IUT for alloimmune anemia, HRQOL and long-term behavioral functioning appears favorable. The clinical significance of our findings should however be validated by further study, as assessment of HRQOL and socioeconomic status in adulthood will provide a clearer representation of the long-term functional outcomes following IUT.

The objective of the study described in *Chapter 3* was to assess the long-term neurodevelopmental outcome in children included in a RCT and treated with either intravenous immunoglobulin (IVIg) or placebo. Sixty-six of the 80 children (82.5%) who were recruited in the initial trial participated in this follow-up study. Children were assessed at a median age of 4 years (range: 2-7 years). The median cognitive score in the IVIg group was 96 (range: 68-118) and 97 (range: 66-118) in the placebo group ($P = .79$). There was no difference in the rate of neurodevelopmental impairment (NDI) between the IVIg and placebo groups (3% (1/34) versus 3% (1/32), $P = 1.00$). In conclusion, long-term neurodevelopmental outcome in children treated with IVIg was not different from children treated with placebo neither after stratification for treatment with or without IUT. Standardized long-term follow-up studies with large enough case series and sufficient power are needed to replicate these findings.

Fetoscopic laser surgery in monochorionic twin pregnancies

Chapter 4 presents an overview of the literature on the long-term neurodevelopmental outcome after 1) serial amnioreduction in TTTS, 2) fetoscopic laser surgery for TTTS and 3) selective feticide in MC pregnancies due to TTTS, twin-reversed arterial perfusion (TRAP), selective intrauterine growth restriction (sIUGR) or congenital anomalies. The review discusses the latest findings on the long-term outcome, risk factors for long-term impairment and provides recommendations for future research. Overall, amnioreduction was associated with an increased risk of long-term impairments compared to fetoscopic laser surgery. Important risk factors for long-term impairment included advanced gestational age at intervention, advanced Quintero stage and low gestational age at birth (and related low birth weight). Unfortunately, accurate and large long-term-follow-up studies on the long-term outcome following selective feticide are limited. All in all, regardless of antenatal treatment, survivors are at risk for NDI and require long-term follow-up.

Chapter 5 reports a systematic review and meta-analysis of studies on cerebral injury and long-term impairment after amnioreduction versus laser surgery for TTTS. The objective was to estimate the odds of severe cerebral injury and long-term NDI in MC

twins treated with amnioreduction versus laser surgery. We found an ample seven-fold higher risk of severe cerebral injury in live-born children treated with amnioreduction compared to laser (Odds Ratio (OR) 7.69, 95% Confidence Interval (CI) 2.78-20.0, *P* = .00). In children surviving the neonatal period, the odds were three-times higher following amnioreduction (OR 3.23, 95% CI 1.45-7.14, *P* = .00). Although not significant, MC twins treated with amnioreduction had higher odds of periventricular leukomalacia and intraventricular hemorrhage (OR 2.08, 95% CI .86-5.00, *P* = .10 and OR 3.56, 95% CI .82-14.29, *P* = .09). Unfortunately, there was insufficient long-term outcome data available to assess the odds of NDI. In conclusion, amnioreduction is associated with an increased risk of severe cerebral injury compared to laser surgery in TTTS. This study highlights a crucial lack of studies focusing on long-term neurodevelopmental outcome. Follow-up into childhood is indispensable to determine outcome in terms of cerebral palsy, cognitive and socio-emotional development.

In *Chapter 6* we compared the neurodevelopmental outcome between the first consecutive cohort of TTTS pregnancies treated with laser surgery from 2000 to 2005, with a cohort treated between 2008 and 2010. Neurological, cognitive and motor development was evaluated using Bayley scales at 2 years of age corrected for prematurity. Overall survival increased from 70% (158/226) to 80% (170/212) (*P* = .014). The incidence of NDI decreased from 18% (28/152) to 6% (10/155) (*P* < .01). In multivariate analysis, severe cerebral injury at birth was independently associated with long-term NDI (OR 34.86, 95% CI 11.83-102.75, *P* < .01). These findings suggest that overall survival in TTTS has improved over time, with a concomitant reduction in the incidence of NDI. Research focused on prevention of cerebral injury is needed to further improve outcomes of these complicated twin pregnancies.

The objective of the study described in *Chapter 7* was to compare the long-term neurodevelopmental outcome in surviving children with TTTS included in the Solomon randomized trial and treated with either the Solomon technique or standard laser surgery technique. Routine standardized follow-up in survivors, at least 2 years after the estimated date of delivery, was performed at two of the five centers participating in the Solomon trial, Buzzi Hospital Milan (Italy) and Leiden University Medical Center (The Netherlands). The primary outcome, survival without long-term NDI, was detected in 95/141 (67%) in the Solomon group and in 99/146 (68%) in the standard group (*P* = .92). NDI in long-term survivors included for follow-up was detected in 12/107 (11%) in the Solomon and in 10/109 (9%) in the standard group (*P* = .61) and was due to: cerebral palsy in 1 (1%) case (spastic unilateral) in the Solomon group and in 2 (2%) cases (spastic unilateral and spastic bilateral) in the standard group (*P* = .58). Cognitive development < 85 was detected in 2/105 (2%) children in the Solomon group and in

6/106 (6%) children in the standard group (P = .23). Motor development < 85 occurred in 8/103 (8%) children in the Solomon group and 3/104 (3%) in the standard group (P = .23). In conclusion, we found no difference in our primary outcome between the Solomon and standard laser technique for TTTS. In view of the reduction of short-term complications and absence of increased adverse long-term effects, these data support the use of the Solomon technique in the treatment of TTTS.

