TWIN ANEMIA POLYCYTHEMIA SEQUENCE

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Twin Anemia Polycythemia Sequence

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Contents

General introduction

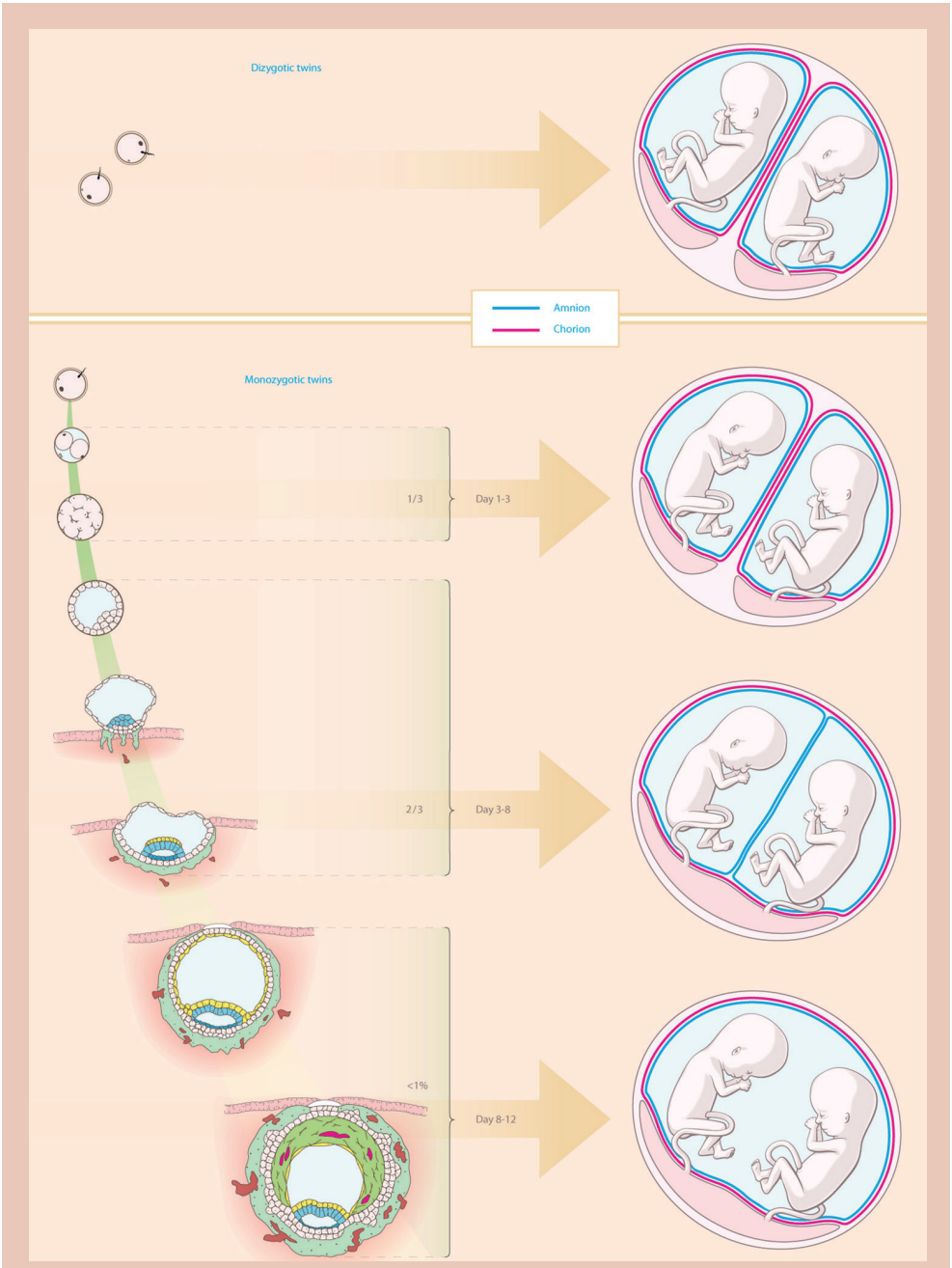

Figure 1. Dizygotic twins versus monozygotic twins resulting in dichorionic and monochorionic twins

General Introduction

Twin pregnancies are at increased risk for adverse outcome compared to singletons. The incidence of twin pregnancies in the Netherlands is approximately 2% [1], of which 1/3 are monozygotic (identical) twins and 2/3 dizygotic (non-identical) twins. Dizygotic twin pregnancies are always dichorionic and each fetus has thus its own placenta. In 1/3 of monozygotic twin pregnancies, dividing into two embryos occurs within three days after fertilization resulting also in a dichorionic twin pregnancy. In 2/3 of monozygotic twins, dividing occurs after three days resulting in monochorionic twins, with both fetuses sharing their placenta. Dividing after 8 days results in monoamniotic twins, besides sharing the placenta these twins also share the amniotic sac (figure 1). With a total of approximately 175.000 pregnancies per year in the Netherlands, approximately 775 twin pregnancies are monochorionic.

Monochorionic twins share their placenta and their blood circulation is connected by vascular anastomoses at the placental surface. In monochorionic placentas three types of anastomoses are seen: artery to artery, vein to vein and artery to vein. An arterio-venous (AV) anastomosis is a “deep hidden” anastomosis. The artery of one twin is corresponding with a vein of the co-twin via a shared cotyledon. Blood flow goes in one direction from artery to vein and therefore unidirectional. Artery to artery (AA) and vein to vein (VV) anastomoses are “superficial” anastomoses since they lie on the placental surface and these anastomoses are bi-directional. In contrast, dichorionic placentas almost never have vascular anastomoses and the circulations of both fetuses are not connected. Dichorionic placentas have a thick intertwin membrane. Figure 2 shows the placenta and ultrasound differences in monochorionic versus dichorionic twins.

Monochorionic twin pregnancies are associated with a perinatal mortality rate of 11% [2;3]. Due to imbalanced blood flow through placental vascular anastomoses, these pregnancies can be complicated by twin-twin transfusion syndrome (TTTS) or twin anemia polycythemia sequence (TAPS).

TTTS

TTTS is caused by an imbalanced blood flow from donor to recipient via placental vascular anastomoses, resulting in hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient twin. The incidence of TTTS is approximately 10% [3]. If left untreated, TTTS can result in an overall mortality rate of 73-100% [4]. In the

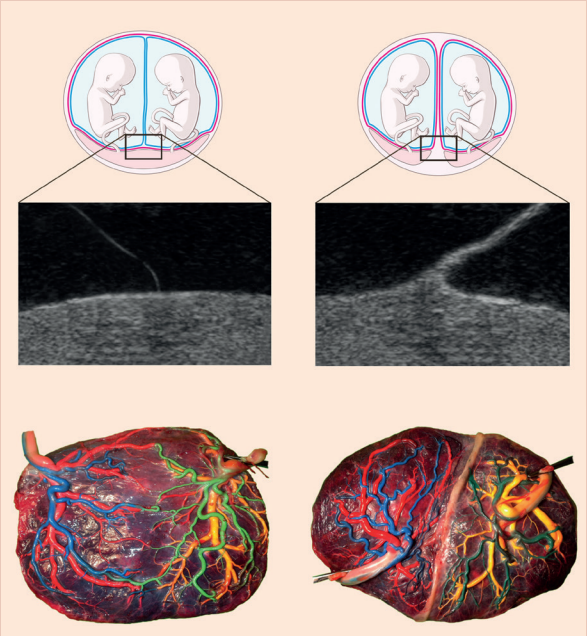

Figure 2. Monochorionic (left) versus dichorionic (right).

In the middle the ultrasound image shows a T-sign in monochorionic twins and a lambda sign for dichorionic twins.

At the bottom placentas after color dye injection are shown. Arteries are injected with green and blue dye and veins with yellow and pink.

A monochorionic placenta is characterized by vascular anastomoses at the placental surface whereas a dichorionic placenta has a thick intertwin membrane and no vascular anastomoses.

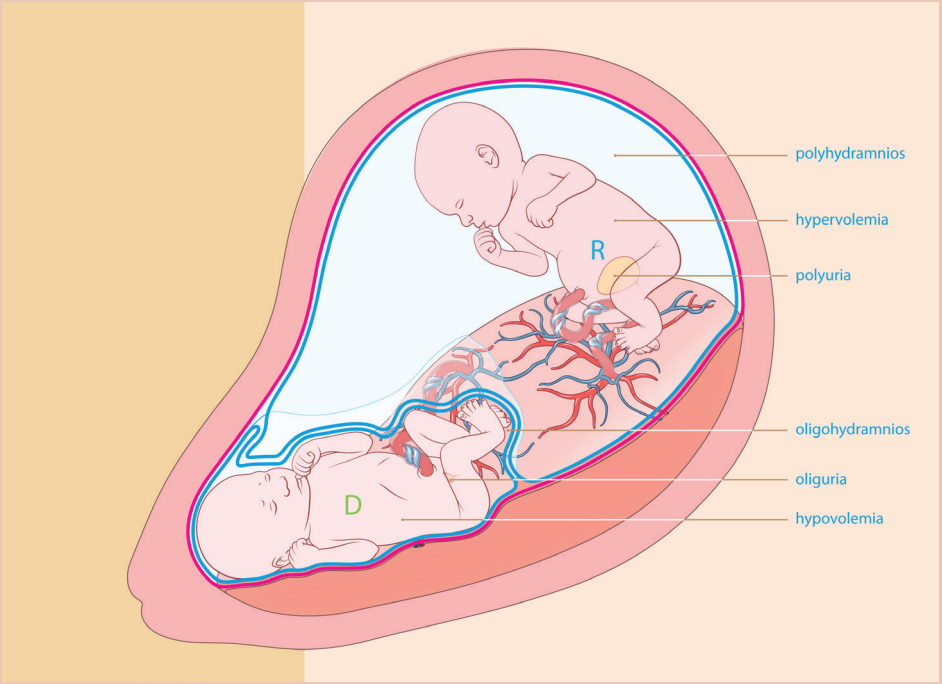

Figure 3. Twin twin transfusion syndrome (TTTS). D is the donor and R is the recipient.

Netherlands, of the approximately 775 monochorionic pregnancies a total of 70 to 80 pregnancies are complicated by TTTS[1]. The preferred treatment option for TTTS is fetoscopic laser coagulation of the vascular anastomoses at the placental surface. The Leiden University Medical Center (LUMC) is the national referral center for fetal therapy, a total of up to 60 patients are treated annually with fetoscopic laser surgery for TTTS.

TAPS

TAPS is a newly described form of chronic feto-fetal transfusion in monochorionic twins. TAPS is characterized by large intertwin hemoglobin (Hb) difference without amniotic fluid differences as in TTTS. TAPS was first described in 2005 by Robyr et al. [5] as a complication after fetoscopic laser surgery for TTTS. In 2007 Lopriore et al. [6] named this complication in monochorionic twins: twin anemia polycythemia sequence, TAPS. Since then TAPS is worldwide accepted as a name for this complication in monochorionic twins. TAPS may occur spontaneous or after laser treatment for TTTS (post-laser TAPS). The incidence of spontaneous TAPS varies between 1-5% [3;7-9] and the incidence of post-laser TAPS is 13% [5]. In the Netherlands up to 40 pregnancies are complicated by spontaneous TAPS and around 8 pregnancies by post-laser TAPS per year.

Solomon study

Post-laser TAPS is caused by residual anastomoses after fetoscopic laser surgery for the treatment of TTTS. In up to 33% after fetoscopic laser surgery for TTTS residual anastomoses are seen [5;10;11]. In order to reduce the number of residual anastomoses, a new laser technique, named the Solomon technique is studied (chapter 7). In the Solomon trial (Selective Or Laser Of the entire equator in MONochorionic twins) we compare the Solomon technique with the standard technique. With the Solomon technique, after identification and coagulation of the individual anastomoses, the whole vascular equator will be coagulated and with the standard technique only the anastomoses will be coagulated. Solomon also refers to the bibliq story of King Solomon. King Solomon was the son of David, and reigned from 970 to 931 BC. He was known for his wisdom. In one account, known as the Judgment of Solomon, two women came before Solomon to resolve a quarrel over whom was the true mother of a baby. When Solomon suggested they should divide the living child in two with a sword, one woman said she would rather give up the child than see it killed. Solomon then declared the woman who showed compassion to be the true mother, and gave the baby to her. In our trial, instead of cutting one baby in two to give two mothers half a baby, we aim to "cut" the placenta in two, to give one mother two babies.

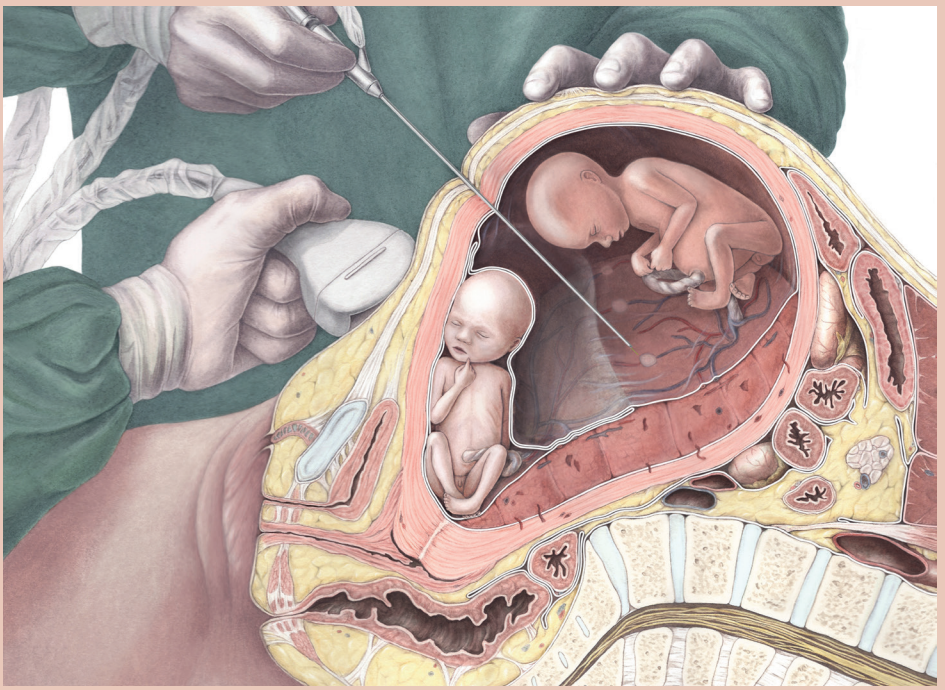

Figure 4. Fetoscopic laser surgery for the treatment of TTTS.

The aim of this thesis is to improve our knowledge on this newly described chronic form of feto-fetal transfusion in monochorionic twins. Improving our knowledge on TAPS is of utmost importance to diagnose, manage and treat this complication in a pregnancy with an increased risk of perinatal mortality and morbidity. Several questions were raised considering pathophysiology, diagnosis, treatment, neonatal and long-term outcome.

Pathophysiology

To understand the pathophysiology of TAPS we studied the placentas from TAPS pregnancies and compared these with placentas of uncomplicated monochorionic twin pregnancies and placentas after fetoscopic laser surgery for TTTS. We formulated the following questions: What are the type and size of anastomoses in TAPS compared to uncomplicated monochorionic twin pregnancies and in placentas after fetoscopic laser surgery for TTTS? Is there a difference in spontaneous TAPS and post-laser TAPS placentas?

Diagnosis

Timely and accurate diagnosis is important for perinatal outcome. Since there is not an

obvious sign as amniotic fluid difference as in TTTS, TAPS can easily be missed in less experienced hands. When TAPS is not timely diagnosed it may result in perinatal mortality or severe morbidity. Middle cerebral artery peak systolic velocity (MCA-PSV) Doppler measurements is proven to be an accurate non-invasive predictor for anemia in red cell alloimmunization [12]. Is MCA-PSV measurement also an accurate predictor of anemia and polycythemia in TAPS? And in postnatal TAPS what are the hematological characteristics in donors and recipients?

Treatment

The best treatment for TTTS is fetoscopic laser surgery of the vascular anastomoses, which is a causal treatment for this form of feto-fetal transfusion [13]. The best treatment option for

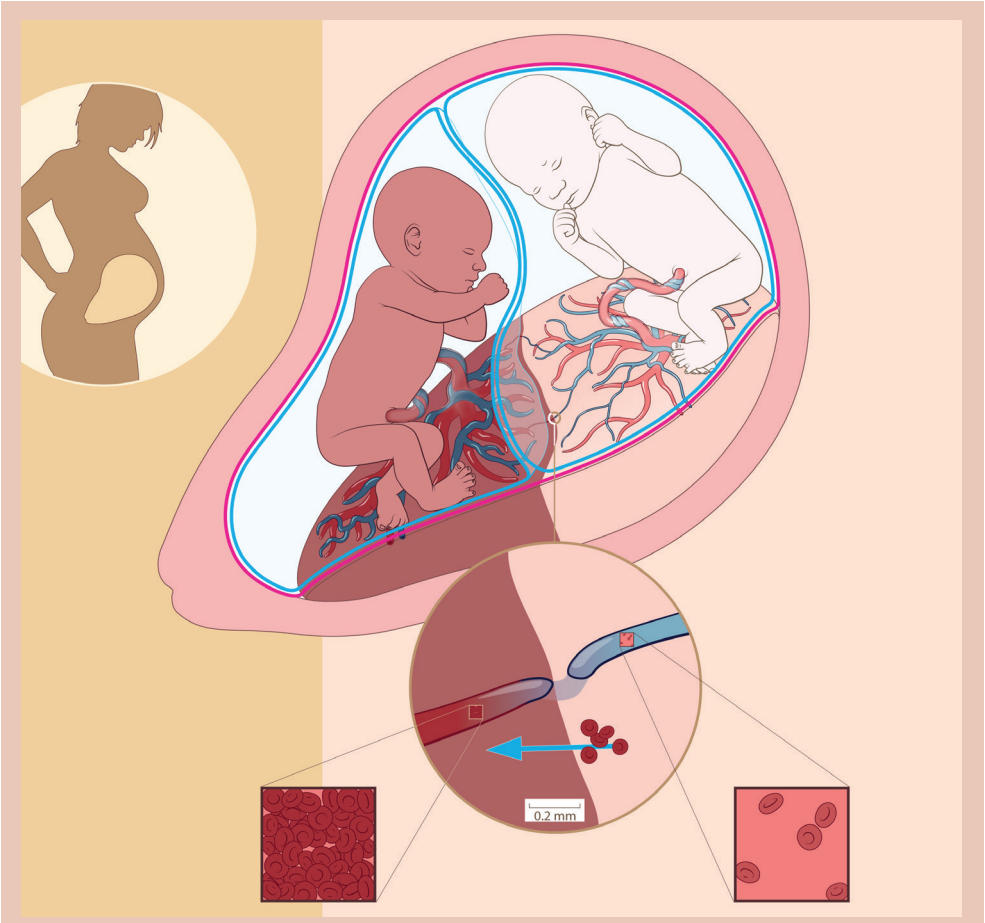

Figure 5. Twin anemia polycythemia sequence (TAPS). The pale donor is anemic and the plethoric recipient is polycythemic, without amniotic fluid differences.