Chapter 8 presents the first study evaluating long-term neurodevelopmental outcome in twin-anemia polycythemia sequence (TAPS) after laser surgery for TTTS. Long-term outcome was assessed in 47/53 (89%) children. The incidence of long-term impairment was 4/47 (9%), occurring in one donor (1/20, 5%) and three recipients (3/27, 11%) (P = .63). Risk factors for low cognitive scores were low gestational age at birth (P = .02) and low birth weight (P = .01). Lowest cognitive scores were detected in the subgroup of TAPS survivors treated with IUT (median score: 82.5). Our results suggest that impairment in post-laser TAPS is frequent, but is within the range of NDI reported in case-series of TTTS treated with laser (range: 6-18%). Consensus on best treatment strategy and ideally prevention of this complication after laser for TTTS is urgently warranted.

Specific complications in monochorionic pregnancies

The objective of the systematic review presented in *Chapter 9* was to estimate the incidence of and risk factors for severe cerebral injury in survivors from MC pregnancies with sIUGR. Eleven articles were included in the systematic review. Analysis was however hampered by different methodology and definitions of cerebral injury. The incidence of severe cerebral injury varied from 0% to 33% (average 8%, 52/661), and was higher in studies including single fetal demise [OR 2.92; 95% CI 0.89- 9.56] and studies with a median gestational age at birth of ≤32 weeks (OR 1.56; 95% CI 1.06–2.27). The risk of severe cerebral injury was higher in pregnancies with abnormal umbilical artery Doppler (13.5% vs 2.5%; OR 7.69; 95% CI 2.56–25.00) and in larger twins (9% vs 5%; OR 1.93; 95% CI 0.95–3.92). In conclusion, the incidence of severe cerebral injury in MC twins with sIUGR is approximately 8% and is associated with abnormal umbilical artery Doppler, larger twins, single fetal demise and low gestational age at birth.

In *Chapter 10* we evaluated the incidence, type and severity of cerebral injury in the surviving MC co-twin after single fetal demise. A total of 49 MC pregnancies with single fetal demise, including one MC pair from a dichorionic triplet, were included in the study (n = 50 co-twins). The median gestational age at single fetal demise of the co-twin was 25 weeks and the median interval between single fetal demise and live birth was 61 days, with a median gestational age at birth of 36 weeks. Severe cerebral injury was diagnosed in 13 (26%) of the 50 co-twins and was detected antenatally in 4/50 (8%) and

postnatally in 9/50 (18%) cases. Cerebral injury was mostly due to hypoxic-ischemic injury resulting in cystic periventricular leukomalacia, middle cerebral artery infarction or injury to basal ganglia, thalamus and/or cortex. Risk factors associated with severe cerebral injury were advanced gestational age at the occurrence of single fetal demise (OR 1.14 for each week of gestation; 95% CI, 1.01-1.29; *P* = .03), TTTS diagnosed prior to single fetal demise, (OR 5.0; 95% CI, 1.30-19.13; *P* = .02) and gestational age at birth (OR 0.83 for each week of gestation; 95% CI, 0.69-0.99; *P* = .04). This study shows that the incidence of severe cerebral injury in MC pregnancies after single fetal demise is high that is, 1 in 4 surviving co-twins. Routine antenatal and postnatal neuroimaging, followed by standardized long-term follow-up, is mandatory.

Chapter 11 presents a retrospective analysis of the perinatal outcome of complicated MC pregnancies treated with selective feticide at the LUMC between June 2000 and November 2011. Overall perinatal survival rate was 67.2% (88/131). Median gestational age at delivery was 34 (interquartile range, 23–38) weeks. Neonatal mortality and morbidity rate in live-born children was 4.3% (4/92) and 12.0 % (11/92), respectively. Severe cerebral injury was detected in three children. The overall incidence of adverse perinatal outcome (fetal demise, neonatal death, termination of pregnancy or severe neonatal morbidity) was 41.2% (54/131). Median gestational age at occurrence of preterm prelabor rupture of membranes (PPROM) was 19.0 weeks and 32.0 weeks in cases with and without adverse perinatal outcome, respectively (*P* = .017). Live-born children with adverse perinatal outcome were born at a lower median gestational age (29.0 weeks) than were children without adverse perinatal outcome (38.0 weeks) (*P* < .001). In conclusion, selective feticide is associated with a high risk of adverse perinatal outcome. Further research is warranted to find ways to minimize the risks of selective feticide, by optimizing indications, timing and methods used. Outcome studies should include long-term follow-up to assess neurodevelopmental outcome in survivors.