TAPS is not known. How should we manage this form of feto-fetal transfusion? Is there a role for fetoscopic laser surgery? If laser is not feasible and intrauterine transfusion is performed should we add partial exchange transfusion for the recipient? Even more important, can we prevent post-laser TAPS with the Solomon technique?

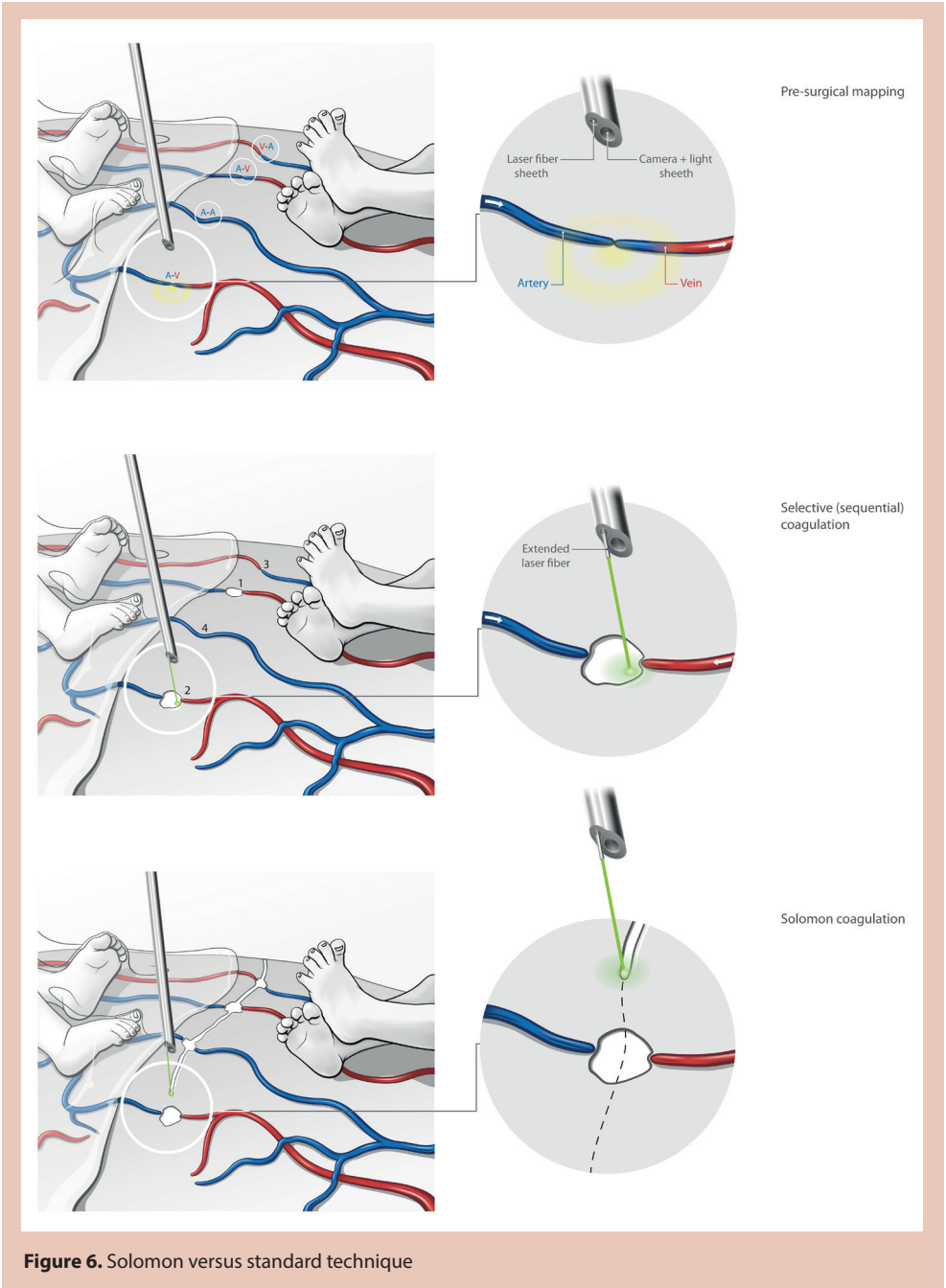

Figure 6. Solomon versus standard technique

Neonatal and long-term outcome

For optimal neonatal care diagnostic criteria for postnatal TAPS and the risk associated with this complication in monochorionic twins should be known. How can we diagnose TAPS postnatally and what should be the criteria? What are the hematological and biochemical characteristics in TAPS donors and recipients? Is TAPS limited to hematological complications? What is the long-term neurodevelopmental outcome in TAPS? And can we identify risk factors for impaired neurodevelopmental outcome?

This thesis will provide answers to the above-mentioned questions and provides diagnostic tools, a stage based classification system, treatment options, how to prevent post-laser TAPS and information on neonatal outcome.

Outline of this thesis

Part I. General introduction

Part II. Review

Chapter 1 – Review of the literature on TAPS. This review focuses on the pathogenesis, incidence, diagnostic criteria, management options and outcome in TAPS. In this review we also proposed a classification system for antenatal and postnatal TAPS.

Part III. Pathogenesis

Chapter 2 – Study on placentas with residual anastomoses after fetoscopic laser surgery for TTTS. Localization, size and consequences of residual anastomoses were studied in this chapter.

Chapter 3 – Study on placenta characteristics in spontaneous versus post-laser TAPS. Localization, size, type, and number of anastomoses were compared between spontaneous TAPS placentas and post-laser TAPS placentas.

Chapter 4 – Study on AA anastomoses in spontaneous TAPS placentas compared to uncomplicated monochorionic twin placentas.

Part IV. Diagnosis

Chapter 5 – This is the first study on MCA-PSV measurements in TAPS. We studied the correlation between MCA-PSV measurements and Hb-deficit.

Chapter 6 – Study on hematological characteristics in neonates with TAPS. In this study we also proposed diagnostic criteria for postnatal detected TAPS.

Part V. Antenatal management and outcome

Chapter 7 – A randomized controlled trial (Solomon trial) on fetoscopic laser surgery for TTTS. We compared the standard selective technique, where only anastomoses were coagulated, with the Solomon technique, where the whole vascular equator was coagulated. We hypothesized that with the Solomon technique the amount of residual anastomoses will be reduced and therefore highly important for the prevention of post-laser TAPS.

Chapter 8 – In this study we reported the secondary outcome of the Solomon trial. We examined and analyzed all placentas that were injected with color dye within the Solomon trial.

Chapter 9 - In this model simulation we show that the addition of PET to IUT, reduces the severity of polycythemia and possible complications as a consequence of hyperviscosity in the recipient.

Chapter 10 – Study on the management options in antenatal detected TAPS. Laser surgery for TAPS appears to improve perinatal survival and neonatal outcome compared to expectant management and intrauterine transfusion.

Part VI. Postnatal management and outcome

Chapter 11 – In this study we compare levels of albumin and total protein in TAPS donors and TAPS recipients. Additionally we looked at placental share in correlation to birth weight.

Chapter 12 – In this case report we showed a case with severe cerebral injury

after post-laser TAPS resulting in neonatal death. This case report shows that TAPS is more than large intertwin Hb difference and also highlights the importance of antenatal Doppler ultrasound monitoring and choice of management.

Chapter 13 – Study on long-term neurodevelopmental outcome in post-laser TAPS. Risk factors were assessed and a subgroup analysis was performed on low cognitive scores.

Part VII. Summary and general discussion

In the summary and general discussion the most important findings of this thesis is outlined and future perspectives are given.

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Chapter 1

Twin anemia polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome

Abstract

Monochorionic twins share a single placenta with intertwin vascular anastomoses, allowing the transfer of blood from one fetus to the other and vice versa. These anastomoses are the essential anatomical substrate for the development of several complications, including twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS). TTTS and TAPS are both chronic forms of fetofetal transfusion. TTTS is characterized by the twin oligopolyhydramnios sequence, whereas TAPS is characterized by large intertwin hemoglobin differences in the absence of amniotic fluid discordances. TAPS may occur spontaneously in up to 5% of monochorionic twins and may also develop after incomplete laser treatment in TTTS cases. This review focuses on the pathogenesis, incidence, diagnostic criteria, management options and outcome in TAPS. In addition, we propose a classification system for antenatal and postnatal TAPS.

Introduction

Almost all monochorionic twins share a single placenta with intertwin vascular anastomoses [1], allowing blood to transfer from one fetus to the other and vice versa. Unbalanced net intertwin blood transfusion may lead to various complications. The best-known clinical syndrome is twin-twin transfusion syndrome (TTTS). TTTS is a chronic form of fetofetal transfusion and affects approximately 9% of monochorionic twins [2]. TTTS leads to hypovolemia, oliguria and oligohydramnios in the one twin (donor) and hypervolemia, polyuria and polyhydramnios in the co-twin (recipient) [3–5]. The main diagnostic criterion for TTTS is the presence of oligohydramnios in the donor twin and polyhydramnios in the recipient twin, the so-called twin oligopolyhydramnios sequence (TOPS).

In 2007, we described a new form of chronic fetofetal transfusion, termed twin anemia-polycythemia sequence (TAPS) [6]. TAPS is characterized by large intertwin hemoglobin (Hb) differences without signs of TOPS. TAPS may occur spontaneously or after laser surgery for TTTS (postlaser form). The spontaneous form complicates approximately 3–5% of monochorionic twin pregnancies [2, 7], whereas the postlaser form occurs in 2–13% of TTTS cases [8, 9]. This review focuses on the pathogenesis, incidence, diagnostic criteria, management options, and short- and long-term outcome in TAPS. In addition, we describe the perinatal outcome in a series of consecutive TAPS cases managed at our center, and propose a staging system to classify TAPS cases diagnosed antenatally and postnatally.

Pathogenesis

TAPS placentas are characterized by the presence of only few, minuscule arteriovenous (AV) vascular anastomoses (fig. 1, 2). The pathogenesis of TAPS is based on this unique angioarchitecture. The few small anastomoses allow a slow transfusion of blood from the donor to the recipient, leading gradually to highly discordant Hb levels. Whether hormonal dysfunction may also play a role in the development of TAPS is not clear. In contrast to TTTS, which results from imbalanced intertwin blood transfusion in combination with imbalanced hormonal regulation, TAPS probably results mainly from slow intertwin blood transfusion without hormonal imbalance [10]. We have recently been able to calculate the actual blood flow through these small AV anastomoses in two postlaser TAPS cases, and found the anastomotic blood flow to be approximately 5–15 ml/24 h [6, 11]. In a recent study on the placental angioarchitecture of 11 consecutive spontaneous TAPS placentas compared to 240 placentas from uncomplicated monochorionic twin pregnancies, we found that TAPS

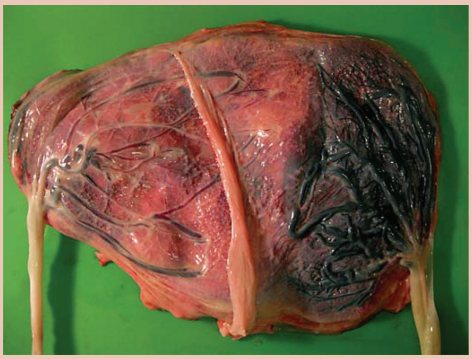

Figure 1. Characteristic placenta in a spontaneous TAPS case, before color dye injection, showing the pale placental share of the donor (left) and the plethoric share of the recipient (right).

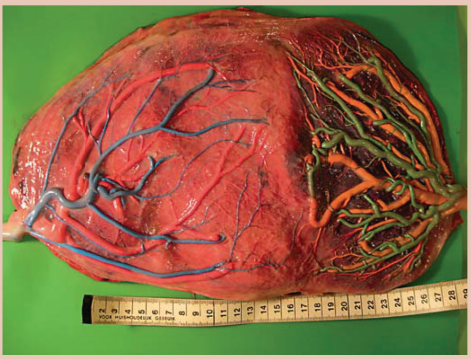

Figure 2. Color dye injection shows the presence of only a few very small anastomoses.

placentas were characterized by the presence of only very few and small unidirectional AV anastomoses in the absence of arterioarterial (AA) anastomoses [12]. Although AA anastomoses are rare in TAPS, the presence of an AA anastomosis does not preclude the development of TAPS. In 2007, we described a case of postlaser TAPS which occurred despite the presence of a small AA anastomosis [11]. Recently, we also detected an AA anastomosis in a spontaneous TAPS case [13]. In a series of 26 TAPS placentas injected at our center to date (from spontaneous TAPS cases and postlaser cases), a small AA anastomosis was found in 3 cases. Thus, the incidence of AA anastomoses in TAPS is approximately 11% (3/26). In comparison, the incidence of AA anastomoses in uncomplicated monochorionic pregnancies and TTTS pregnancies is 80 and 25%, respectively. Importantly, in these 3 cases of TAPS, the diameter of the AA anastomosis was very small (<1 mm).

It is not clear why the donor twin in TAPS cases does not develop oligohydramnios and the recipient twin does not develop polyhydramnios, such as in TTTS. The absence of amniotic fluid discordances in TAPS may be related to the very slow intertwin blood transfusion, allowing more time for hemodynamic compensatory mechanisms to take place [12]. Another explanation is that in postlaser TAPS cases, the colloid osmotic pressure of the ex-recipient is strongly increased prior to and shortly after laser therapy, which attracts excess fluid from the maternal blood to the recipient's fetal blood. This source of increased fetal plasma volume followed by amniotic fluid production delays the development of oligohydramnios in the ex-recipient [10]. Some monochorionic twins may first develop severe Hb discordance followed by amniotic fluid discordance. We were able to reproduce this chain of events in a computerized model in which incomplete laser therapy was simulated and one patent AV anastomosis was left open [14]. In some circumstances, e.g. in case of small unidirectional

AV anastomoses, TAPS may thus precede the onset of TOPS. Interestingly, in postlaser TAPS, it is usually the former recipient who becomes anemic, whereas the former donor becomes polycythemic [8, 15–17]. In a series of 22 postlaser TAPS cases detected at our center, in the majority (77%, 17/22) of cases the previous recipient became the new donor.

Incidence

Only a few studies have reported on the incidence of postlaser TAPS and spontaneous TAPS. The incidence of postlaser TAPS ranges from 2 to 13% depending on the criteria and definitions used [8, 9]. Robyr et al. [8] reported on late complications of 101 TTTS cases treated with fetoscopic laser surgery and found a 13% incidence of postlaser TAPS. Habli et al. [9] report a lower incidence of TAPS (2%). The incidence of TAPS in TTTS cases treated with laser at our center is 8% (22/276) (unpublished data). Occurrence of TAPS after laser treatment may be regarded as treatment failure, when the perceived goal of the operation is complete dichorionization of the placenta and division of the fetal circulations [8, 15–17]. However, care should be taken when comparing the different reported rates between the various centers. A difference in the incidence of TAPS may be due to several factors including placenta injection technique, completeness of follow-up, selection bias and different criteria for TAPS. We use antenatal as well as postnatal criteria and measure Hb levels in all monochorionic twins at birth (see diagnostic criteria below) and inject all monochorionic placentas as described below. The TAPS criteria used by Robyr et al. [8] and Habli et al. [9]

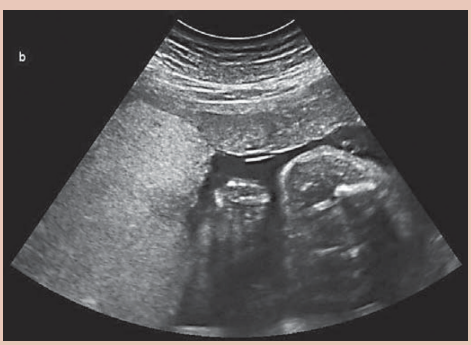

Figure 3. Ultrasound image of a TAPS placenta showing the difference in echodensity and thickness.
On the left side the hydropic placenta part of the anemic donor twin and on the right side the normal aspect of the placenta of the recipient can be seen.

Figure 4. Spontaneous TAPS twin pair at birth: a pale anemic donor (left) and a plethoric polycythemic recipient (right).

were based only on antenatal findings. More restrictive criteria for TAPS may automatically lead to a lower incidence of TAPS.

The incidence of spontaneous TAPS varies between 3 and 5%. In a series of 113 monochorionic twin pairs without TTTS delivered at our center we registered 3 cases of TAPS [2, 7]. Our findings are in accordance with the recently reported findings in the prospective study from Lewi et al. [2] in which they found an incidence of 5% (10/202).

Diagnosis

Absence of antenatal ultrasound signs of oligohydramnios and polyhydramnios is a conditio sine qua non for the diagnosis of TAPS [18]. The presence of TOPS is pathognomonic for TTTS and requires a different management. In contrast with TTTS, TAPS can be diagnosed either before or after delivery [18]. Since TAPS has just recently been described, uniform criteria still need to be clearly established.

Antenatal criteria	Postnatal criteria
MCA-PSV >1.5 MoM in the donor *and*	Intertwin Hb difference >8.0 g/dl *and* at least one of the following:
MCA-PSV <1.0 MoM in the recipient	• Reticulocyte count ratio >1.7 • Placenta with only small (diameter <1 mm) vascular anastomoses

Table 1. Antenatal and postnatal criteria for TAPS.