The objective of the study described in *Chapter 12* was to assess the incidence and risk factors for adverse long-term neurodevelopmental outcome in complicated MC pregnancies treated with selective feticide at our center between 2000 and 2011. Children, at least two years of age, underwent an assessment of neurologic, motor and cognitive development using standardized psychometric tests and the parents completed a behavioral questionnaire. Long-term outcome was assessed in 74/88 (84%) survivors. NDI was detected in 5/74 (6.8%, 95%CI 1.1 to 12.5). Overall adverse outcome, including perinatal mortality or NDI was 48/131 (36.6%). In multivariate analysis, parental educational level was associated with cognitive test scores (regression coefficient B 3.9; 95% CI 1.8 to 6.0). Behavioral problems were reported in 10/69 (14.5%). Our results suggest that long-term NDI in survivors of MC pregnancies treated with selective feticide appears slightly more prevalent compared to general populations.

Long-term cognitive test scores were associated with the level of education of the parents. Multi-center efforts are required to entangle those factors leading to adverse perinatal and long-term outcome.

In conclusion, we believe long-term follow-up should be an integral component of fetal therapy in all fetal medicine centers around the world. Although fetal therapy resulted in an impressive increase in overall survival after various fetal diseases, perinatal morbidity and long-term neurodevelopmental impairment in surviving children are still significant. More research and new developments are required to improve short- and long-term outcome after fetal therapy. International collaboration between fetal medicine centers around the world is necessary to reliably investigate outcome and, in particular, risk factors for adverse long-term outcome.

Nederlandse samenvatting

Een toenemend aantal foetale aandoeningen wordt al voor de geboorte gediagnosticeerd dankzij belangrijke verbeteringen in prenataal echoscopisch onderzoek en de brede implementatie van screeningsprogramma's.[26] Voor verschillende ziekten is foetale therapie een levensreddende optie of een alternatief voor postnatale behandeling om permanente orgaanschade (inclusief het zich ontwikkelende foetale brein) te kunnen voorkomen. Een grote doorbraak in de foetale therapie was de introductie van de intra-uteriene bloedtransfusie (IUT) ter behandeling van ernstige foetale bloedarmoede (anemie). Deze interventie werd voor het eerst beschreven in de vroege jaren '60 door Sir William Liley in Nieuw Zeeland.[27] Sindsdien heeft de foetale therapie zich geleidelijk doorontwikkeld met als resultaat een significante toename in de overleving na uiteenlopende foetale aandoeningen. Wereldwijd werden gespecialiseerde foetale therapie centra opgezet en een nieuwe medische discipline deed zijn intrede.
In Nederland, is de foetale therapie geconcentreerd in één centrum, het Leids Universitair Medisch Centrum (LUMC). Het LUMC is een tertiair medisch centrum en het nationale kennis-en verwijscentrum voor invasieve behandeling van het ongeboren kind.

Intra-uteriene bloedtransfusie bij foetale anemie

Intra-uteriene bloedtransfusie kan sterfte voorkomen bij foetale anemie.[28] De bekendste oorzaak van deze aandoening is de hemolytische ziekte door maternale alloimmunisatie tegen rode bloedcelantigenen (zoals Rhesus immunisatie). Bij alloimmunisatie tijdens de zwangerschap maakt de moeder afweerstoffen tegen een bloedgroepeiwit, dat niet op haar eigen bloedcellen aanwezig is. Deze antistoffen kunnen de placenta passeren, in de bloedsomloop van de foetus terecht komen, en foetale rode bloedcellen afbreken (hemolyse). Progressieve hemolyse kan, indien niet behandeld, leiden tot ernstige foetale anemie, hydrops en perinatale sterfte.[29] Andere oorzaken van foetale anemie kunnen zijn een infectie met humaan Parvovirus B19 of (chronische of acute) foetomaternale transfusie.
In Nederland werd in 1986 de eerste intravasculaire IUT toegepast. Sinds 2000 is het overlevingspercentage na een IUT als behandeling voor foetale anemie vanwege alloimmunisatie gestegen tot > 95%.[28] In het LUMC worden jaarlijks ongeveer 30 foetussen behandeld met een IUT. Gemiddeld blijkt 3 maal een transfusie nodig (uitersten: 1-8 maal). Dit maakt dat er jaarlijks ongeveer 90 tot 100 IUTs worden uitgevoerd.

Foetoscopische laserbehandeling bij monochoriale tweelingen

Een andere, belangrijke interventie in de foetale therapie betreft de foetale behandeling van eeneiige of monochoriale (MC) tweelingen. MC tweelingen hebben een zogenaamde gezamenlijke placenta die vrijwel altijd vaatverbindingen bevat, waardoor de bloedsomlopen van beide foetussen met elkaar verbonden zijn. Ten gevolge van deze vaatverbindingen kan in 15% van de MC tweelingen het tweelingtransfusie syndroom (TTS) ontstaan. Door de vaatverbindingen stroomt bloed van de ene foetus (de donor) naar de andere foetus (de ontvanger of recipiënt) en de donor krijgt hiervoor maar weinig bloed terug. Bij de donor ontstaat een tekort aan bloed, waardoor hij minder en, in een later stadium, helemaal niet meer plast en uiteindelijk geen vruchtwater meer heeft (oligohydramnion). De ontvanger krijgt juist te veel bloed en gaat steeds meer plassen. De recipiënt krijgt daardoor te veel vruchtwater in zijn vruchtzak (polyhydramnion). Dit kan leiden tot een acute toename van de buikomvang van de moeder en tot vroegtijdige weeën en vroeggeboorte. Indien niet behandeld kan TTS leiden tot een sterfte 73-100%.[30] De beste behandeling voor TTS is de foetoscopische laserbehandeling waarbij de bloedvatverbindingen op de gezamenlijke placenta worden dicht gebrand (gecoaguleerd).[23]

Sinds 2000 wordt de foetoscopische laserbehandeling in het LUMC toegepast. Jaarlijks worden ongeveer 60 MC zwangerschappen behandeld, met een overlevingspercentage >74%.[11] In een recent beschreven complicatie bij MC tweelingen, tweeling anemie-polycythemie sequentie (TAPS), kunnen verschillende foetale interventies worden overwogen zoals de IUT en de foetoscopische laserbehandeling. De beste therapeutische optie voor TAPS dient nog te worden bepaald.