Antenatal Criteria

Antenatal diagnosis of TAPS can be reached based on Doppler ultrasound abnormalities showing an increased peak systolic velocity in the middle cerebral artery (MCA-PSV) in the donor twin (suggestive of fetal anemia) and a decreased MCA-PSV in the recipient twin (suggestive of polycythemia). Robyr et al. [8] proposed the use of a MCA-PSV >1.5 multiples of the median (MoM) for the donor twin and <0.8 MoM in the recipient. However, the sensitivity, specificity and predictive value of these criteria have not yet been studied. In our experience, the MCA-PSV in the recipient may not always drop below the 0.8 MoM. In a recent TAPS case detected at our center, although the MCA-PSV of the donor was >1.5 MoM, the MCA-PSV of the recipient remained around 1.0 MoM. Nevertheless, the recipient twin was polycythemic at birth and required a partial exchange transfusion (fulfilling the criteria for postnatal TAPS). In another similar case with one fetus with an MCA-PSV >1.5 MoM and the co-twin's MCA-PSV remaining around 1.0 MoM no intervention was performed but unexpected double fetal demise occurred 2 weeks later. We therefore suggest using a new cutoff level of the MCA-PSV of the recipient <1.0 MoM instead of <0.8 MoM. The proposed antenatal TAPS diagnostic criteria are summarized in table 1. These different cutoff levels

for MCA-PSV in TAPS still need to be validated and compared to the postnatal Hb levels in a larger series.

Antenatal stage	Findings at doppler ultrasound examination
Stage 1	MCA-PSV donor >1.5 MoM and MCA-PSV recipient <1.0 MoM, without other signs of fetal compromise
Stage 2	MCA-PSV donor >1.7 MoM and MCA-PSV recipient <0.8 MoM, without other signs of fetal compromise
Stage 3	As stage 1 or 2, with cardiac compromise of donor, defined as critically abnormal flow[a]
Stage 4	Hydrops of donor
Stage 5	Intrauterine demise of one or both fetuses preceded by TAPS

[a] Critically abnormal Doppler is defined as absent or reversed end-diastolic flow in umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in ductus venosus.

Table 2. Antenatal TAPS classification.

For timely detection and eventually treatment of TAPS cases, we recommend routine measurement of MCA-PSV with Doppler ultrasound on a regular basis (at least once every 2 weeks) in all monochorionic twins, in particular after laser treatment. However, the antenatal diagnosis of TAPS can be missed in later stages of pregnancy because of difficulty in measuring MCA-PSV when one twin is in cephalic position. Interestingly, in several cases of spontaneous TAPS detected antenatally at our center, we found a striking difference in placental thickness and echodensity on ultrasound examination (fig. 3). The placental part of the anemic twin was hydropic and had an increased echodensity while the placental part of the polycythemic twin appeared normal. More studies are required to investigate the validity and significance of these antenatal (Doppler) ultrasound findings.

Postnatal Criteria

Postnatal diagnosis of TAPS is based on the presence of (chronic) anemia in the donor and polycythemia in the recipient (fig. 4), in association with typical placental angioarchitecture as identified by injection with colored dye. Uniform criteria for hematological values in TAPS have not yet been established and several different postnatal criteria have been proposed.

Lewi et al. [2] defined TAPS as the presence of an Hb level <11 g/dl in the anemic twin and >20 g/dl in the polycythemic co-twin. Although the use of fixed cutoff levels for Hb values has clear practical advantages, it does not take into account the correlation of Hb levels with gestational age. Fetal Hb concentration is known to increase linearly with gestation [19–21].

In previous studies, we used gestational-age-independent cutoff levels to define anemia in

the donor (Hb <5th centile) [20] and polycythemia in the recipient (hematocrit >65%) [6]. However, this definition has a practical disadvantage in that it requires the use of a specific normogram related to gestational age. Several normograms have been published and differ slightly from one another [19, 20]. Our criteria are therefore less practical compared to the fixed Hb levels proposed by Lewi et al. [2].

A logical and pragmatic alternative would be to use fixed intertwin Hb differences instead of specific cutoff levels for anemia and polycythemia. In a recent case-control study on hematological values in TAPS compared to a control group of uncomplicated monochorionic twins, we found that all TAPS cases had an intertwin Hb difference >8.0 g/dl [22]. However, large intertwin Hb differences may also sporadically occur in uncomplicated monochorionic twin pairs due to acute intrapartum TTTS or due to acute placentofetal transfusion after delivery of the first twin [23]. An additional criterion is therefore required to differentiate a chronic intertwin transfusion phenomenon (TAPS) from acute forms of intertwin transfusion. According to our findings, determination of reticulocyte count has an important diagnostic value in TAPS. Since blood loss in acute peripartum TTTS or acute placentofetal transfusion occurs rapidly, the reticulocyte count in the anemic co-twin is not increased. In contrast, in TAPS cases, the reticulocyte count in the anemic co-twin is always increased, reflecting chronic blood loss, and it is always significantly higher than in its co-twin. In a recent case-control study on hematological values in TAPS, we found that an increased intertwin reticulocyte count ratio >1.7 was pathognomonic for TAPS (the intertwin reticulocyte ratio was calculated by dividing the reticulocyte count of the donor by the reticulocyte count of the recipient) [22].

Postnatal stage	Intertwin Hb difference, g/dl
Stage 1	> 8.0
Stage 2	> 11.0
Stage 3	> 14.0
Stage 4	> 17.0
Stage 5	> 20.0

Table 3. Postnatal TAPS classification.

Besides high Hb discordances and high intertwin reticulocyte count difference or ratio, a third postnatal criterion for TAPS is based on placental findings. TAPS is characterized by the presence of a small number of minuscule (diameter <1 mm) anastomoses. Accurate injection studies of each monochorionic placenta are crucial to help differentiate TAPS from acute fetofetal transfusion, which in contrast is thought to be mediated through large superficial low-resistance AA and venovenous anastomoses [3]. The proposed postnatal TAPS diagnostic criteria are summarized in table 1.

Since anastomoses in TAPS cases are small and, in postlaser cases, most residual anastomoses are localized at the margin of the placenta [7], accurate color dye injection of the placenta and

meticulous inspection of the vascular equator are of paramount importance. The color dye needs to be massaged along the whole vascular equator with special attention given to the margins of the placenta. A detailed video explaining the technique used at our center to inject a monochorionic placenta can be found on:

http://www.youtube.com/watch?v=Qm4bdLkl9BE.

TAPS Classification

Since TAPS is a heterogeneous disease, a staging system can be helpful to discriminate between the various forms. In addition, a staging system may also prove to be useful in the future to compare and analyze TAPS cases (including effect of treatment) between the various centers. We therefore developed a TAPS staging system based on pathophysiological grounds, to classify TAPS cases using the Doppler ultrasound findings at first detection (table 2). The aim of this staging system is to reflect the increasing severity of anemia and polycythemia in twin pairs with TAPS, including ultrasound signs of clinical deterioration related to increasing fetal anemia. We defined stage 1 as MCA-PSV >1.5 MoM in the donor and <1.0 MoM in the recipient. Stage 2 is defined as MCAPSV >1.7 MoM in the donor and <0.8 MoM in the recipient. In stage 3, in addition to abnormal MCA-PSV values (such as in stage 1 or 2), signs of cardiac compromise of the donor (defined as absent or reversed end-diastolic flow in umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in ductus venosus) must be present. Stage 4 is based on the presence of hydrops fetalis in the donor due to severe anemia. Stage 5 represents the worst stage when intrauterine demise occurs in 1 or both fetuses preceded by TAPS.

Since the diagnosis of TAPS is often not reached antenatally (in cases where Doppler ultrasound measurements were not performed or in cases where MCA-PSV were falsely negative), we also propose a postnatal staging system to determine the severity of TAPS. The postnatal stage classification is based solely on the intertwin Hb difference on day 1. The larger the difference is, the higher is the stage of TAPS (table 3).

Perinatal Management and Outcome

The perinatal mortality and morbidity rate in TAPS is not known. The range of outcome in TAPS is wide and is probably due to the heterogeneity of the disease. TAPS may remain undetected during pregnancy (in cases where Doppler measurements are not performed)

and result in 2 healthy neonates without major morbidity at birth besides large intertwin Hb discordance. On the other side of the spectrum, TAPS may also lead to double intrauterine fetal demise, particularly when undetected or untreated.

To date only case reports or small series have been reported on the management and perinatal outcome in TAPS [22, 24–27]. There are several treatment options in TAPS, including expectant management, induction of labor, intrauterine blood transfusion (IUT; intravenous and/or intraperitoneal), selective feticide or (repeat) fetoscopic laser surgery.

Although treatment with IUT has often been reported, it is only a temporary symptomatic treatment (for the donor) and not a causal treatment. The effect of the IUT may be of short duration since the chronic intertwin transfusion is not interrupted. Moreover, a potential side effect of IUT treatment is worsening of the polycythemia-hyperviscosity syndrome in the recipient. Robyr et al. [8] reported a postlaser TAPS case treated with several IUTs in which the recipient twin developed skin necrosis of the leg (Hb concentration in the recipient twin at birth was 28 g/dl). In a recent report, Herway et al. [25] suggested that intraperitoneal transfusion might be superior to intravenous blood transfusion in TAPS cases. Intraperitoneal transfusion may allow a slower absorption of red blood cells into the fetal circulation preventing rapid loss of transfused blood into the circulation of the recipient twin [25]. Our thus far limited experience with this method confirms this theory.

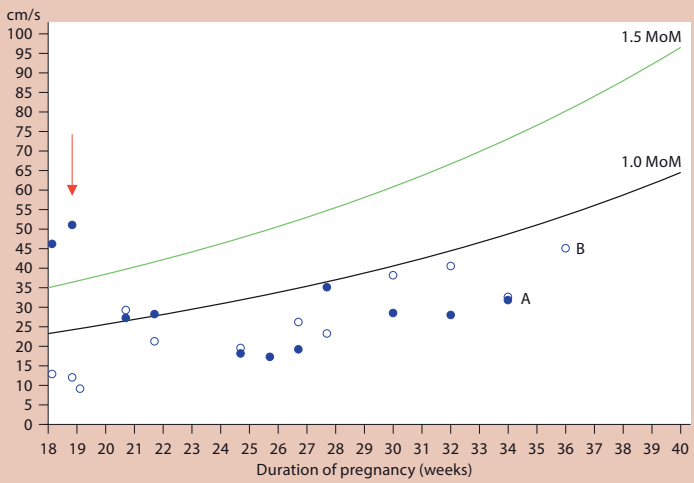

Figure 5. Measurements of MCA-PSV with Doppler ultrasound in a spontaneous TAPS case. Primary fetoscopic laser surgery was successfully performed at 19 weeks (arrow). Two small AV anastomoses were coagulated.
A= Donor twin ●
B= Recipient twin ○

The only causal treatment for TAPS is fetoscopic laser coagulation of the vascular anastomoses. However, laser surgery in TAPS can be technically more challenging than in TTTS because

of the absence of polyhydramnios and a stuck twin, which makes the visualization of the vascular equator more difficult. Moreover, placental anastomoses in TAPS are known to be only few and minuscule and may therefore be missed during fetoscopy.

Spontaneous resolution of antenatal TAPS has also been reported. In a previously published case report, evident ultrasound signs of TAPS persisted for several weeks but eventually resolved without any therapeutic intervention. Spontaneous resolution likely resulted from spontaneous thrombosis of the residual AV anastomosis [28]. Whether expectant management would lead to spontaneous resolution in other TAPS cases is not known and should be considered to be unlikely.

More studies (ideally a multicenter randomized trial) are needed to determine the optimal management option for TAPS. Independently of the treatment of choice for TAPS, it is even better if it can be prevented. Prevention of postlaser TAPS can be achieved by reducing the rate of residual anastomoses. An alternative laser surgery technique (so-called 'Solomon technique') may help reduce the risk of missing a small anastomosis during surgery. With the Solomon technique the entire vascular equator is coagulated on the chorionic surface instead of selectively coagulating each visible anastomosis. We recently initiated a randomized controlled trial (Solomon trial, http://www.trialregister.nl, trial No.: NTR1245), to investigate if this technique reduces the rate of recurrence of TTTS and postlaser TAPS (http://www.studies-obsgyn.nl/solomon/page.asp?page_id = 786).

Perinatal Outcome in TAPS Cases Managed at Our Center

Between June 2002 and December 2009 30 patients were diagnosed with TAPS, of whom 18 (60%) were diagnosed antenatally and 12 (40%) were diagnosed only postnatally. Overall, 66% (20/30) of the TAPS cases fulfilled the postnatal TAPS diagnostic criteria. Ten TAPS twin pairs detected antenatally did not fulfill the postnatal criteria because of single or double fetal demise (n = 6), spontaneous antenatal resolution (n = 1), resolution after laser intervention (n = 2), and ongoing pregnancy (n = 1).

Out of the 18 patients prenatally diagnosed with TAPS at our center, TAPS occurred spontaneously in 4 (22%) cases and in 14 (78%) cases after laser surgery. Management consisted of expectant management (close monitoring with frequent cardiotocography and

ultrasound) (n = 10), IUT (n = 4), fetoscopic laser surgery (n = 1) and IUT followed by fetoscopic laser surgery (n = 1). One couple requested termination of pregnancy and one couple requested selective feticide of the new donor (ex-recipient). Details on the characteristics and management of the 18 prenatally diagnosed TAPS cases are presented in table 4.

	Expectant management	IUT	IUT + laser	Laser	Selective feticide	TOP
Pregnancies, n	10	4[a]	1	1	1	1
GA at diagnosis, wks	24 (20-29)	24 (21-28)	24	18	19	18
GA at delivery, wks	34 (32-41)	29 (26-29)	32	36	28	18
Perinatal survival	15/20 (75%)	8/8 (100%)	2/2 (100%)	2/2 (100%)	1/2 (50%)	0
Postnatal treatment[b]	7/15 (47%)	8/8 (100%)	0	0	0	0

[a] Including 1 patient treated with intraperitoneal transfusion at 26 weeks' gestation (pregnancy still ongoing).
[b] Postnatal treatment is defined as blood transfusion due to neonatal anemia and/or partial exchange transfusion due to polycythemia-hyperviscosity syndrome.
TOP = Termination of pregnancy. GA = Gestational age (median, range); n/N = number per total number.

Table 4. Perinatal management and outcome in 18 antenatal TAPS cases detected at our center

In the group managed expectantly (n = 10), perinatal survival was 75% (15/20). Five fetal deaths occurred, among which there was one double demise (spontaneous TAPS) at 22 weeks preceded by signs of fetal hydrops in one twin (anemic twin) and three single fetal demises (all postlaser TAPS); in all 10 cases the former recipient became the new anemic donor. In 2 cases there were signs of fetal hydrops. One postlaser TAPS case in the expectant management group has been reported previously and showed complete spontaneous resolution without intervention [28]. Seven of the 15 surviving twins had hematological complications requiring partial exchange transfusion or blood transfusion (47%).

In the group treated with IUT (n = 4), perinatal survival was 100%. Two postlaser cases treated with IUTs were reported previously [6, 29]. In both cases, treatment with IUT allowed us to temporarily prolong the pregnancy and avoid extremely preterm delivery. However, although treatment with IUT initially improved the clinical condition of the donor, TAPS reoccurred in both cases within 1 week. In the third case transfusion was given intraperitoneally and pregnancy was prolonged with this treatment from 22 to 26 weeks. In the fourth case an intraperitoneal transfusion was performed at 26 weeks' gestation; this pregnancy is still ongoing.

In the group treated with fetoscopic laser surgery (n =2), perinatal survival was also 100%. Both were spontaneous TAPS cases. One case was treated first with IUT at 24 weeks'

gestation, but as TAPS rapidly reoccurred, fetoscopic laser surgery was performed at 25 weeks' gestation. The second case was treated primarily with fetoscopic laser surgery at 19 weeks. A diagram showing the course of the MCA-PSV before and after laser surgery is shown in figure 5. Both pairs of twins were alive and well at discharge and had no hematological complications. Placental injection in both cases showed no residual anastomoses.

The stage distribution in the 18 cases with antenatal TAPS according to our new antenatal classification was 17% (3/18) stage 1, 28% (5/18) stage 2, 8% (1/18) stage 3, 33% (6/18) stage 4, and 17% (3/18) stage 5.

Perinatal survival per fetus was 83% (5/6) in stage 1, 80% (8/10) in stage 2, 100% (2/2) in stage 3, 92% (11/12) in stage 4, and 33% (2/6) in stage 5. The postnatal stage distribution in the 20 cases fulfilling the postnatal TAPS criteria was: 25% (5/20) stage 1, 30% (6/20) stage 2, 25% (5/20) stage 3, 15% (3/20) stage 4, and 5% (1/20) stage 5. Neonatal survival was 98% (39/40). One neonatal death occurred in a postnatal stage 4 TAPS donor after withdrawal of life support because of antenatally acquired severe brain injury. Postnatal treatment (blood transfusion or partial exchange transfusion) per neonate was required in 40% (4/10) of neonates with postnatal stage 1, 67% (8/12) of neonates with postnatal stage 2, 90% (9/10) of neonates with postnatal stage 3, 100% (6/6) of neonates with postnatal stage 4, and 100% (2/2) in postnatal stage 5. Thus, an increasing postnatal stage is positively correlated with an increased need for postnatal treatment.

Neonatal and Pediatric Outcome

Knowledge on the neonatal and pediatric morbidity in TAPS is scarce and based on case reports and small series. Neonatal morbidity in TAPS appears to be mainly limited to hematological problems at birth. Donor twins may be severely anemic and require blood transfusions, whereas recipient twins may be severely polycythemic and require partial exchange transfusion. As reported above, polycythemia-hyperviscosity syndrome in recipient twins with TAPS may even lead to skin necrosis of the leg [8].

We recently performed a case-control study of 19 TAPS cases with double survivors at birth (38 infants) and compared the short-term outcome with a control group of uncomplicated monochorionic twins matched for gestational age [30]. The incidence of neonatal mortality and morbidity was similar in the TAPS group and control group, 3% (1/38) versus 1% (1/76) and 24% (9/38) versus 28% (21/76), respectively. Severe cerebral injury was detected in 1

(5%) infant in the TAPS group and 1 (2%) infant in the control group. In the TAPS group, blood transfusion at birth was required in 80% (15/19) of donors, and partial exchange transfusion for polycythemia-hyperviscosity syndrome was required in 68% (13/19) of recipients compared to 5% (2/38) and 3% (1/38) in the control group, respectively ($p < 0.01$).

Another important finding reported in our recent case series is that TAPS neonates, in particular recipients, are at risk of thrombocytopenia [22] . Thrombocytopenia occurred more often in the TAPS group than in the control group, 45% (17/38) versus 11% (11/38), respectively ($p < 0.01$). Mean platelet count was significantly lower in recipients than in donors, 133 versus 218 $<10^9$/l, respec tively ($p < 0.01$). The cause of thrombocytopenia in recipients is related to the polycythemia-hyperviscosity syndrome. Platelet count in recipients was negatively correlated with the Hb level at birth (Spearman correlation coefficient = –0.698, $p = 0.001$).