Als bij een MC tweeling één foetus een ernstige afwijking heeft, kan dit de gezonde foetus bedreigen.[28] Soms wordt dan gekozen voor selectieve reductie door middel van navelstrengcoagulatie. Indicaties voor selectieve reductie kunnen zijn een ernstige selectieve intra-uteriene groeirestrictie (sIUGR), twin reversed arterial perfusion (TRAP) en/of een ernstige congenitale afwijking. Het overlevingspercentage voor de gezonde foetus betreft 65-92% afhankelijk van de indicatie en de techniek die wordt toegepast.[31]

Lange termijn uitkomsten na foetale therapie

Met een toenemend aantal kinderen dat overleefd na behandeling met een foetale therapie, verschuift de aandacht van de korte termijn uitkomst naar de ontwikkeling van de kinderen op de lange termijn. Echter, betrouwbare informatie over de lange termijn uitkomst van kinderen behandeld met een foetale therapie is nog altijd schaars. Vervolgstudies zijn kostbaar, niet gemakkelijk uitvoerbaar (planning en organisatie) en zodoende moeilijk te realiseren. Daarbij komt dat het opzetten van voldoende

grote vervolgstudies wordt belemmerd door de relatieve zeldzaamheid van foetale ziekten. Desalniettemin zijn vervolgstudies naar de ontwikkeling van kinderen na foetale therapie van groot belang voor het optimaliseren van foetale behandeling als ook kwaliteitsborging. Onderzoek naar de ontwikkelingsuitkomsten van kinderen behandeld met een foetale therapie is cruciaal; op deze manier kunnen risicofactoren voor een suboptimale ontwikkeling van het kind worden achterhaald en zo de foetale therapie verder worden verbeterd. Daarnaast is gedetailleerde en adequate informatie over de lange termijn uitkomst nodig om toekomstige ouders van betrouwbare informatie te kunnen voorzien tijdens antenatale en postnatale counseling. Tenslotte kunnen ouder en (het vaak prematuur geboren) kind aan de hand van deze informatie tijdig van gepaste ondersteuning worden voorzien.

Vervolgstudies met aandacht voor de motorische, cognitieve en sociaal-emotionele ontwikkeling van het kind zijn essentieel voor het opzetten van gerandomiseerde trials (RCT) in de foetale therapie, met als doel nieuwe of aangepaste technieken te kunnen introduceren. Dit vereist samenwerking tussen gynaecologen-perinatologen, neonatologen, kinderpsychologen en andere experts in de (vroege) ontwikkeling van het kind om verder te kijken dan perinatale overleving. Daarbij is samenwerking tussen internationale foetale therapie centra geboden om betrouwbaar data te kunnen verzamelen aan de hand van voldoende grote case series. Dit stelt ons in staat om potentiële risicofactoren voor ontwikkelingsproblemen op de lange termijn te achterhalen.

Voortgaand onderzoek naar de lange termijn ontwikkeling van kinderen behandeld met een foetale therapie is noodzakelijk. Daarbij dient gebruikt te worden gemaakt van formele, gestandaardiseerde tests van voldoende psychometrische kwaliteit. Hierbij is het belangrijk om op te merken dat de betrouwbaarheid van de verkregen testresultaten toeneemt naarmate de kinderen ouder worden. Om de klinische significantie van de verkregen resultaten te kunnen valideren, dient vervolgonderzoek plaats te hebben tot tenminste de schoolleeftijd en bij voorkeur tot en met de volwassen leeftijd aangezien het evalueren van de kwaliteit van leven en de verworven socio-economische status op de volwassen leeftijd een duidelijker representatie geven van de functionele lange-termijn uitkomst na foetale therapie.

Het doel van onderhavig proefschrift is om de kennis over de lange termijn ontwikkeling van kinderen behandeld met een foetale therapie te vergroten alsmede potentiele risico factoren voor ontwikkelingsproblemen te kunnen identificeren.

Intra-uteriene bloedtransfusie bij foetale anemie

Hoofdstuk 1 bevat een samenvatting van de literatuur betreffende de lange termijn uitkomst na IUT vanwege foetale anemie. Mogelijke risicofactoren voor ernstige neurologische, motorische en cognitieve ontwikkelingsproblemen op de lange termijn worden beschreven als ook aanbevelingen voor toekomstig vervolgonderzoek. Uit het literatuuroverzicht komt naar voren dat de meerderheid van de kinderen behandeld met IUT vanwege foetale alloimmuun anemie een gunstige lange-termijn ontwikkeling laat zien. Risicofactoren voor ontwikkelingsproblemen blijken: ernstige foetale hydrops, het aantal IUTs verricht, ernstige neonatale morbiditeit en het opleidingsniveau van de ouders. De studie toont aan dat er dringend behoefte is aan lange termijn studies na foetale behandeling van Parvovirus B19 infectie en foetomaternale transfusie.

In *Hoofdstuk 2* hebben we de gezondheidsgerelateerde kwaliteit van leven (KvL) en de gedragsuitkomsten onderzocht van 285 kinderen en adolescenten behandeld met IUT vanwege foetale alloimmuun anemie. In vergelijking met een Nederlandse norm-groep werden significant lagere KvL-scores gerapporteerd door de ouders van kinderen 6-11 jaar op 3 van de 7 schalen: cognitief functioneren, sociaal functioneren en positieve emoties ($P < .00$, $P = .02$, $P = .04$). De kinderen van 8-11 jaar rapporteerden eveneens lagere scores op de schaal cognitief functioneren vergeleken met de Nederlandse norm-groep ($P = .01$). De kinderen van 12-15 jaar rapporteerden hogere scores op de schaal negatieve emoties ($P = .02$). Na correctie voor het aantal testen dat werd uitgevoerd (Bonferroni correctie), bleek alleen de verschilscore op de schaal cognitief functioneren zoals gerapporteerd door de ouders nog significant ($P < .001$). Er werden geen verschillen gevonden tussen de KvL-scores van de adolescenten ≥ 16 jaar en de Nederlandse norm-groep. Gedragsproblemen werden gerapporteerd bij 37/246 (15%) kinderen 3-16 jaar. Een laag opleidingsniveau van de moeder bleek geassocieerd met meer gedragsproblemen ($P < .001$). Over het geheel genomen, tonen deze bevindingen aan dat de lange-termijn uitkomst (de gezondheidsgerelateerde KvL en de gedragsuitkomsten) gunstig lijkt voor de meerderheid van de kinderen en adolescenten behandeld met IUT vanwege foetale alloimmuun anemie.

In *Hoofdstuk 3* hebben we de motorische en cognitieve ontwikkeling van kinderen, geïncludeerd in een gerandomiseerde studie en behandeld met intraveneuze immunoglobuline (IVIg) of placebo vanwege hemolytische ziekte van de foetus en pasgeborene (HZFP), onderzocht. Primaire uitkomstmaat was een ernstig ontwikkelingsprobleem of 'neurodevelopmental impairment' (NDI), een samengestelde uitkomstmaat gedefiniëerd als de aanwezigheid van één of meer van de volgende uitkomsten: cerebrale parese, blindheid, doofheid, een ernstige motorische en/of cognitieve ontwikkelingsachterstand. De kinderen (66/80, 83%) werden onderzocht op een mediane leeftijd van 4 jaar (uitersten: 2-7 jaar) met gestandaardiseerde testen.

De mediane cognitieve score van de kinderen in de IVIg groep was 96 (uitersten: 68-118) en 97 (uitersten: 66-118) in de placebo groep (*P* = .79). Er bleek geen verschil in het voorkomen van een ernstig ontwikkelingsprobleem (NDI) tussen de IVIg- en placebo groep (3% (1/34) versus 3% (1/32), *P* = 1.00). De lange termijn uitkomst van de kinderen behandeld met IVIg bleek dan ook niet te verschillen van de kinderen behandeld met placebo. Behandeling met IVIg bij HZFP lijkt geen positief (noch een negatief) effect te hebben op de lange termijn uitkomst. Deze bevindingen dienen echter gerepliceerd te worden aan de hand van gestandaardiseerde vervolgstudies met een voldoende grote onderzoeksgroep.

Foetoscopische laserbehandeling bij monochoriale tweelingen

Hoofdstuk 4 bevat een literatuuroverzicht betreffende de lange termijn uitkomst na 1) amniodrainage voor TTS, 2) de foetoscopische laserbehandeling voor TTS 3) selectieve reductie bij gecompliceerde MC zwangerschappen (ernstige sIUGR, TRAP en/of een ernstige congenitale afwijking). Mogelijke risicofactoren voor ernstige neurologische, motorische en cognitieve ontwikkelingsproblemen op de lange termijn worden beschreven als ook aanbevelingen voor toekomstig vervolgonderzoek. Uit het literatuuroverzicht blijkt dat amniodrainage geassocieerd wordt met een verhoogd risico op ernstige ontwikkelingsproblemen op de lange termijn in vergelijking met de foetoscopische laserbehandeling voor TTS. Belangrijke risicofactoren voor ontwikkelingsproblemen zijn: Quintero stadium (hoe verder gevorderd het stadium hoe hoger de kans op beperkingen op de lange termijn) en een lage zwangerschapsduur bij geboorte. Onderzoek naar de lange termijn uitkomst na selectieve reductie blijkt schaars; Adequaat opgezette en voldoende grote vervolgstudies worden dan ook dringend geadviseerd.