The incidence of long-term neurodevelopmental impairment in TAPS is not known. In a recent multicenter follow-up study in TTTS cases treated with laser, we were able to determine the long-term outcome in a small group of 6 pairs of twins with postlaser TAPS. Neurodevelopmental outcome was within normal ranges in the 12 surviving infants [31]. Larger studies are required to determine the risk of neurodevelopmental impairment in survivors after spontaneous TAPS and postlaser TAPS. Ideally, international collaboration between fetal centers using a web-based registry is needed to gather information on the short- and long-term outcome in TAPS cases.

Conclusions

TAPS is a newly described form of fetofetal transfusion through small (<1 mm) anastomoses that may occur in monochorionic twins spontaneously or after laser treatment for TTTS. Increasing awareness of TAPS is crucial in order to improve the detection and treatment in these cases. Further studies are required to determine the accuracy of the diagnostic criteria, the optimal treatment and the (long-term) prognosis in TAPS.

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Chapter 2

Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences

Abstract

Objective:

To study the localization and size of residual anastomoses in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery and correlate the findings with outcome.

Study design:

Placental injection in twin-to-twin transfusion syndrome placentas treated with laser was performed by using colored dye.

Results:

A total of 77 twin-to-twin transfusion syndrome placentas were included in the study. Residual anastomoses (n=48) were found in 32% (25/77) of lasered placentas. Most residual anastomoses were localized near the margin of the placenta. The majority of residual anastomoses (67%; 32/48) were very small (diameter, < 1 mm). Eleven of the 25 cases (44%) in the residual anastomoses group developed twin anemia-polycythemia sequence.

Conclusion:

Most residual anastomoses in twin-to-twin transfusion syndrome placentas treated with laser are very small and localized near the placental margin. Almost half of cases with residual anastomoses developed twin anemia-polycythemia sequence after laser surgery.

Introduction

Twin-to-twin transfusion syndrome (TTTS) affects approximately 15% of monochorionic twin pregnancies and results from unbalanced intertwin blood flow between the donor and the recipient twin through placental vascular anastomoses. Untreated, TTTS is associated with high perinatal mortality and morbidity.[1] Fetoscopic laser occlusion of the vascular anastomoses is currently the best treatment option for TTTS.[2] The aim of laser surgery is to separate completely both fetal circulations by occluding all placental vascular anastomoses. However, several studies have shown that residual anastomoses (RA) may still be present after laser surgery and can be detected in up to 33% of lasered placentas.[3,4] RA may lead to recurrence of TTTS in 14% of cases and twin anemia polycythemia sequence (TAPS) in 13% of cases.[5]

The aim of this study was to measure the size of the RA and determine the localization of the RA in relation to the margin of the placenta. A secondary aim of this study was to determine the association between RA and hematologic complications at birth.

Materials and methods

TTTS placentas treated with laser at the Leiden University Medical Center and consecutively examined at our center between June 1, 2002, and Aug. 1, 2008, were included in this study. Leiden University Medical Center is the national referral center for in utero management of TTTS in The Netherlands. Part of the placental data in this study was included in a previous report on RA.[4] We excluded TTTS cases with intrauterine fetal demise (because of placental maceration) and TTTS cases treated with an alternative laser technique that comprises coagulation of the placental surface along the entire vascular equator. Damaged placentas were excluded if deterioration was too extensive to allow adequate and complete injection study. Furthermore, TTTS cases in which laser surgery was interrupted because of poor visibility or other reasons, were also excluded. Diagnosis of TTTS was based on internationally accepted standardized antenatal ultrasound criteria.[6]

Placental injection with colored dye was performed to determine the localization, size, type, and number of RA. Details on the technique used for placental injection have been described previously.[4] Digital pictures of the placentas were taken perpendicularly to the placenta. We measured the length of the vascular equator on the digital picture of the placenta using Image Tool for Windows version 3.0 (Image Tool, San Antonio, TX). We measured the

distance between each RA and the margin of the placenta and expressed this distance as a percentage of the distance between margin and center of the vascular equator. We divided the distance between margin and center of the vascular equator into 5 equal segments (of 20%).

The following obstetric and neonatal data were retrieved from our databases, including gestational age at laser surgery and at birth, localization of the placenta on the uterine wall (anterior or posterior), recurrence of TTTS, TAPS, hemoglobin levels and reticulocyte count at birth, need for blood transfusion or partial exchange transfusion at birth. For the purpose of this study, diagnosis of TAPS was based on postnatal criteria.7 Postnatal criteria were chronic anemia in the donor (hemoglobin, < 2 standard deviations [SD])[8,9] and polycythemia in the recipient (hematocrit, > 65%) on day 1. Chronic anemia was differentiated from acute anemia based on the presence of a reticulocyte count > 2 SD.[9]

Results of categorical variables were compared with the x^2 test. Continuous variables were analyzed with the independent samples *t* test. A P value less than .05 was considered to indicate statistical significance. Analysis was performed by using SPSS version 14 (SPSS Inc, Chicago, IL).

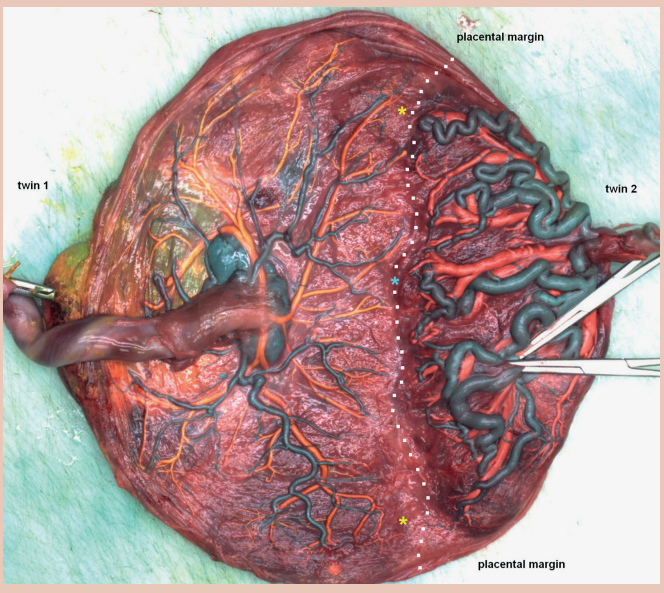

Figure 1. TTTS placenta treated with laser surgery at 17 weeks' gestation and delivered at 33 weeks' gestation. The white dots show the vascular equator and the light-blue star indicates the center of the vascular equator. The yellow stars indicate 2 small RA near the margins of the placenta: 1 arteriovenous anastomosis (top of the picture) at 27% distance from the placental margin (in relation to the placental center) and 1 venovenous anastomosis (bottom of the picture) at 17% distance from the placental margin.

Results

A total of 123 TTTS placentas treated with laser were consecutively examined at our center. Forty-one placentas were excluded because of intrauterine fetal demise (n=22), laser coagulation of the entire vascular equator (n=14), or damaged placenta (n=6). Four TTTS cases were excluded because laser surgery was incomplete because of a "stucktwin" on the vascular equator

(n=2) or blurred vision (n=2). In 1 case, a large arterioarterial anastomosis was detected during fetoscopy but could not be coagulated because of its size. This placenta was included in the analysis but the arterioarterial anastomosis was excluded from the calculations.

A total of 77 placentas were thus analyzed. RA were found in 25 (32%) placentas.
These 25 placentas showed 48 anastomoses, a mean number of 2 RA per placenta (range,

	RA group (n=25)	No RA group (n=52)	p value
Gestational age at laser (wk) [a]	20.3±3.7	20.6±3.1	0.62
Gestational age at birth (wk) [a]	31.9±3.1	32.9±3.7	0.26
Anterior placenta, n(%)	10 (40%)	19 (37%)	0.77
TAPS after laser surgery, n(%)	11 (44%)	0 (0%)	<0.001
Intertwin hemoglobin difference at birth (g/dL) [a]	10.0±5.5	2.1±2.5	<0.001
Intertwin reticulocyte count difference at birth (‰) [a]	68.3±74.1	8.9±10.5	<0.001
Neonates requiring a blood transfusion at birth, n(%)	16 (64%)	5 (10%)	<0.001
Neonates requiring a partial exchange transfusion, n(%)	11 (44%)	0 (0%)	<0.001

RA, residual anastomoses; SD, standard deviation; TAPS, twin anemia-polycythemia sequence.
[a] Values given as mean ± SD.

Table 1: Differences between the group with and without residual anastomoses

1-5). Most RA were arteriovenous anastomoses (85%; 41/48), of which 41% (17/41) were from donor to recipient and 59% (24/41) from recipient to donor. Residual arterioarterial anastomoses were found in 10% (4/48) of cases and venovenous anastomoses in 7% (3/48). The majority of RA (67%, 32/48) were very small (diameter, < 1 mm). An example of RA detected near the placental margin is shown in Figure 1. The localization of RA on the vascular equator is shown in Figure 2. Most RA were localized close to the placental margin.

Differences in perinatal outcome between the group with and without RA are

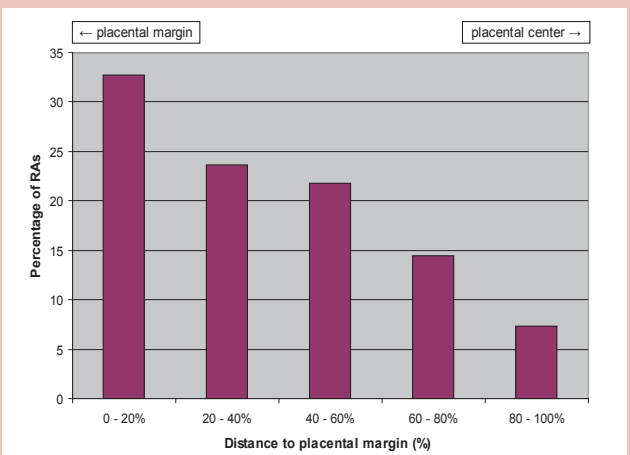

Figure 2. Localization of residual anastomoses in relation to the placental margin

shown in table 1. Presence of RA was associated with a higher incidence of TAPS. Recurrent TTTS after laser was not found in this cohort. Twins in the RA group were more often anemic or polycythemic at birth requiring a blood transfusion (64%) or a partial exchange transfusion (44%) compared with infants in the no-RA group (10% and 0%, respectively).

Comment

This study shows that RA after laser are a common finding and that most RA are situated near the margin of the placenta. This finding is in accordance with a previous study from Lewi et al.[3] in which they found that most missed anastomoses (56%) were within 2 cm of the placental margin.

The reason for the clustering of RA near the placental margins is not clear. Two explanations may be envisaged: first, placental margins may be more difficult to visualize during fetoscopic surgery because of technical reasons associated with the position and the angle of the fetoscope. Ideally, the fetoscope should be inserted perpendicularly to the vascular equator to allow a clear view of the whole vascular equator. However, this is not always possible, particularly when the placenta is placed on the anterior uterine wall. A suboptimal angle of the fetoscope may prevent a complete view of the vascular equator, and in particular of the placental margin. A second possible explanation may that placental margins may be less well scrutinized during fetoscopic laser surgery, even in the absence of previously mentioned technical problems related to the angle of the fetoscope.

Second, as shown in this and other studies, RA are clearly associated with TAPS or recurrent TTTS after laser surgery.[3-5] Whether more careful scrutinization of the placental margins can lead to reduction of RA and its associated complications needs further study.

A third important result of this study is that most RA are very small (diameter, < 1 mm). This finding is in agreement with previous reports.[3,4] Various reasons may explain the high rate of these hair-thin anastomoses. First, anastomoses that are barely visible on placental examination without colored dye may obviously be even more difficult to detect during fetoscopy that took place several weeks/months before. Second, placental vessels of the donor twin may have been collapsed at the time of fetoscopy because of hypovolemia and vasoconstriction.

Further research to find a way to reduce the rate of RA and associated complications is

warranted. A possible solution for this problem could be to draw a laser coagulation line across the entire vascular equator from one placental margin to the other, instead of coagulating only the visible anastomoses. Hypothetically, this technique may be more effective in coagulating all vascular anastomoses, in particular the very small anastomoses that may be difficult to identify during fetoscopy. We have recently started a randomized control trial (named Solomon trial; www.trialregister.nl, trial ID: NTR1245) to determine whether this alternative laser technique (so-called Solomon technique) reduces the incidence of RA and consequently the incidence of TAPS and recurrent TTTS.

The incidence of RA (32%) found in this study was similar to our previous report.[4] Lewi et al.[3] also reported a 32% (16/50) incidence of RA by using a similar placenta injection technique with colored dye. In contrast, Quintero et al.[10,11] report a much lower incidence of RA (4%; 3/75 and 6%; 4/62) in their series of lasered placentas by using air injection. Differences in incidence of RA could theoretically be explained by differences in surgical techniques and expertise. However, care should be taken when interpreting the various results. Difference in incidence of RA could also be related to methodologic differences, including the injection technique used (colored dye injection vs air injection) and selection bias. We were not able to examine a consecutive series of all placenta injected at our center as not all placentas delivered elsewhere are shipped back to our department. A selection bias is thus introduced because uncomplicated cases are more likely to deliver elsewhere and this could explain the higher incidence of RA reported in our studies. We have shown in a previous study that complicated TTTS-cases after laser surgery were more commonly delivered at our tertiary care center.[12]

An important limitation of the measurements performed in this study warrants further attention. In vitro examination of the placenta on a table/flat surface is a simplification of the more complex in vivo situation in which the placenta is attached on a concave uterus. Care should thus be taken when extrapolating our in vitro measurements to the in vivo situation.

Although laser surgery has been shown to be the best available treatment for TTTS, results are still suboptimal, leaving room for improvement. This study shows that, as most RA are situated near the placental margin, fetal surgeons may need to inspect the margins of the vascular equator more carefully. Whether this knowledge can lead to reduction of RA by more careful surgical technique needs further study.

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Chapter 3

Placental characteristics in monochorionic twins with spontaneous versus post-laser twin anemia polycythemia sequence

Abstract

Introduction:

Twin anemia-polycythemia sequence (TAPS) may occur in monochorionic twins either spontaneously or after laser surgery for twin-twin transfusion syndrome. Our aim was to analyze the placental angioarchitecture in spontaneous versus post-laser TAPS.

Methods:

We included all monochorionic twin placentas with spontaneous or post-laser TAPS injected at our center between 2002 and 2012. Placental angioarchitecture was evaluated using colored dye injection.

Results:

A total of 600 monochorionic placentas were injected during the study period of which 43 (7.2%) with TAPS (spontaneous TAPS, n=16; post-laser TAPS, n=27). Almost all anastomoses (96%; 119/124) were very small (diameter <1 mm) and the majority was localized near the placental margin. The median number of anastomoses per placenta was 4 (interquartile range (IQR): 3-5) in the spontaneous TAPS group and 2 (IQR: 1-3) in the post-laser TAPS group ($p = 0.003$). Arterio-arterial (AA) anastomoses were detected in 14.0% (6/43) of TAPS placentas and were all minuscule (diameter <1 mm). The rate of AA anastomoses in the spontaneous TAPS group and post-laser TAPS group was 18.8% (3/16) and 11.1% (3/27), respectively ($p = 0.184$).

Discussion:

Spontaneous TAPS placentas have a significantly higher total number of anastomoses compared to post-laser TAPS placentas. Most anastomoses were localized near the margins of the placenta. Minuscule AA anastomoses were detected sporadically in both groups and the rate of AA anastomoses is slightly higher in the spontaneous TAPS group than in the post-laser group.

Conclusion:

Spontaneous TAPS placentas have a different placental angioarchitecture than post-laser TAPS placentas in terms of number and type of vascular anastomoses.

Introduction

Twin anemia-polycythemia sequence (TAPS) is a rare form of feto-fetal transfusion and can be diagnosed ante- and postnatally. Antenatal diagnosis is based on predefined Doppler ultrasound criteria [1]. Postnatal diagnosis uses hematological criteria (chronic anemia with an increased reticulocyte count in the donor and polycythemia in the recipient) in combination with placental injection studies [2,3].

The typical angioarchitecture in TAPS placentas demonstrates only a few, minuscule and mostly unidirectional arterio-venous (AV) anastomoses [2,4]. Arterio-arterial (AA) anastomoses are rare and have, when present, a very small diameter [2,5]. TAPS can occur spontaneously in uncomplicated monochorionic (MC) twin pregnancies or after laser treatment for twin-to-twin transfusion syndrome (TTTS). The spontaneous form occurs in 3-5% of monochorionic pregnancies, while the post-laser form complicates 2-13% of TTTS pregnancies treated with laser coagulation [2,6-9]. Both forms of TAPS are characterized by the presence of large intertwin hemoglobin discordances without amniotic fluid discordances that are needed to diagnose TTTS [2]. Whether both forms have different clinical outcomes and different placental angioarchitecture has not been studied yet.

The objective of this study was to estimate the number and types of placental anastomoses in placentas with spontaneous TAPS compared to placentas with post-laser TAPS.

Methods

All consecutive TAPS placentas examined at our center between June 2002 and October 2012 were included in this study. Our center is a tertiary national referral center for the management of complicated monochorionic twin pregnancies, including TTTS and TAPS. Cases with an incomplete placental injection study were excluded from the study. We compared the placental angioarchitecture in spontaneous TAPS cases with post-laser TAPS cases. Some of the placental data has been published previously [4,5,10].

For the purpose of this study, TAPS was diagnosed using the following proposed postnatal criteria. An inter-twin hemoglobin difference >8.0 g/dl and at least one of the following: reticulocyte count ratio >1.7 or placenta with only small (diameter < 1 mm) vascular anastomoses [2]. Hemoglobin levels and reticulocyte count are routinely measured at birth in all monochorionic twins.

Each monochorionic placenta examined at our center is routinely injected with colored dye according to a previously described protocol [11]. After colored dye injection, placentas are photographed in a plain view, and digital pictures are saved for computer analysis. Data on placenta angioarchitecture, including the number, type and size of anastomoses and the percentage of placental territory are recorded and entered in a dedicated database. We also recorded the type of abnormal umbilical cord insertion, velamentous or marginal insertion (within 1 cm of the placental margin). Combination insertions are the combination of cord insertions of one placenta. The term peripheral is used in this context to indicate both marginal and velamentous insertions. The insertion-diameter ratio is the ratio between the distance between the two cord insertions and the maximum diameter of the placenta.