Hoofdstuk 5 betreft een samenvatting en meta-analyse van studies naar het voorkomen van cerebrale schade en ernstige ontwikkelingsproblemen in TTS tweelingen na behandeling met amniodrainage versus foetoscopische laserbehandeling. De kans op ernstige cerebrale schade bij levendgeboren kinderen bleek na behandeling met amniodrainage significant hoger dan na laserbehandeling (Odds Ratio (OR) 7.69, 95% betrouwbaarheidsinterval 2.78-20.0, *P* = .00). Zo ook de kans op cerebrale schade bij kinderen die de neonatale periode hadden overleefd (OR 3.23, 95% betrouwbaarheidsinterval 1.45-7.14, *P* = .00). Helaas bleek er onvoldoende lange-termijn data beschikbaar om het risico op een ernstig ontwikkelingsprobleem in TTS tweelingen na behandeling met amniodrainage versus foetoscopische laserbehandeling betrouwbaar te kunnen onderzoeken. De conclusie is dan ook dat de behandeling van TTS met amniodrainage geassocieerd is met een verhoogd risico op het ontwikkelen van ernstige cerebrale schade in vergelijking met de foetoscopische laserbehandeling.

De studie onderstreept een belangrijk gebrek aan gedegen studies naar de lange termijn uitkomst van TTS tweelingen. Gestandaardiseerd vervolgonderzoek tot tenminste de schoolleeftijd is noodzakelijk om de lange termijn uitkomst in termen van de neurologische, motorische, cognitieve en sociaal emotionele ontwikkeling betrouwbaar in kaart te kunnen brengen.
In *Hoofdstuk 6* hebben we de ontwikkelingsuitkomsten van TTS tweelingen behandeld met foetoscopische laser sinds de start van het laserprogramma in 2000 tot en met 2005 vergeleken met een recent cohort TTS tweelingen behandeld tussen 2008 en 2010. De neurologische, motorische en cognitive ontwikkeling werd onderzocht op de leeftijd van 2 jaar. Het overlevingspercentage bleek toegenomen van 70% (158/226) in de periode 2000-2005 naar 80% (170/212) in de periode 2008-2010 (P = .014). Het voorkomen van een ernstig ontwikkelingsprobleem bleek daarentegen afgenomen van 18% (28/152) naar 6% (10/155) in 2008-2010 (P < .01). In een multivariate analyse, bleek ernstige cerebrale schade bij geboorte geassocieerd met een ernstig ontwikkelingsprobleem op de lange termijn (OR 34.86, 95% betrouwbaarheidsinterval 11.83-102.75, P < .01). Deze bevindingen suggereren dat de kans op overleving zonder een ernstig ontwikkelingsprobleem significant is toegenomen sinds de introductie van het foetoscopische laserprogramma in het LUMC. Onderzoek naar preventie van cerebrale schade is noodzakelijk om de uitkomst van TTS tweelingen nog verder te kunnen verbeteren.

In *Hoofdstuk 7* hebben we de lange termijn uitkomst van TTS tweelingen, geïncludeerd in de Solomon RCT en behandeld met de Solomon of de standaard laserbehandeling, beschreven. Gestandaardiseerd ontwikkelingsonderzoek op de leeftijd van 2 jaar werd systematisch uitgevoerd in 2 van de 5 deelnemende centra te weten, V Buzzi Children's Hospital Milaan (Italië) en het LUMC. De primaire uitkomst, overleving zonder een ernstig ontwikkelingsprobleem (NDI), werd vastgesteld bij 95/141 (67%) in de Solomon groep en bij 99/146 (68%) in de standaard groep (P = .92) Een ernstig ontwikkelingsprobleem werd gediagnosticeerd bij 12/107 kinderen (11%) in de Solomon en bij 10/109 kinderen (9%) in de standaard groep (P = .61) en was gebaseerd op: cerebrale parese (n=1 in de Solomon versus n=2 in de standaard groep (P = .58)), cognitieve ontwikkeling < 85 (n=2 in de Solomon versus n=6 in de standaard groep (P = .23)) en motorische ontwikkeling < 85 (n=8 in de Solomon versus n=3 in de standaard groep (P = .23)). De conclusie is dan ook dat er geen verschil in primaire uitkomst werd gevonden tussen de Solomon en standaard laserbehandeling. Met het oog op de significante reductie in complicaties op de korte termijn en de afwezigheid van een verhoogd risico op ernstige ontwikkelingsproblemen op de lange termijn, ondersteunen deze bevindingen het gebruik van de Solomon techniek voor de behandeling van TTS.

De eerste studie naar de lange termijn uitkomst van post-laser TAPS kinderen wordt beschreven in *Hoofdstuk 8.* In totaal werden 47/53 (89%) kinderen teruggezien voor ontwikkelingsonderzoek. Een ernstig ontwikkelingsprobleem werd vastgesteld bij 4/47 kinderen (9%), daarbij werd geen verschil gezien tussen donoren en recipiënten (1/20 (5%) versus 3/27 (11%), $P = .63$). Het voorkomen van een ernstig ontwikkelingsprobleem bij post-laser TAPS kinderen lijkt vergelijkbaar met het voorkomen bij post-laser TTS kinderen (6-18%). In een univariate risico-analyse bleek een lage zwangerschapsduur bij geboorte ($P = .02$) als ook een laag geboortegewicht ($P < .01$) geassocieerd met lagere cognitieve scores. In een subgroep analyse waarbij TAPS voor de geboorte ontdekt en behandeld was, werd in de gevallen behandeld met IUT, een lagere mediane cognitieve testscore (82.5) gezien in vergelijking met de andere subgroepen (afwachtend beleid, laserbehandeling en selectieve reductie). Een mogelijke verklaring voor de lagere testscores kan zijn dat deze kinderen bij een lagere zwangerschapsduur worden geboren, omdat de behandelaar beslist om de kinderen geboren te laten worden vanwege de ernst van de anemie en polycythemie. De resultaten suggereren dat een ontwikkelingsprobleem niet ongewoon is bij tweelingen die TAPS ontwikkelen na laserbehandeling voor TTS, maar vergelijkbaar lijkt met het voorkomen bij TTS tweelingen behandeld met laser. Studies met grote aantallen zijn nodig om risicofactoren voor ernstige ontwikkelingsproblemen op de lange termijn betrouwbaar te kunnen onderzoeken. Er is dringende behoefte aan overeenstemming voor wat betreft de best beschikbare therapeutische optie voor TAPS en, idealiter, preventie van deze complicatie na laserbehandeling voor TTS.

Specifieke complicaties bij monochoriale zwangerschappen

Het doel van het systematische literatuuroverzicht beschreven in *Hoofdstuk 9* was om het voorkomen van en de risicofactoren voor ernstige cerebrale schade bij MC zwangerschappen met sIUGR te onderzoeken. Hiertoe werden elf studies geïncludeerd. De data-analyse werd echter belemmerd door grote verschillen in methodologie als ook in de definitie van ernstige cerebrale schade. Het voorkomen van ernstige cerebrale schade varieerde van 0% tot 33% (gemiddelde 8%, 52/661), en bleek hoger in studies met zwangerschappen waarbij één van de tweeling intra-uterien was overleden (OR 2.92; 95% betrouwbaarheidsinterval 0.89- 9.56) en in studies met een mediane zwangerschapsduur ≤ 32 weken (OR 1.56; 95% betrouwbaarheidsinterval 1.06–2.27). Het risico op ernstige cerebrale schade bleek hoger in zwangerschappen met afwijkende echo Doppler bevindingen (14% vs 3%; OR 7.69; 95% betrouwbaarheidsinterval 2.56–25.00) en bij het 'grote' kind (9% vs 5%; OR 1.93; 95% betrouwbaarheidsinterval 0.95–3.92). Samenvattend, het voorkomen van ernstige cerebrale schade bij MC tweelingzwangerschappen gecompliceerd door sIUGR is ongeveer 8% en geassocieerd

met intra-uterien overlijden van één van de tweeling, een lage zwangerschapsduur bij geboorte, afwijkende echo Doppler bevindingen en het 'grote' kind. De optimale benadeling van MC zwangerschappen gecompliceerd door sIUGR is nog onbekend en internationale overeenstemming over de best beschikbare therapeutische optie ontbreekt.

In *Hoofdstuk 10* hebben we het voorkomen, type en de ernst van cerebrale schade onderzocht bij de overlevende tweeling na plotseling intra-uterien overlijden van de co-tweeling. In totaal werden 49 MC zwangerschappen, inclusief een MC tweeling deel uitmakend van een dichoriale drieling zwangerschap, geïncludeerd ($n = 50$ co-tweelingen). De mediane zwangerschapsduur ten tijde van intra-uterien overlijden van de co-tweeling was 25 weken met een mediaan interval tussen overlijden en het levend geboren worden van de co-tweeling van 61 dagen (mediane zwangerschapsduur bij geboorte 36 weken). Ernstige cerebrale schade werd vastgesteld bij 13/50 co-tweelingen (26%); antenataal bij 4/50 (8%) and postnataal bij 9/50 (18%) kinderen. Cerebrale schade bleek met name gebaseerd op hypoxisch-ischemische schade resulterend in periventriculaire leucomalacie met cystevorming, een arterieel infarct en/of schade aan de basale ganglia, de thalamus en cortex. Risicofactoren geassocieerd met ernstige cerebrale schade bleken een gevorderde zwangerschapsduur bij intra-uterien overlijden van één van de tweeling (OR, 1.14; 95% betrouwbaarheidsinterval, 1.01-1.29; $P = .03$), TTS gediagnosticeerd alvorens het intra-uterien overlijden van één van de tweeling (OR, 5.0; 95% betrouwbaarheidsinterval, 1.30-19.13; $P = .02$) en de zwangerschapsduur bij geboorte van de overlevende co-tweeling (OR, 0.83; 95% betrouwbaarheidsinterval, 0.69-0.99; $P = .04$). Gezien de hoge incidentie van ernstige cerebrale schade na het plotseling intra-uterien overlijden van één van de tweeling adviseren wij om routinematig antenataal en postnataal echografisch onderzoek van de hersenen (en indien nodig MRI) te laten verrichten gevolgd door gestandaardiseerd vervolgonderzoek naar de ontwikkeling van deze kinderen.