We measured the distance between each anastomosis and the margin of the placenta, and expressed this distance as a percentage of the distance between margin and center of the vascular equator. We divided the distance between margin and center of the vascular equator into 5 equal segments (of 20%), as previously reported [10]. Diameters of the anastomoses and individual placental territories were measured using ImageJ 1.45s (ImageJ, National Institutes of Health, USA).

Results of continuous variables were analyzed using ManneWhitney U test and categorical variables were analyzed with Fischer exact test. A p-value <0.05 was considered to indicate statistical significance. All statistical data were analyzed using IBM SPSS Statistics v20.0 (SPSS Inc., an IBM Company, Chicago, IL, USA).

Results

During the 10-year study period, 410 TTTS cases were treated with laser surgery at our center and we were able to examine 65% (265/410) of the lasered placentas. In the TTTS group treated with laser, 27 cases (10%) fulfilled the postnatal criteria for TAPS and were included in the post-laser group. During the same study period, 335 monochorionic placentas without TTTS were also examined at our center. In this group, 16 cases (5%) fulfilled the postnatal criteria for TAPS and were included in the spontaneous TAPS group. In total, 43 placentas fulfilled the inclusion criteria for postnatal TAPS and were included in the study (post-laser TAPS group, n=27; spontaneous TAPS group, n=16). Sixteen (37%) of these cases were also detected antenatally.

In the post-laser TAPS group, 5/27 (18.5%) pregnancies were treated with intrauterine

transfusion (IUT) or intraperitoneal transfusion. In the spontaneous TAPS group, 3/16 (18.8%) pregnancies were treated with intrauterine transfusion or intraperitoneal transfusion. Median gestational age at delivery was 33.5 weeks (interquartile range (IQR): 31-35 weeks) and 33 weeks (IQR: 29-34 weeks) in the spontaneous TAPS group and post-laser TAPS group, respectively (p = 0.038). Median inter-twin hemoglobin difference in the spontaneous and post-laser TAPS group was 14.2 g/dL and 12.2 g/dL, respectively (p = 0.784). Further details on the baseline characteristics are shown in Table 1.

	MC twins with spontaneous TAPS (n=32)	MC twins with post-laser TAPS (n=54)
Female n/N(%)	14/32 (44)	18/54 (33)
Gestational age at delivery - weeks [a]	33.5 (31-35)	33 (29-34)
Birth weight - grams [a]	1905 (1489-2109)	1701.5 (1189-2001)
Birth weight smaller twin - grams [a]	1750 (1181-1968)	1520 (1156-1770)
Birth weight larger twin - grams [a]	2020 (1556-2346)	1880 (1237-2140)
Birth weight discordance - % [a]	15.6 (8.3-26)	13.6 (4-20.8)
Caesarean delivery - n/N(%)	14/32 (44)	30/54 (56)
Inter-twin hemoglobin difference - g/dl [a]	14.2 (11.8-19.4)	12.2 (10.3-15.8)

[a] Value given as median (IQR).

Table 1: Baseline characteristics in MC placentas with spontaneous and post-laser TAPS.

A total of 124 anastomoses were detected. Of all anastomoses, the vast majority 96% (119/124) had a diameter <1 mm. Median total number of anastomoses in the spontaneous TAPS group and post-laser TAPS group was 4 anastomoses (IQR: 3-5) and 2 anastomoses (IQR: 1-3), respectively (p = 0.003). AV anastomoses were present in all (43/43) placentas. AA anastomoses were present in only 14% (6/43) of TAPS placentas and were detected in 18.8% (3/16) of spontaneous TAPS placentas compared to 11.1% (3/27) of postlaser TAPS placentas.

Median diameter of the AA anastomosis diameter in the spontaneous and post-laser TAPS groups was 0.4 mm and 0.6 mm, respectively (p = 0.184). All AA anastomoses (6/6) had a diameter <1 mm. Veno-venous (VV) anastomoses were detected in only 2 post-laser TAPS placentas (2/27; 7.4%) and in none of the spontaneous TAPS placentas. Detailed information of placental angioarchitecture is shown in Table 2.

In the spontaneous TAPS group, we found that in 75% (12/16) of placentas, AV anastomoses from donor to recipient were accompanied by VA anastomoses in opposite direction (from

	MC placentas with spontaneous TAPS (n=16)	MC placentas with post-laser TAPS (n=27)	p value
Number of anastomoses per placenta [a]	4(3-5)	2 (1-3)	0.003
AA anastomoses present (%)	3 (18.8)	3 (11.1)	0.184
VV anastomoses present (%)	0 (0)	2 (7.4)	0.522
AV anastomoses present (%)	15 (100)	27 (100)	1.00
Diameter of AA anastomosis (mm) [b]	0.4 (0.3-0.6)	0.6 (0.5-0.8)	0.184
Placental share discordance (%) [a]	29.7 (12-46)	20.1 (10.7-37)	0.688
Velamentous cord insertion - n(%) [c]	3/32 (9.4)	12/54 (22)	0.153
Marginal cord insertion - n(%) [c]	11/32 (34.4)	19/54 (35.2)	1.00
Velamentous or marginal cord insertion - n(%) [c]	14/32 (43.8)	31/54 (57.4)	0.267
Combination insertion [d]			
Peripheraleperipheral present (%)	5 (31.3)	6 (22.2)	0.719
Peripheralecentral present (%)	5 (31.3)	19 (70.4)	0.025
Centralecentral present (%)	6 (37.5)	2 (7.4)	0.037
Insertion-diameter ratio [a]	74 (60.1-87.6)	70 (59.3-76.7)	0.291

AA: arterio-arterial; VV: veno-venous; AV: arterio-venous.
[a] Value given as median (IQR).
[b] Value given as median (range).
[c] Refers to the type of cord insertion per fetus.
[d] Refers to the combination of cord insertion on one placenta.

Table 2: Placental characteristics in MC placentas with spontaneous and post-laser TAPS.

recipient to donor) or by bidirectional AA anastomosis. In the postlaser TAPS group, this combination occurred in only 37% (10/27) of cases. Compensating VA anastomoses or AA anastomoses occurred significantly more frequently in the spontaneous TAPS group than post-laser TAPS group (75% versus 37%, $p < 0.01$).

Velamentous or marginal cord insertions were present in 43.8% (14/32) and 57.4% (31/54) of spontaneous and post-laser TAPS twins respectively. The combinations of umbilical cord insertions and insertion-diameter ratio in both groups are presented in Table 2.

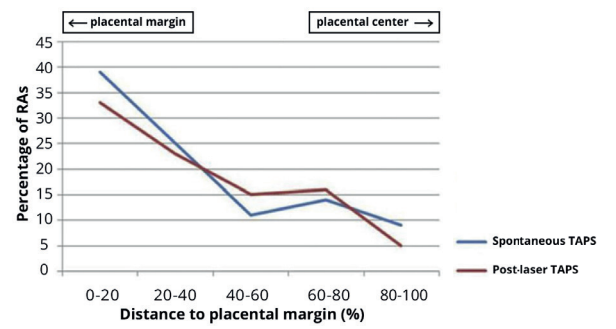

Figure 1. Distance of the anastomoses in relation to the margin of the placenta.

In both the post-laser TAPS group and spontaneous TAPS group, the localization of anastomoses was mostly close to the placental margin (Fig. 1). An example of a post-laser and a spontaneous TAPS placenta is shown in Figs. 2 and 3.

Discussion

TAPS placentas are characterized by the presence of few minuscule AV anastomoses and the rare occurrence of AA anastomoses [2]. This study shows that the unique placental angioarchitecture involved in the pathogenesis of TAPS is present in both spontaneous and post-laser TAPS. However, spontaneous TAPS placentas have a significantly higher total number of anastomoses compared to post-laser TAPS placentas. The discordance in number of anastomoses is probably related to the difference between both TAPS groups. Post-laser TAPS is an iatrogenic de novo event developing because one or two small anastomoses are left patent during surgery.

In addition, the rate of AA anastomoses appeared to be higher in the spontaneous TAPS group (18.8%) than in the post-laser group (11.1%), although the difference was not significant. Given the small numbers of included cases (which is inherent to the rarity of TAPS in general), lack of significance could be due to the fact that the study may be underpowered. Larger studies are required to determine reliably whether there is a difference in percentage

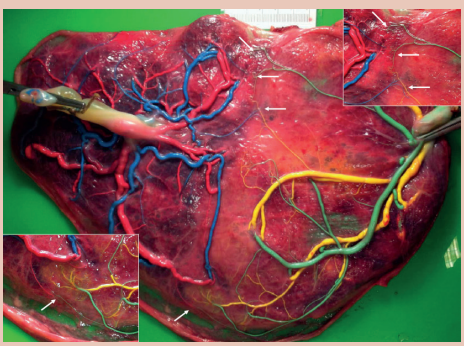

Figure 2. Spontaneous TAPS placenta after colored dye injection (blue or green for arteries and pink or yellow for veins). The placenta share on the right side of the picture belongs to the anemic donor and the placenta share on the left side belongs to the recipient. The white arrows indicate the AV and VA anastomoses. Details of the anastomoses are shown in the top-right and bottom-left corner.

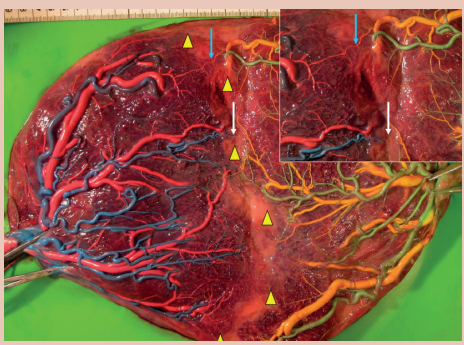

Figure 3. Post-laser TAPS placenta after colored dye injection lasered at 24 weeks' gestation and delivered at 37 weeks' gestation. The placenta share on the right side of the picture belongs to the anemic donor and the placenta share on the left side belongs to the recipient. The white arrow indicates an AV anastomosis and the blue arrow shows a VV anastomosis. The yellow cones show laser spots. Details of the anastomoses are shown in the top-right corner.

of AA between spontaneous TAPS cases and post-laser TAPS cases.
This study confirms that AA anastomoses are detected only in a minority of TAPS cases (14%, 6/43) and all AA anastomoses had a minuscule diameter (1 mm). The combination of few small AV anastomoses and sporadic small AA anastomoses is of paramount importance in the understanding of the pathophysiology of TAPS. These small anastomoses allow only a limited and slow transfer of blood from donor to recipient causing the large inter-twin hemoglobin difference in TAPS, but no oligo-polyhydramnios sequence. The volume of fluid passing through small anastomoses is less than in larger anastomoses as a result of an increased vascular resistance (Poisseuille's law). Discordant hemoglobin levels are therefore caused by the slow inter-twin blood transfusion (as low as 5-15 ml/ 24 h) [12-14]. This allows more time for compensatory hemodynamic regulation systems to act and probably prevents the development of hormonal imbalance and twin oligo-polyhydramnios sequence such as in TTTS [12-14].

We found that AV anastomoses in both directions (from donor to recipient and vice versa) or bidirectional anastomoses are more frequently present in spontaneous TAPS than post-laser TAPS placentas. This may explain why spontaneous TAPS cases may remain stable and undetected until the third trimester, whereas in postlaser TAPS a rapid decompensation occurs a few weeks after the intervention.

This study also shows that (residual) anastomoses (RA) in postlaser TAPS cases are usually found on the margin of the placenta, as previously also reported [10]. Interestingly, we found the same trend of higher rates of anastomoses nearer to the placental margin compared to the placental center in the spontaneous TAPS group. The increased rate of residual anastomoses in the post-laser group is thought to result from increased technical difficulties during fetoscopy to accurately visualize the complete vascular equator. The reason for the increased rate of anastomoses near the placental margin in the spontaneous TAPS group is not known. Whether this unequal spread of anastomoses differs from other monochorionic placentas with or without TTTS is not known either, as the localization of anastomoses has not yet been studied in monochorionic placentas.

The optimal management of TAPS is not clear and includes expectant management, intrauterine blood transfusion and fetoscopic intervention to coagulate the vascular anastomoses [1,2,8,15-17]. The clinical implication of the findings in this study is that if fetoscopic laser coagulation is envisaged, most of the few anastomoses in post-laser TAPS and spontaneous TAPS cases are found near the margins of the placenta. Since these anastomoses are extremely small and difficult to detect, laser coagulation of the complete

equator (Solomon technique) [2] would probably be the preferred laser technique. However, more studies (ideally randomized controlled trials) are required to determine the best treatment intervention in TAPS cases.

In conclusion, this comparative study between spontaneous TAPS and post-laser TAPS cases shows that post-laser TAPS cases have fewer anastomoses, less AA anastomoses and most anastomoses are localized near the margins of the placenta.

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Chapter 4

Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia polycythemia sequence

Abstract

We performed a matched case-control study to analyze the placental angioarchitecture, in particular the diameter of arterio-arterial (AA) anastomoses in monochorionic placentas from pregnancies with spontaneous twin anemia-polycythemia sequence (TAPS) compared to a control group of uncomplicated monochorionic placentas. Placental angioarchitecture was analyzed using colored dye injection. AA anastomoses were detected in 20% (3/15) of spontaneous TAPS placentas. The median diameter of AA anastomoses in the group with and without TAPS was 0.4 mm and 2.2 mm, respectively ($p = 0.01$). In conclusion, AA anastomoses are rarely detected in TAPS placentas. When present, the AA anastomosis is very small, preventing equilibration of hemoglobin levels between both twins.

Introduction

Feto-fetal blood transfusion occurs in all monochorionic (MC) twins because of the invariable presence of placental vascular anastomoses and may lead to the development of twin anemia-polycythemia sequence (TAPS) [1]. TAPS is characterized by large inter-twin hemoglobin differences in the absence of amniotic fluid discordances [1]. The typical angio-architecture in TAPS placentas after colored dye injection demonstrates only a few minuscule arterio-venous (AV) anastomoses [2]. Arterio-arterial (AA) anastomoses are reported to be rare (10%) in TAPS [1]. In contrast, AA anastomoses are almost ubiquitous in normal monochorionic placentas [2], suggesting that AA anastomoses may play a protecting role against the development of TAPS [2].

We previously hypothesized that the diameter of AA anastomoses in TAPS may be smaller, preventing adequate inter-twin blood volume equilibration [3,4]. However, evidence to support this hypothesis is currently lacking. The objective of this study was to compare the diameter of AA anastomoses in MC twin placentas with and without TAPS.

Methods

All consecutive TAPS placentas examined at our center between June 2002 and November 2011 were included in this study. For the purpose of this study we excluded TAPS placentas without AA anastomoses. We also excluded TAPS cases that occurred after laser treatment for twin-twin transfusion syndrome (TTTS). Each spontaneous TAPS placenta was compared with 3 control placentas from uncomplicated MC twin pregnancies and matched for gestational age at birth (+/-1 week gestation). TAPS was diagnosed using standard antenatal ultrasound criteria and/or postnatal criteria [1].

Each MC placenta examined at our center is routinely injected with colored dye according to a previously described protocol [5]. After colored dye injection, placentas are photographed in a plain view, and digital pictures are saved for computer analysis. Data on placenta angioarchitecture, including the number and type of anastomoses, the percentage of placental territory and the type of umbilical cord insertion are prospectively entered in a dedicated database.

The primary outcome was the diameter of the AA anastomoses. Diameters of the AA anastomoses and individual placental territories were measured using Image Tool for

Windows version 3.0 (Image Tool, San Antonio, Texas, USA, http://ddsdx.uthscsa.edu/dig/itdesc.html). Part of the placental data reported in this study was included previously in a case report on AA anastomoses in TAPS [1,4].

Results of continuous and categorical variables were analyzed using the Mann Whitney U test and Fisher exact test. A p-value <0.05 was considered to indicate statistical significance. All statistical data were analyzed using SPSS statistics version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 491 MC placentas were injected during the study period, of which 44 (9%) fulfilled the diagnostic criteria for antenatal or postnatal TAPS. TAPS cases occurred after incomplete laser treatment for TTTS in 66% (29/44) of cases and occurred spontaneously in 34% (15/44) of cases. In this subgroup of 15 cases with spontaneous TAPS, an AA anastomosis was identified in 3 placentas (20%, 3/15). None of these TAPS cases were treated antenatally with fetoscopic laser coagulation. Each TAPS placenta (n = 3) was matched with 3 control MC placentas (n = 9). Mean gestational age at delivery was 32.3 weeks in both groups (range 29-36 weeks).

The median diameter of AA anastomoses in the group with and without TAPS was 0.4 mm (range: 0.3-0.6) and 2.2 mm (range: 0.7-3.5), respectively (p = 0.01). The diameter of the AA anastomosis was 1 mm in each TAPS placenta whereas only 2 AA anastomoses in the

	Study group (n=3)	Control group (n=9)	p value
Number of anastomoses per placenta [a]	5(3-6)	10 (3-16)	0.32
VV anastomoses present (%)	0 (0)	2 (22)	0.55
AV anastomoses present (%)	3 (100)	9 (100)	1.0
Diameter of AA anastomosis (mm) [a]	0.4 (0.3-0.6)	2.2 (0.7-3.5)	0.01
Placental share discordance (%) [a]	42 (24-64)	26 (11-43)	0.08
Unequal placental sharing>20% - n(%)	3 (100)	2 (22)	0.11
Velamentous cord insertion - n(%) [b]	2/6 (33)	6/18 (33)	0.70
Marginal cord insertion - n(%) [b]	2/6 (33)	2/18 (11)	0.25
Velamentous or marginal cord insertion - n(%) [b]	4/6 (66)	8/18 (44)	0.32

AA: arterio-arterial; VV: veno-venous; AV: arterio-venous.
[a] Value given as median (range).
[b] Refers to the type of cord insertion per fetus.

Table 1: Placental characteristics in the study group (TAPS placentas with an AA anastomosis) and control group (uncomplicated MC placentas with an AA anastomosis).

control group had a diameter 1 mm (22.2%, 2/9), leading to a sensitivity of 100% (3/3) and a specificity of 77.8% (7/9). A total of 11 AV anastomoses were detected in the 3 TAPS cases.

The direction of the AVs in the TAPS cases was mostly (64%, 7/11) from the anemic fetus to the polycythemic one. Further details on placental characteristics in both groups are shown in Table 1. An example of a TAPS placenta with a small AA anastomosis is shown in Fig. 1.