Hoofdstuk 11 betreft een retrospectieve analyse van de perinatale uitkomst van gecompliceerde MC zwangerschappen behandeld met selectieve reductie tussen Juni 2000 en November 2011 in het LUMC. Het perinatale overlevingspercentage bleek 67.2% (88/131). De mediane zwangerschapsduur bij geboorte was 34 weken. Neonatale mortaliteit en morbiditeit in levend geboren kinderen was, respectievelijk, 4% (4/92) en 12 % (11/92). Ernstige cerebrale schade werd gedetecteerd bij drie kinderen. Het voorkomen van een slechte perinatale uitkomst (gedefiniëerd als intra-uterien overlijden, neonataal overlijden, zwangerschapsafbreking of ernstige neonatale morbiditeit) was 41% (54/131). De kinderen met een slechte perinatale uitkomst bleken geboren bij een lagere mediane zwangerschapsduur (29 weken) dan de kinderen zonder slechte perinatale uitkomst (38 weken) ($P < .001$). Wij concluderen dat selectieve

reductie geassocieerd is met een hoog risico op een slechte perinatale uitkomst. Nader onderzoek naar mogelijkheden om de risico's van selectieve reductie te minimaliseren is geboden, door het optimaliseren van indicaties, timing en procedures.
In *Hoofdstuk 12* hebben wij het voorkomen van en de risicofactoren voor een ernstig ontwikkelingsprobleem onderzocht bij MC tweelingen behandeld met selectieve reductie tussen 2000 en 2011 in het LUMC. De neurologische, motorische en cognitieve ontwikkeling van 74/88 (84%) kinderen werd onderzocht met gestandaardiseerde testen en de ouders van de kinderen vulden een gedragsvragenlijst in. Een ernstig ontwikkelingsprobleem werd gedetecteerd bij 5/74 kinderen (7%, 95% betrouwbaarheidsinterval 1.1 - 12.5). Een slechte uitkomst in het algemeen, te weten perinataal overlijden of een ernstig ontwikkelingsprobleem, werd vastgesteld in 48/131 (37%). In een multivariate analyse, bleek het opleidingsniveau van de ouder geassocieerd met de cognitieve test scores van het kind (regressie coëfficiënt B 3.9, 95% betrouwbaarheidsinterval 1.8 - 6.0). Gedragsproblemen werden gerapporteerd bij 10/69 kinderen (15%). Deze bevindingen suggereren dat ontwikkelingsproblemen bij tweelingen behandeld met selectieve reductie vaker voor lijken te komen in vergelijking met de algemene bevolking. Studies met grotere aantallen, bij voorkeur in internationaal samenwerkingsverband, zijn nodig om risicofactoren voor ontwikkelingsproblemen betrouwbaar te kunnen onderzoeken. Wij adviseren gestandaardiseerd pre- en postnataal echografisch onderzoek van de hersenen (en indien nodig MRI) bij alle overlevende kinderen na selectieve reductie van de co-tweeling gevolgd door gestandaardiseerd vervolgonderzoek naar de ontwikkeling van deze kinderen op de lange termijn.

Samenvattend kunnen we stellen dat, hoewel foetale therapie voor verschillende ziekten een levensreddende optie betreft met een toenemend aantal overlevende kinderen, de lange termijn uitkomsten voor verschillende foetale therapieën nog onvoldoende in kaart zijn gebracht. Internationale samenwerking is vereist om aan de hand van voldoende grote groepen patiënten betrouwbaar de ontwikkelingsuitkomsten te onderzoeken tot minimaal de schoolleeftijd en, bij voorkeur, tot in de volwassen leeftijd.

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PART VI

APPENDICES

Publications

1. Neurodevelopmental outcome at 2 years in twin-twin transfusion syndrome survivors randomized for the Solomon trial. van Klink JM, Slaghekke F, Balestriero M, Scelsa B, Introvini P, Rustico M, Middeldorp JM, Oepkes D, Lopriore E. Am J of Obstet Gynecol. Accepted Aug 2015. doi: 10.1016/j.ajog.2015.08.033. [Epub ahead of print]
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List of abbreviations

BSID	Bayley Scales of Infant and Toddler Development
CBCL	Child Behavioral Checklist
CP	Cerebral Palsy
FD	Fetal Demise
GA	Gestational Age
Hb	Hemoglobin
HRQOL	Health Related Quality Of Life
IUT	Intra Uterine Transfusion
IVH	Intraventricular Hemorrhage
IVIg	IntraVenous Immunoglobulin
LOTUS	LOng-Term follow-up after intra-Uterine transfusionS
LUMC	Leiden University Medical Center
MC	MonoChorionic
MCA-PSV	Middle Cerebral Artery - Peak Systolic Velocity
NND	NeoNatal Death
NDI	NeuroDevelopmental Impairment
PVL	PeriVentricular Leukomalacia
sIUGR	Selective Intra Uterine Growth Restriction
SOLOMON	Selective Or Laser Of the entire equator in MONochorionic twins
SDQ	Strengths and Difficulties Questionnaire
TAPS	Twin Anemia Polycythemia Sequence
TACQOL	TNO-AZL Child Quality of Life Questionnaire
TAAQOL	TNO-AZL Adult Quality of Life Questionnaire
TOP	Termination Of Pregnancy
TOPS	Twin Oligo Polyhydramnios Sequence
TTTS	Twin-Twin Transfusion Syndrome
WPPSI	Wechsler Preschool and Primary Scale of Intelligence
WISC	Wechsler Intelligence Scale for Children