Discussion

This study shows that in the rare TAPS cases with an AA anastomosis, the diameter of the AA anastomosis is extremely small (≤1 mm), in accordance with our hypothesis [4]. In addition, this study confirms that only a minority of TAPS placentas (20%) contains an AA anastomosis [1], a significantly lower incidence compared to uncomplicated MC pregnancies (89%) [2]. Although the presence of an AA anastomosis may have an important protective effect, it does not preclude the development of TAPS [3,4,6].

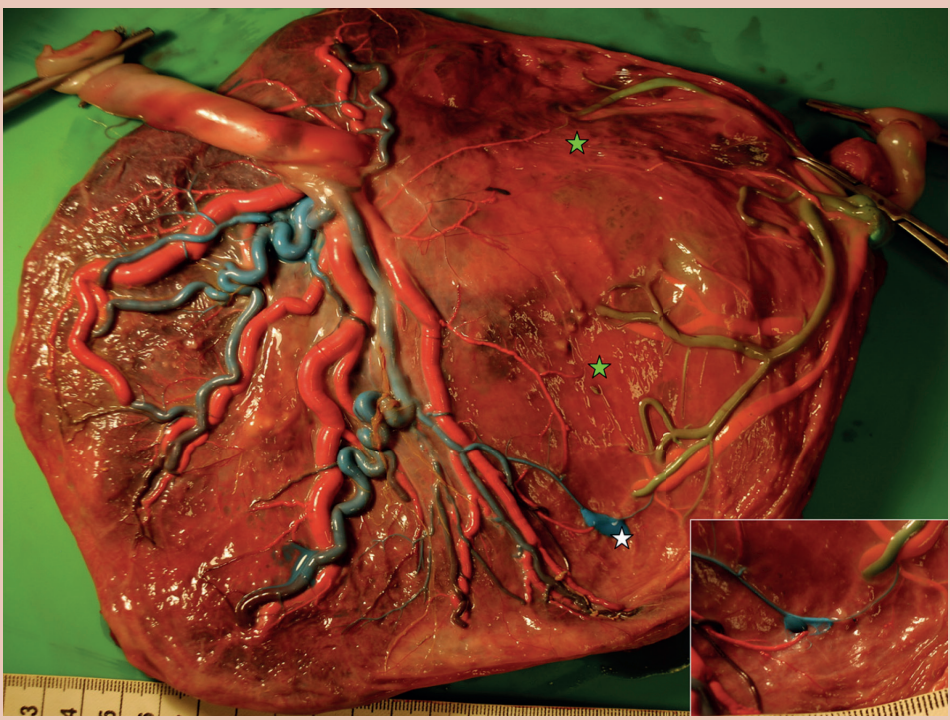

Figure 1. TAPS placenta after colored dye injection (blue and green for arteries, pink and orange for veins) with a small AA anastomosis (white star)(diameter of anastomosis: 0.3 mm). Detail of the AA anastomosis is shown in the bottom-right corner. The two green stars indicate two small AV anastomoses.

The exact pathophysiologic role of the small AA anastomosis in the development of TAPS is not entirely clear. As previously shown, the pathogenesis of TAPS seems to be based on a unique angioarchitecture characterized by the presence of a paucity of minuscule vascular anastomoses [2]. The few small anastomoses allow a slow transfusion of blood (as low as 5-15 ml/24h) from the donor to the recipient, leading gradually to highly discordant hemoglobin levels [6,7].

AA anastomoses are bidirectional anastomoses and are thought to have protective properties against hemodynamic imbalance caused by AV anastomoses [8-10], as confirmed by a mathematical computer model [11]. However, the blood flow through a minuscule AA anastomosis is probably extremely low and insufficient to allow for adequate equilibration of the blood volumes between both twins. Inter-twin blood flow volume and velocity is known to be strongly correlated to the diameter of an anastomosis, according to Pouiseuille's equation. Flow resistance depends linearly upon the viscosity and the length of a vessel, but depends to the fourth power upon the radius. Minuscule AA anastomoses may thus have a high flow resistance and thus fail to prevent the development of TAPS.

In conclusion, AA anastomoses in placentas with TAPS are rare. When present, the diameter of the AA anastomosis is small (≤1 mm), subsequently inhibiting adequate compensatory mechanisms and allowing development of TAPS. Disclosure All authors report no conflict of interest.

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Chapter 5

Middle cerebral artery peak systolic velocity to predict fetal hemoglobin levels in twin anemia polycythemia sequence

Abstract

Objective:

Our aim was to evaluate the diagnostic accuracy of middle cerebral artery peak systolic velocity (MCA-PSV) Doppler measurements in twin anemia polycythemia sequence (TAPS).

Methods:

In a consecutive cohort of monochorionic twin pregnancies with TAPS between 2005 and 2013 in three European fetal therapy centers, the accuracy to predict anemia and polycythemia of MCA-PSV measured just prior to fetal hemoglobin (Hb) measurement by fetal or cord blood sampling was assessed using 2x2 tables.

Results:

A total of 116 measurements (74 in donors and 42 in recipients) from 43 TAPS cases could be used for analysis. MCA-PSV multiples of the mean (MoM) values correlated well with Hb levels ($R = -0.86$ $P < 0.001$). The sensitivity of the MCA-PSV > 1.5 MoM to predict severe anemia (Hb deficit >5 standard deviations (SD)) in TAPS donors was 94% (95%CI 85-98%), specificity 74% (95%CI 62-83%), positive predictive value 76% (95%CI 65-85%), negative predictive value 94% (95%CI 83-98%). The sensitivity of MCA-PSV < 1.0 MoM to predict polycythemia (Hb >5SD above the mean) in TAPS recipients was 97% (95%CI 87-99%), specificity is 96% (95%CI 89-99%), positive predictive value 93% (95%CI 81-97%), negative predictive value 99% (95%CI 81-97%).

Conclusion:

This retrospective study shows a high diagnostic accuracy of MCA-PSV measurements for abnormal Hb levels in TAPS.

Introduction

Twin anemia polycythemia sequence (TAPS) is caused by chronic inter-twin blood transfusion through few minuscule placental anastomoses leading to large inter-twin hemoglobin (Hb) difference without signs of twin oligo-polyhydramnios sequence (TOPS) [1]. TAPS may occur spontaneously, or after laser treatment for twin-twin transfusion syndrome (TTTS) (post-laser TAPS). 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 signs of TOPS. These findings are often accompanied by a distinct difference in placenta echodensity [1]. Postnatal diagnosis is based on inter-twin Hb difference of ≥ 8g/dL, with at least one of the following: reticulocytosis in the donor with a reticulocyte count ratio ≥ 1.7 or small anastomoses (<1 mm) at the placental surface [2].

Middle cerebral artery peak systolic velocity (MCA-PSV) measurement, a non-invasive test, has become the standard test for the diagnosis of fetal anemia in singletons in a variety of fetal diseases [3]. Normal ranges for MCA-PSV for monochorionic diamniotic twins have been reported by Klaritsch et al. [4]. MCA-PSV measurements in monochorionic twin pregnancies with TAPS have not been evaluated before. The aim of this study was to evaluate the diagnostic accuracy of MCA-PSV measurements in TAPS.

Methods

All TAPS cases, in which fetal or neonatal blood sampling for Hb measurement was done, preceded by a MCA-PSV measurement at most 24 hours prior to the procedure, were included in this retrospective study. Fetal blood sampling was routinely performed in TAPS cases managed with intrauterine blood transfusion or partial exchange transfusion. Neonatal blood samplings for Hb measurements were routinely performed in all TAPS twins at birth. Postnatal blood sampling was done after cord clamping and after the delivery of the placenta. Samples were obtained from the umbilical vein. Cord blood samples were not compared to neonatal blood samples. The study cohort consisted of consecutive cases managed between 2005 and 2013 in three European fetal therapy centers (Leiden University Medical Center, the Netherlands; University Hospitals KU Leuven, Belgium and University Hospital - Centre Medico Chirurgical Obstetrical (CMCO) Strasbourg, France).

TAPS was identified using previously published criteria[1]. 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 was based on inter-twin Hb difference of ≥ 8g/dL, with at least one of the following: reticulocytosis in the donor with a reticulocyte count ratio ≥ 1.7 or small anastomoses (<1 mm) at the placental surface[1]. Intrauterine treatment was offered in TAPS stage 3 and 4. In TAPS stage 1 or 2, intrauterine treatment was offered in case TAPS was quickly progressing (within days) or other signs of severe anemia not meeting criteria for stage 3 (such as increasing heart size or prehydropic signs) were present.

Hb and MCA-PSV values were retrospectively obtained from the medical records. MCA-PSV was measured according to previously described technique by Mari et al. [5]. MCA-PSV values were recalculated to MoM using the reference ranges for monochorionic diamniotic twin pregnancies described by Klaritsch et al. [4]. In normal singleton pregnancies hemoglobin levels increase with gestation. Reference values for fetal hemoglobin according to gestational age have been published by Nicolaides et al. [6]. Hemoglobin deficit was calculated by the difference between the measured Hb value and the mean value for the corresponding gestational age. The definition of severe anemia and polycythemia was based on previous studies on fetal anemia based on Rhesus alloimmunization [3]. Severe anemia or polycythemia was defined as Hb value of more than 5 standard deviations (SD) below the mean in TAPS donors, and more than 5 SD above the mean in TAPS recipients.

	Fetal blood sampling (n=30)	**Postnatal blood sampling (n=13)**
GA at diagnosis (median)	25 (19-30)	26.5 (19-32)[a]
GA in weeks at cordocentesis (median)	26 (±3)	
GA in weeks at birth (median)	31 (26-34)[b]	30 (27-36)
Delivery mode (caesarean section)	19/25 (76%)[b]	11/13 (85%)
Diagnosis made only after birth		3/13 (23%)

[a] n=10, three were diagnosed after birth
[b] n=25, Termination of pregnancy n=1, Lost to follow-up n=4, including cord occlusion in 3 cases

Table 1: Patient characteristics in fetal blood sampling and postnatal blood sampling

Statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA). Correlation between continuous variables was analyzed using Pearson linear regression analysis. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihoodratio were calculated using 2x2 tables and standard

formulas for binominal proportions. To calculate the 95% confidence interval (CI) the Wilson's interval method was used [7].

Results

During the study period, 43 patients were diagnosed with TAPS and met the inclusion criteria. In 31/43 (72%) of the cases, TAPS occurred after laser surgery for TTTS (post-laser TAPS) and 12/43 (28%) were spontaneous TAPS cases. A total of 116 complete sets of both MCA-PSV and Hb measurements were available for analysis. In 55/116 (47%) cases, the Hb measurements were obtained by fetal blood sampling. In the other 61/116 (53%) the MCA-PSV measurements were done within 24 hours before birth and Hb measurements were performed in cord blood at birth. 74 of the 116 (64%) measurements belonged to donors and 42/116 (36%) belonged to recipients. Patient characteristics in fetal and postnatal blood sampling and neonatal outcome in donors and recipients are reported in tables 1 and 2.

	Donor	Recipient	p value
Overall survival [a]	29/41 (71%)	40/41 (98%)	0.002
Birth weight	1370 (±409) [b]	1644 (±441) [c]	0.02
Hemoglobin at birth	8.2 (±3.5) [d]	21.6 (±2.7) [e]	<0.001

[a] n=41, 2 patients lost to follow-up for survival
[b] n=27, 16 neonates lost to follow-up for birth weight
[c] n=28, 15 neonates lost to follow-up for birth weight
[d] n=34, 9 neonates lost to follow-up for hemoglobin at birth
[e] n=36, 7 neonates lost to follow-up for hemoglobin at birth

Table 2: Neonatal outcome in donors and recipients

MCA-PSV measurements and Hb levels were highly correlated, R = -0.86 P < 0.001 (Figure 1). In 16 measurements, the MCA-PSV measurement was above 1.5 MoM but the Hb levels were not below more than 5 SD of the mean. The majority of these measurements (11/16, 69%) were done in fetuses that already received an intrauterine transfusion. The sensitivity of the MCA-PSV measurements in the TAPS donor as a test for Hb levels below 5 SD of the mean was 94% (95%CI 85-98%), specificity was 74% (95%CI 62-83%), the positive predictive value 76% (95%CI 65-85%), negative predictive value 94% (95%CI 83-98%), positive

	Hb-deficit <- 5 SD	Hb-deficit >- 5 SD	Total
MCA-PSV > 1.5 MoM	51	16	67
MCA-PSV < 1.5 MoM	3	46	49
Total	54	62	116

Sensitivity=94% (95%CI 85-98%), specificity=74% (95%CI 62-83%), positive predictive value=76% (95%CI 65-85%), negative predictive value=94% (95%CI 83-98%), positive likelihood ratio=3.66, negative likelihood ratio=0.07

Table 3: MCA-PSV Doppler measurements to predict severe fetal anemia (Hb-deficit below -5 SD of the mean) in TAPS donors.

likely hood ratio is 3.66 and negative likelihood ratio is 0.07 (Table 3). Excluding the measurements done after an intrauterine transfusion, sensitivity was 97% (95%; CI 84-99%), specificity was 79% (95%; CI 59-91%), positive predictive value was 86% (95%CI 71-94%), negative predictive value was 95% (95%CI 76-99%), positive likely hood ratio is 4.65 and negative likelihood ratio is 0.04 (Table 4).

	Hb-deficit <- 5 SD	Hb-deficit >- 5 SD	Total
MCA-PSV > 1.5 MoM	30	5	35
MCA-PSV < 1.5 MoM	1	19	20
Total	31	24	55

Sensitivity=97% (95%CI 84-99%), specificity=79% (95%CI 59-91%), positive predictive value=86% (95%CI 71-94%), negative predictive value=95% (95%CI 76-99%), positive likelihood ratio=4.65, negative likelihood ratio=0.04

Table 4: MCA-PSV Doppler measurements to predict severe fetal anemia (Hb-deficit below -5 SD of the mean) in TAPS donors, measurements prior to IUT

In 5 recipients Hb was measured before birth (as part of partial exchange transfusion). In total 11 measurements were performed. In two cases PET was performed once, in one case twice, in one case three times and in the last case four times. In one recipient (postnatal detected TAPS), MCA-PSV before birth was not below 1.0 MoM but neonatal sample of Hb was above 5 SD of the mean, resulting in a sensitivity of 97% (95% CI 87-99%). Three MCA-PSV measurements in recipients were below 1.0 MoM although Hb levels where less than 5 SD above the mean, which resulted in a specificity of MCA-PSV to predict polycythemia of 96% (95% CI 89-99%). The positive predictive value was 93%(95% CI 81-97%) and the negative predictive value was 99% (95% CI 93-100%), positive likely hood ratio is 25 and negative likelihood ratio is 0.03 (Table 5). The mean of MCA-PSV measurements in recipients was 0.70 (±0.17).

	Hb-deficit > 5 SD	Hb-deficit < 5 SD	Total
MCA-PSV < 1.0 MoM	38	3	41
MCA-PSV > 1.0 MoM	1	74	75
Total	39	77	116

Sensitivity=97% (95%CI 87-99%), specificity=96% (95%CI 89-99%), positive predictive value=93% (95%CI 81-97%), negative predictive value=99% (95%CI 93-100%), positive likelihood ratio=25, negative likelihood ratio=0.03

Table 5: MCA-PSV Doppler measurements to predict severe fetal polycythemia (Hb-deficit above 5 SD of the mean) in TAPS recipients.

Discussion

This is the first study reporting on the diagnostic accuracy of MCA-PSV measurements to predict abnormal fetal hemoglobin levels in TAPS. We found high sensitivities and

specificities of MCA-PSV both for anemia and for polycythemia, confirming the clinical usefulness of this noninvasive test in yet another pregnancy complication.

The most common application of MCA-PSV Doppler measurements is in the management of pregnancies with red cell alloimmunization [3]. Obviously, the pathophysiology underlying the development of fetal anemia in this disease differs completely from anemia in TAPS. Still, the predictive values of the Doppler measurements are remarkably similar in both diseases. Interestingly, just as in alloimmune anemia, the accuracy of MCA-PSV measurements was lower after an intrauterine transfusion was performed [8-10].

Our study is the first to evaluate MCA-PSV measurements for the prediction of fetal polycythemia. Recently, a study on the use of MCA-PSV in neonates showed a similar correlation between low peak systolic velocities and polycythemia [11]. The positive predictive value for TAPS donors was 76% and for TAPS recipients 93%. The lower positive predictive value in donors compared to recipients is mainly due to a reduced accuracy for measurements performed when anemia again developed following an intrauterine transfusion. Using only measurements performed prior to the first transfusion, the positive predictive value increased to 86%.

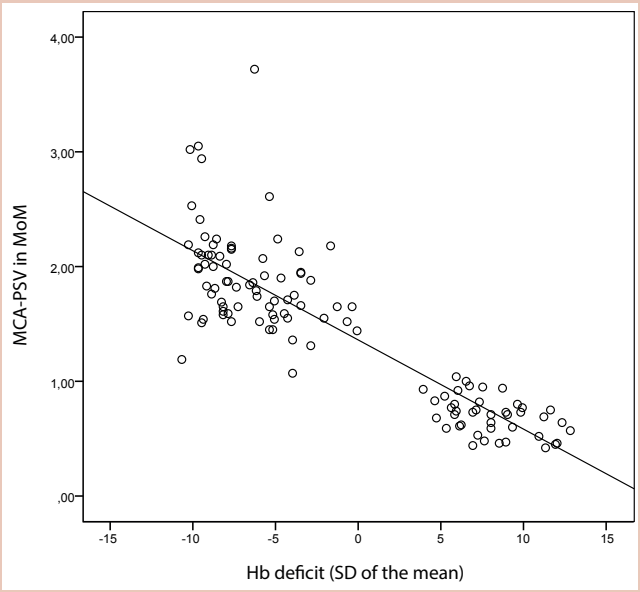

Figure 1. MCA-PSV (MoM) versus Hb deficit (standard deviations (SD) of the mean).
On the x-axis the Hb deficit in SD of the mean. Hb levels below -5 SD are severe anemic donors and Hb above 5 SD are severe polycythemic recipients. On the y-axis Middle cerebral artery peak systolic velocity (MCA-PSV) measurements in multiples of the mean (MoM).

Our results strengthen our previous findings and proposal to use 1.0 MoM as cut-off level for the TAPS recipient, as described in our proposed staging system [1]. With the previously suggested cut-off level of 0.8 MoM, a significant number of cases of severe polycythemia will be missed [12]. As shown in figure 1 there are more outliners in high MCA-PSV measurements compared to low MCA-PSV

measurements. Whether delta MCA-PSV MoM is a more accurate predictor for TAPS should be studied in future research.

One of the limitations of this study is the retrospective nature of this study. The highly selected patient group, referred to specialized centers and predefined as suspected to have TAPS, influences in particular the positive and negative predictive values, since these parameters strongly depend on prevalence. In a general practice with uncomplicated monochorionic twins, MCA-PSV measurements may perform less well. To evaluate the use of MCA-PSV as a screening tool to timely detect TAPS, only large-scale prospective studies in cohorts of monochorionic twins would provide adequate data. Such studies are, apart from being time-consuming and expensive, impossible to perform in specialized fetal therapy centers, given the pre selection of referred high-risk cases. In addition, fetal blood sampling is an invasive procedure with inherent risks, which we cannot perform on a large scale in pregnancies without a strong suspicion for TAPS, thus limiting our ability to confirm negative predictive values.

In summary, in selected pregnancies at increased risk for TAPS, managed in fetal medicine centers, MCA-PSV Doppler is a powerful tool in the prediction of both fetal anemia as well as fetal polycythemia.

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Chapter 6

Hematological characteristics in neonates with twin anemia polycythemia sequence

Abstract

Objective:

To evaluate the neonatal hematological features of monochorionic twins with twin anemia-polycythemia sequence (TAPS) and to determine the additional diagnostic value of reticulocyte count measurement.

Methods:

A cohort of consecutive monochorionic twins with TAPS (n = 19) was included in the study and each twin pair was compared with two monochorionic twin pairs (n = 38) unaffected by TAPS or twin–twin transfusion syndrome (TTTS), matched for gestational age at birth. We measured full blood counts on day 1 and determined the incidence of anemia, polycythemia, reticulocytosis and thrombocytopenia.

Results:

Median inter-twin hemoglobin (Hb) difference in monochorionic twins with and without TAPS was 13.7 g/dL and 2.4 g/dL, respectively ($p < 0.01$). Median inter-twin reticulocyte count ratio in twins with and without TAPS was 3.1 and 1.0, respectively ($p < 0.01$). Thrombocytopenia (platelet count $<150 \times 10^9$ /L) occurred more often in the TAPS group than in the control group, 45% (17/38) versus 11% (11/38), respectively ($p < 0.01$). In the TAPS group, mean platelet count was significantly lower in recipients than in donors, 133×10^9/L versus 218×10^9/L, respectively ($p < 0.01$).

Conclusions:

TAPS twins have a large inter-twin Hb difference in combination with a large intertwin reticulocyte count ratio. Recipients are more often thrombocytopenic than donors, probably due to polycythemia.

Introduction

Twin anemia–polycythemia sequence (TAPS) is an atypical form of chronic feto-fetal blood transfusion which may occur in uncomplicated monochorionic twin pregnancies (spontaneous form) or after laser surgery for twin–twin transfusion syndrome (TTTS) (post-laser form) [1-3]. Both forms are characterized by the presence of large intertwin hemoglobin (Hb) difference without the degree of amniotic fluid discordance that is required for the diagnosis of TTTS [1-3]. The post-laser form of TAPS is reported to occur in up to 13% of TTTS patients treated with laser [1]. The spontaneous form of TAPS complicates approximately 3 to 5% of monochorionic twin pregnancies [4;5].

Both spontaneous and post-laser TAPS have a similar anatomic substrate which is based on the presence of only few minuscule arterio-venous anastomoses, allowing a very slow inter-twin blood transfusion [6]. The optimal management of TAPS (both for the spontaneous form and for the post-laser form) is not clear and may include expectant management, intrauterine blood transfusion or laser coagulation of the vascular anastomoses [7].

Diagnosis of TAPS can be reached after birth based on the presence of chronic anemia in one twin and polycythemia in the co-twin, in association with typical placental angioarchitecture following colored dye injection [5]. Diagnosis of TAPS can also be reached prenatally but requires different criteria based on Doppler ultrasound measurements [1-3]. Our knowledge of hematological features in TAPS is sparse and based on only a few casuistic reports [2;3]. Large cohort or case–control studies evaluating hematological aspects in TAPS are required to further develop accurate diagnostic criteria for TAPS.

The aim of this study was to report the hematological characteristics of neonates with TAPS and to determine the additional diagnostic value of reticulocyte count measurement in TAPS.

Methods

All consecutive monochorionic twin pairs with TAPS admitted to our neonatal nursery at the Leiden University Medical Centre (The Netherlands) between June 2002 and February 2009 were included in the study. The Leiden University Medical Centre is an academic referral center managing all types of complications of monochorionic pregnancies. Absence of prenatal signs of TTTS (i.e. absence of oligohydramnios–polyhydramnios sequence on ultrasound) is a conditio sine qua non for the diagnosis of TAPS.

For the purpose of this study, diagnosis of TAPS was based on postnatal criteria. Postnatal criteria were met if hematological investigations on day one showed chronic anemia in the donor (Hb <5th centile for gestational age) [8] and polycythemia in the recipient (venous hematocrit >65%) in combination with placental injection studies showing only small arterio-venous anastomoses (diameter <1 mm) and no superficial arterio-arterial anastomoses. Details on the technique used for placental injection and placental territory measurement have been described previously [6]. Part of the placental data reported in this study was included in a previous report on placental angioarchitecture in TAPS [6]. Two spontaneous TAPS cases and one TAPS case after laser surgery were previously published [2;9].

We excluded all twin pregnancies with single or double intrauterine death and patients with an incomplete placental injection. Patients with TAPS (spontaneous TAPS cases as well as post-laser TAPS cases) that were treated after diagnosis with fetoscopic laser surgery were excluded from the study.

Each twin pair with TAPS was compared with two control monochorionic twin pairs unaffected by TAPS or TTTS, matched by gestational age at birth (±1 week gestation). All monochorionic twins (with and without complications) delivered at our center are entered prospectively in a "monochorionic twins" database. The control population in this study was obtained from this dedicated database.

We recorded the gestational age at birth, birth weight and inter-twin birth weight discordance. Full blood count and reticulocyte count were measured routinely on day one in all monochorionic twins. Blood samplings were obtained primarily from umbilical cord blood. If cord blood was not available, samplings were routinely obtained on day one through heel stick or venous puncture. Anemia on day one was defined as Hb level <5th centile for gestational age [8]. Polycythemia on day one was defined as a venous hematocrit >65% [10]. Reticulocytosis was defined as a reticulocyte count >95th centile for gestational age [8].

We calculated the inter-twin Hb difference and intertwin reticulocyte count ratio. The inter-twin reticulocyte ratio was calculated by dividing the reticulocyte count of the infant with the lowest Hb by the reticulocyte count of its co-twin. Hematological characteristics were compared between the TAPS group and the control group. We also compared the hematological results between donors and recipients in the TAPS group and between the twin infant with the lower Hb level and its co-twin in the control group. In this study, the term "recipient" is used to define the twin infant with polycythemia, whereas the term "donor" is used to define the anemic twin infant.

Data are reported as means and standard deviations (SD) or as medians and interquartile range (IQR), as appropriate. The unpaired Student t-test was used for parametric and the Mann–Whitney U test for non-parametric comparisons for continuous variables. Results of categorical variables were compared using Fisher's exact test. For comparisons within twin pairs, the paired Student t-test was used for continuous variables, and the Mc Nemar test was used for the analysis of paired nominal variables. Sensitivity and specificity were calculated by standard formulas for a binominal proportion. The Spearman rank correlation was used to study the relationship between polycythemia and thrombocytopenia. A p-value <0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Results

A total of 22 consecutive TAPS patients were identified during the study period. Two spontaneous TAPS patients were treated with fetoscopic laser surgery after diagnosis and were thus excluded from the study. One post-laser TAPS patient was excluded because of the presence of a (small) superficial arterio-arterial anastomosis on placental injection (Lopriore et al., 2007c). Nineteen twin pairs thus fulfilled the inclusion criteria for this study. Five (26%) were spontaneous TAPS and 14 (74%) were post-laser TAPS cases. Doppler measurements of the middle cerebral artery-peak systolic flow velocity (MCA-PSV) from both fetuses were available from 79% (15/19) of patients in the TAPS group (MCA-PSV measurements were performed in two of the five spontaneous TAPS cases and in 13 of the 15 postlaser TAPS cases). Increased MCA-PSV (>1.5 multiples of the median) in the donor in combination with a decreased MCA-PSV (<0.8 multiples of the median) in the recipient was detected prenatally in 60% (9/15) of these patients (all in the post-laser TAPS cases). Two post-laser TAPS patients were treated prenatally with intrauterine blood transfusion(s), one was recently published as a case report [11].

	TAPS group (n=19 pregnancies; 38 neonates)	**Control group (n=38 pregnancies; 76 neonates)**
Gestational age at birth (weeks) [a]	32±2.8	32.2±2.7
Female n(%)	16 (42)	40 (53)
Vaginal delivery n (%)	10 (53)	18 (47)
Birth weight g [a]	1591±415	1780±521
Birth weight discordance (%) [a]	14±14	15±14
Small for gestational age n (%)	2 (5)	4 (5)

[a] Value given as mean (±SD), Birth weight discordance was calculated as follows: [(birth weight larger twin - birth weight smaller twin)/birth weight larger twin]×100%.

Table 1: Baseline characteristics in TAPS twin pairs and control twin pairs

	TAPS group (n=38)	Controls (n=76)	p value
Hemoglobin level (g/dL) [a]	15.3 (8.6–23.1)	16.4 (14.8–18.5)	0.86
Hematocrit (%) [a]	53 (29–74)	47 (42–52)	0.99
Inter-twin hemoglobin difference (g/dL) [a]	13.7 (12.2–16.9)	2.4 (0.6–3.9)	<0.01
Anemia n(%)	19 (50)	3 (4)	<0.01
Polycythemia n(%)	19 (50)	4 (5)	<0.01
Reticulocyte count (%) [a]	7.3 (3.6–14.5)	6.1 (5.2–7.0)	0.34
Reticulocytosis n/N (%) [b]	19/36 (53)	32/66 (48)	0.68
Inter-twin reticulocyte count difference (%) [a]	10.6 (5.7–14.4)	2.4 (0.6–3.9)	<0.01
Inter-twin reticulocyte count ratio [a,c]	3.1 (2.3–5.8)	1.0 (1.0–1.1)	<0.01
Platelet count (10^9/L) [a]	155 (112–231)	216 (174–259)	<0.01
Thrombocytopenia (<150) n (%)	17 (45)	11 (14)	<0.01
Thrombocytopenia (<100) n (%)	7 (18)	1 (1)	<0.01
Thrombocytopenia (<50) n (%)	1 (3)	0 (0)	0.33

[a] Value given as median (interquartile range).
[b] Reticulocytosis: reticulocyte count >95th centile.
[c] Reticulocyte count of infant with lower Hb level divided by the reticulocyte count of the co-twin.

Table 2: Hematological characteristics in TAPS neonates and controls at birth

Baseline characteristics in the groups with and without TAPS are presented in Table 1. Details on prenatal findings and neonatal morbidities in both groups are presented separately [12]. Other possible causes for neonatal anemia at birth, such as Rhesus incompatibility, partial abruption, infection and fetomaternal hemorrhage were ruled out with clinical, ultrasound and/or laboratory examinations. Similarly, chronic hypoxia as a possible cause of polycythemia at birth was ruled out in plethoric neonates. Anemia in one twin in combination with polycythemia in the co-twin occurred (by definition) in all TAPS twins, but also in one twin pair in the control group. In this control twin pair delivered at 35 weeks' gestation, the first-born twin infant was anemic (Hb 12.5 g/dL) but did not require a blood transfusion and the second-born twin was polycythemic (Hb 23.8 g/dL, venous hematocrit 73%) and needed a partial exchange transfusion. The anemic twin had no reticulocytosis (reticulocyte count value of 5.7%) and placental injection showed a large arterio-arterial anastomosis. Both factors precluded TAPS and were more suggestive of an acute form of peripartum feto-fetal transfusion.

Details on hematological characteristics in the TAPS group and control group are shown in Table 2. The median inter-twin Hb difference at birth in the group with and without TAPS was 13.7 g/dL (IQR 12.2–16.9; range 8.9–20.3 g/dL) and 2.4 g/dL (IQR 0.6–3.9; range 0–11.3 g/dL), respectively ($p < 0.01$). An inter-twin Hb difference <8 g/dL was found in all TAPS twin

pairs and in one control twin pair (3%, 1/38), leading to a sensitivity of 100% (19/19) and a specificity of 97% (37/38).

Reticulocyte count was measured in 95% (18/19) of twin pairs in the TAPS group and in 87% (33/38) of control twin pairs. The median inter-twin reticulocyte count ratio in twins with and without TAPS was 3.1 (IQR 2.3–5.8; range 1.8–10.0) and 1.0 (IQR 1.0–1.1; range 1–1.5), respectively ($p < 0.01$). All TAPS twins had an inter-twin reticulocyte count ratio >1.7, whereas this never occurred in the control twin pairs (sensitivity and specificity of 100%).

Thrombocytopenia (defined as a platelet count $< 150 \times 10^9$ /L) occurred more often in the TAPS group than in the control group, 45% (17/38) and 11% (11/38) ($p < 0.01$). In the TAPS group, platelet count was significantly lower in recipients than in donors, 133×10^9 /L and 218×10^9 /L, respectively ($p < 0.01$). One recipient twin had a clinically significant severe thrombocytopenia (24×10^9 /L) requiring a platelet transfusion at birth. Platelet count in recipient twins was negatively correlated to the Hb level at birth (Spearman correlation coefficient = −0.698, $p = 0.001$) (Figure 1).

Differences in hematological characteristics between donors and recipients in the TAPS group and between the twin infant with the lower Hb level and the co-twin in the control group are presented in Table 3. In the TAPS group, Hb values in the anemic donors ranged from 4.5 to 11.7 g/dL (mean value of 8.9 g/dL), whereas Hb values in the polycythemic recipient ranged from 19.0 to 26.6 (mean value of 23.0 g/dL). Mean reticulocyte count in donor twins in the TAPS group was 16.3% (range 7.1–27.9). Reticulocytosis (reticulocyte count <95th centile) was present in 94% (17/18) of donor twins, 11% (2/18) of recipient twins, 48% (16/33) of twin infants with the lowest Hb in the control group and 48% (16/33)

	TAPS group (N=38)			Control group (N=76)		
	Donor (n=19)	Recipient (n=19)	p value	Twin A (n=38)	Twin B (n=38)	p value
Hemoglobin level (g/dL) [a]	8.9±2.1	23.0±2.1	<0.01	15.3±2.2	18.0±2.5	<0.01
Hematocrit (%) [a]	29±6	74±7	<0.01	44±5	52±9	<0.01
Reticulocyte count (%) [a]	16.3±7.8	4.6±3.6	<0.01	6.4±1.6	6.2±1.4	0.09
Reticulocytosis n/N (%) [b]	17/18 (94)	2/18 (11)	<0.01	16/33 (48)	16/33 (48)	1.0
Platelet count (10^9/L) [a]	218±101	133±51	<0.01	213±64	221±53	0.20
Thrombocytopenia n(%)	5 (26)	12 (63)	0.02	6 (16)	5 (13)	0.51

[a] Value given as mean (±SD)
[b] Reticulocytosis: reticulocyte count >95th centile

Table 3: Hematological characteristics in TAPS neonates (donor vs recipients) and in controls [twin A is the infant with lower Hb (Hb) level and twin B is the co-twin with higher Hb level] at birth

of their co-twins, leading to a sensitivity of reticulocytosis in TAPS of 94% (17/18) and a specificity of 60% (50/84).

Discussion

This study reports detailed hematological features which are characteristic of TAPS, including a large intertwin Hb difference and a large inter-twin reticulocyte count difference. These hematological results can be used to establish clear criteria for postnatal diagnosis of TAPS. Since TAPS has just recently been described [1;2], uniform criteria for hematological values have not yet been established. Strict diagnostic criteria for TAPS are also required to allow comparison of outcome data between centers performing laser treatment in TTTS pregnancies. Occurrence of TAPS after laser treatment is often due to residual anastomoses [9;13] and is regarded as treatment failure [1;14-16].

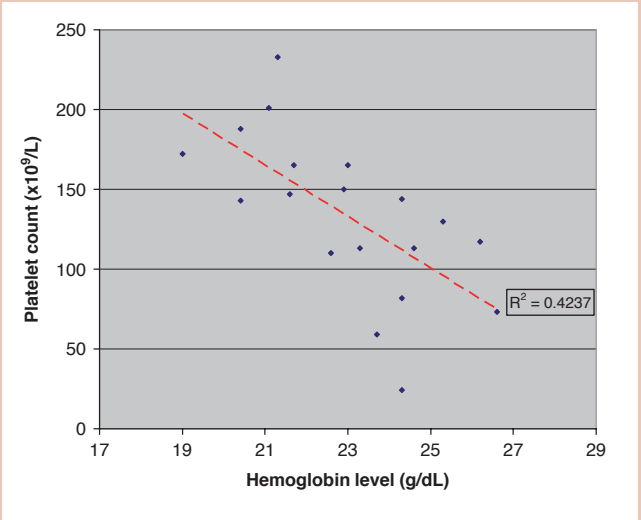

Figure 1. Negative correlation in recipient twins between hemoglobin level and platelet count at birth

Several different diagnostic criteria for TAPS have been proposed using different cut-off values. Specific cut-off levels for MCA-PSV measured with Doppler ultrasound have been used to define prenatal criteria for TAPS. Frequently reported criteria are an increased MCA-PSV >1.5 multiples of the median in one fetus (suggestive of fetal anemia) with a concurrent decrease in MCA-PSV <0.8 multiples of the median (suggestive of polycythemia) in the co-twin [1]. However, the sensitivity and specificity of these prenatal criteria have not yet been demonstrated.

Several postnatal criteria for TAPS have been proposed. Lewi et al. defined TAPS as the presence of a Hb level <11 g/dL in the anemic twin and >20 g/dL in the polycythemic

co-twin[4]. Although the use of fixed cut-off levels for Hb values has clear practical advantages, it does not take the correlation of Hb levels with gestational age into consideration. Hb concentration is known to increase linearly with gestation [8;17]. We therefore chose to use gestational age-dependent cutoff levels for Hb concentration [2]. However, this definition has the practical disadvantage in that it requires the use of specific normograms for Hb in relation to gestational age. Several different normograms have been published [8;17]. Moreover, there is little consensus between neonatologists on the definition of neonatal anemia at birth (using different cut-off values for Hb levels) and international guidelines on when to transfuse anemic infants are also highly discordant [18]. Similarly, although most neonatologists define neonatal polycythemia as a hematocrit 65%, other definitions are also being used and consensus on the optimal management for polycythemia is highly controversial [10]. The criteria used in this study to define TAPS may therefore seem less practical compared to the fixed Hb levels proposed by Lewi et al. [4].

An alternative and more pragmatic method would be to use inter-twin Hb differences instead of specific cutoff levels for anemia and polycythemia. Since TAPS is a form of inter-fetal transfusion, the use of intertwin Hb difference seems a logical alternative. As shown in this study, all TAPS twins have a high intertwin Hb difference (>8 g/dL). However, large intertwin Hb difference may also occur in uncomplicated monochorionic twin pairs due to acute peripartum TTTS or due to acute placento-fetal transfusion after delivery of the first twin [19].

Differentiation between these two types of inter-twin transfusions is difficult to reach solely on the basis of physical examination. In both situations, neonatologists are confronted at birth with a pale anemic infant and a plethoric polycythemic co-twin. Although the absence of symptoms of shock in the pale anemic twin would argue in favor of TAPS, an additional objective criterion to guide the clinicians would be very useful. Since blood loss in acute peripartum TTTS or acute placento-fetal transfusion occurs rapidly, the reticulocyte count in the anemic co-twin is not increased. In contrast, the reticulocyte count in the anemic co-twin in TAPS is almost always increased, reflecting chronic blood loss. However, reticulocytosis (reticulocyte count >95th centile for gestational age) in the anemic twin was not highly discriminative and does not appear to be an adequate criterion for TAPS. Although the sensitivity of reticulocytosis in the anemic twin was 95%, its specificity was only 60%. The lack of specificity may also be explained by the fact that reticulocytosis reflects not only anemia but also chronic fetal distress. In addition, reference ranges for reticulocyte count may not be easily available. Moreover, in analogy to Hb concentration, the reticulocyte count is known to decrease linearly with advancing gestation [8]. Determination of

reticulocytosis thus requires appropriate graphs. We therefore propose, for practical reasons (see previous arguments for Hb levels), to use an inter-twin reticulocyte count ratio instead of a fixed reticulocyte count. As shown in this study, an increased inter-twin reticulocyte count ratio >1.7 appears to be pathognomonic for TAPS (sensitivity and specificity of 100%).

Another important finding of this study is the increased incidence of thrombocytopenia in the TAPS group, related principally to a decreased platelet count in recipient twins. Recipient twins with TAPS are per definition polycythemic. Polycythemia is known to be associated with thrombocytopenia [10]. Low platelet counts in infants with polycythemia may occur due to impaired production secondary to tissue hypoxia, slow spleen blood flow and decreased plasma fraction with normal concentrations [10]. Thrombocytopenia in recipient twins was mostly self-limiting, but one of the recipient twins required a platelet transfusion at birth due to severe thrombocytopenia.

In conclusion, TAPS twins have a large inter-twin Hb difference in combination with a large inter-twin reticulocyte count ratio. Determination of reticulocyte count in monochorionic twins with discordant Hb levels at birth is important to differentiate between chronic fetofetal transfusion (TAPS) and acute feto-fetal transfusion (such as peripartum TTTS) or placento-fetal transfusion at birth. A high inter-twin reticulocyte count ratio (>1.7) appears to be pathognomonic for TAPS. We propose that postnatal hematological criteria for TAPS should include an inter-twin Hb difference (>8 g/dL) in association with a high inter-twin reticulocyte count ratio.

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Chapter 7

Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial

Abstract

Background:

Monochorionic twin pregnancies complicated by twin-to-twin transfusion syndrome are typically treated with fetoscopic laser coagulation. Postoperative complications can occur due to residual vascular anastomoses on the placenta. We aimed to assess the efficacy and safety of a novel surgery technique that uses laser coagulation of the entire vascular equator (Solomon technique).

Methods:

We undertook an open-label, international, multicentre, randomised controlled trial at five European tertiary referral centres. Women with twin-to-twin transfusion syndrome were randomly assigned by online randomisation (1:1) with permuted blocks to the Solomon technique or standard laser coagulation. The primary outcome was a composite of incidence of twin anaemia polycythaemia sequence, recurrence of twin-to-twin transfusion syndrome, perinatal mortality, or severe neonatal morbidity. Analyses were by intention to treat, with results expressed as odds ratios (ORs) and 95% CIs. This trial is registered with the Dutch Trial Registry, number NTR1245.

Findings:

Between March 11, 2008, and July 12, 2012, 274 women were randomly assigned to either the Solomon group (n=139) or the standard treatment group (n=135). The primary outcome occurred in 94 (34%) of 274 fetuses in the Solomon group versus 133 (49%) of 270 in the standard treatment group (OR 0·54; 95% CI 0·35–0·82). The Solomon technique was associated with a reduction in twin anaemia polycythaemia sequence (3% vs 16% for the standard treatment; OR 0·16, 95% CI 0·05–0·49) and recurrence of twin-to-twin transfusion syndrome (1% vs 7%; 0·21, 0·04–0·98). Perinatal mortality and severe neonatal morbidity did not differ significantly between the two groups. Outside of the common and well-known complications of twin-to-twin transfusion syndrome and its treatment, no serious adverse events occurred.

Interpretation:

Fetoscopic laser coagulation of the entire vascular equator reduces postoperative fetal morbidity in severe twin-to-twin transfusion syndrome. We recommend that fetoscopic surgeons consider adopting this strategy for treatment of women with twin-to-twin transfusion syndrome.

Introduction

Monochorionic twin pregnancies are associated with a perinatal mortality rate of 11%.[1,2] Most complications, such as twin-to-twin transfusion syndrome, can be attributed to the presence of inter-twin vascular anastomoses on the monochorionic placenta. If left untreated, twin-to-twin transfusion syndrome can result in an overall mortality rate of 73–100%.[3]

The preferred treatment for twin-to-twin transfusion syndrome is fetoscopic laser coagulation of the vascular anastomoses, which is associated with survival rates of both fetuses of 35–67%.[4–9] Among the surviving children, 4–16% have signs of cerebral injury and 13–17% have neurodevelopmental morbidity.[10–12]

The goal of fetoscopic laser surgery is to coagulate all of the placental vascular anastomoses. However, in up to 33% of treated pregnancies, some inter-twin vascular connections remain patent.[13,14] These residual patent anastomoses can cause severe complications such as twin anaemia polycythaemia sequence (13%) or recurrent twin-to-twin transfusion syndrome (14%).[15]

To minimise the occurrence of residual anastomoses and their complications, we developed a modified fetoscopic laser surgery technique called the Solomon technique, in which the entire vascular equator is coagulated. The rationale of the Solomon technique is coagulation of the whole vascular equator (including tiny anastomoses that might not be visualised). Laser coagulation (in both the selective and Solomon techniques) occurs only at the chorionic surface. In the Solomon trial, we compared the efficacy and safety of the Solomon technique with standard laser coagulation and examined whether the Solomon technique can prevent the occurrence of residual anastomoses and their associated complications.

Methods

Study design and participants

We did an open-label, international, multicentre, randomised controlled trial at five European tertiary referral centres (University Hospital Leuven, Leuven, Belgium; University Hospital of Strasbourg, Strasbourg, France; Birmingham Women's Hospital, University of Birmingham, Birmingham, UK; Buzzi Hospital Milan, Milan, Italy; and Leiden University Medical Centre, Leiden, Netherlands). Women with monochorionic, diamniotic twin pregnancies up to 26

weeks' gestation complicated by twin-to-twin transfusion syndrome (Quintero stage [16] 2, 3, or 4) and women with Quintero stage 1 with clinical symptoms from polyhydramnios

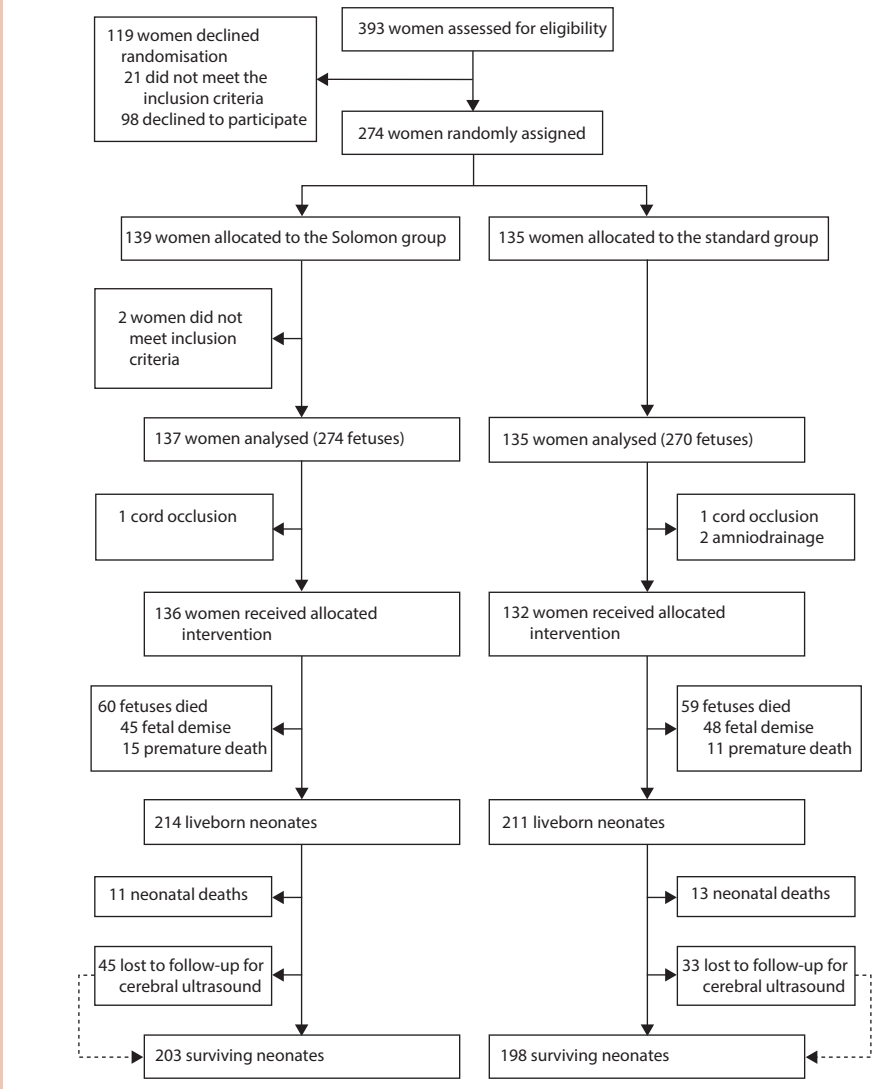

Figure 1. Trial profile

were eligible for participation in the trial. We defined twin-to-twin transfusion syndrome with the Eurofoetus criteria,[4] with a cutoff at a deepest vertical pocket of amniotic fluid in the donor of 2 cm or smaller; additionally, we used a cutoff for the deepest vertical pocket in the recipient of at least 8 cm within the first 20 weeks of gestation or at least 10 cm after

week 20. Exclusion criteria consisted of triplet pregnancies and mothers who were unable to provide informed consent.

Each woman who was included in the study provided written informed consent in accordance with institutional and national guidelines. The study was approved by the Leiden University Medical Centre Medical Ethics Committee (MEC P07.261) and each centre's respective institutional review board. The trial was registered with the Dutch trial registry, number NTR1245.

Randomisation and masking

Women were informed about the study by their obstetrician after they were planned for fetoscopic laser therapy. After written informed consent was obtained, women were randomly assigned (1:1) to the Solomon technique or standard laser coagulation by online randomisation (with the program ALEA, TENALEA, Amsterdam, Netherlands) with permuted blocks with a maximum block size of four (stratified by centre). The randomisation sequence was computer generated with an online randomisation program and was not accessible by the recruiters, (ie, fetal surgeons who did the laser surgery) or the trial coordinator (FS). The allocation code was disclosed after a participating woman's initials and date of birth were entered and a unique number was generated. Data collection was web based. The trial was open label because masking of the surgical intervention was not possible.

Procedures

Women were scheduled to undergo the procedure within 48 h of random assignment. All fetoscopic laser procedures were undertaken by experienced operators (12 surgeons), each of whom had done at least 60 previous laser procedures and were competent to undertake the Solomon technique. This technique is a minor modification of the selective fetoscopic procedure, and pilot studies of the technique were done in all centres. All five participating centres used the same fetoscopic laser coagulation technique as described previously.[17,18] Twin-to-twin transfusion syndrome cases were stratified by centre. In centres with more than one surgeon, we ensured that all surgeons were suffciently experienced and used an identical technique before starting the trial. The following steps were identical for both the experimental and the standard procedure. By continuous ultrasound visualisation, a cannula was introduced transabdominally into the amniotic cavity of the recipient twin either by the Seldinger technique or by sharp trocar insertion. Depending on the gestational age, a 1·3 mm or 2·0 mm fetoscope (Karl Storz, Tuttlingen, Germany) and 7–10 Fr cannula was used.

After identifying the vascular anastomoses, a 400 μm or 600 μm laser fibre connected to a diode or Nd:YAG laser device (Dornier MedTech, Wessling, Germany) was introduced through the operating sheath. All visible anastomoses were coagulated using one to three bursts of a few seconds each at a power setting of 20–70 W, depending on the vessel diameter.

For women in the Solomon technique group, after coagulation of all visible anastomoses, a thin line of tissue at the placental surface was coagulated from one edge of the placenta to the other, with a power setting of 20–50 W, to connect the white areas that resulted from coagulation of the anastomoses. The purpose of coagulating along this line was to completely separate the two parts of the chorionic surface of the placenta at the level of the vascular equator. In both groups, the laser procedure was followed by draining of the excess amniotic fluid to a deepest vertical pocket of 6 cm. Both groups received identical postoperative monitoring and peripartum management. Follow-up visits, including an ultrasound assessment, were scheduled a minimum of once every 2 weeks.

The timing and mode of delivery were at the discretion of each woman's obstetric caregiver. After birth, venous blood samples were taken from each umbilical cord to measure the haemoglobin concentration and reticulocyte count. The placentas were examined for the presence of residual anastomoses by colour dye injection within 1 week.[14,19]

The primary outcome was a composite endpoint consisting of the following four items: incidence of twin anaemia polycythaemia sequence, recurrence of twin-to-twin transfusion syndrome, perinatal mortality, or severe neonatal morbidity. The primary outcome was scored in case one of the endpoints was reached. The presence or absence of twin anaemia polycythaemia sequence was identified using previously published criteria.[15] In brief, antenatal twin anaemia polycythaemia sequence was defined as present when doppler ultrasound examination revealed an increase in middle cerebral artery peak systolic velocity (MCA-PSV) of over 1·5 multiples of the median in one fetus that coincided with a decrease in MCA-PSV of less than 0·8 multiples of the median in the cotwin. Postnatal twin anaemia polycythaemia sequence was defined as both an inter-twin haemoglobin difference of at least 80 g/L at birth and at least one of the following: reticulocytosis in the donor with an inter-twin reticulocyte count ratio greater than 1·7 or the presence of only small (<1 mm in diameter) residual anastomoses seen at the time of postnatal placental injection assessments.[20]

The definition of recurrent twin-to-twin transfusion syndrome was based on the same parameters used to define twin-to-twin transfusion syndrome. Recurrent twin-to-twin transfusion syndrome includes cases of reversal of twin-to-twin transfusion syndrome. Severe

neonatal morbidity was defined as the presence of at least one of the following: chronic lung disease (defined as oxygen dependency at 36 weeks gestational age), patent ductus arteriosus needing medical therapy or surgical closure, necrotising enterocolitis grade 2 or higher, retinopathy of prematurity stage 3 or higher, ischaemic limb injury, amniotic band syndrome, or severe cerebral injury. Severe cerebral injury includes at least one of the following: intraventricular haemorrhage grade 3 or higher, cystic periventricular leukomalacia grade 2 or higher, ventricular dilatation greater than the 97th percentile, porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome.[21] Neonatal follow-up was done by the treating neonatologist. Follow-up was until term age or at discharge (whichever came first); neonatal outcome in this study only included short-term outcome.

The secondary outcomes consisted of residual anastomoses, gestational age at birth, birthweight, and complications such as iatrogenic monoamnionicity, membrane separation, bleeding at the introduction site or at the placental surface, intrauterine infection, and preterm pre-labour rupture of membranes. The following procedure characteristics were assessed: number of anastomoses detected, complete laser (opinion of the surgeon), visibility score ranging from 1 to 10 (with 1 for no visibility and 10 excellent visibility), procedure time, total energy, and maximum power used.

Statistical analysis

Each participating centre uploaded their data prospectively to a secure web-based database. Analyses were done by intention to treat. Continuous variables are reported as the mean (SD); group differences were compared using the Student's *t* test. Proportions were compared using the χ^2 test or the Fisher's exact test, where appropriate. Differences with a p value of less than 0·05 were regarded as statistically significant. Results are expressed as odds ratios (ORs) and 95% CIs. All analyses per fetus or neonate were done by the generalised estimated equation module to account for the effect that findings between cotwins are not independent. All statistical data were analysed using SPSS version 20.0 (IBM, Chicago, IL, USA).

Sample size and power were calculated based on a bilateral test of two proportions. Observational pilot data were used to compute the required intra-cluster correlation coeffcient ρ=0·58, with an expected decrease in the primary outcome of 15%. In a classic test based on a type 1 error rate (α) of 5% and a power (1–β) of 80%, we calculated that 173 women needed to be included. The estimated high correlation coeffcient showed an expected correlation between the twin fetuses in each pregnancy. Power calculations were done using the formula

for the variance inflation factor (VIF = 1+ρ × [k – 1] and N = VIF × n), where n is the number of women per arm in the classic test, k is the cluster size (k=2), and ρ is the intra-cluster correlation coeffcient. With α=0·05, 1–β=0·8, P1=45%, and P2=60%, we set our target sample size to 137 pregnant women in each group.

	Solomon group (n=137 women)	Standard group (n=135 women)
Maternal age (years)	30·0 (5·3)	30·7 (4·9)
Gestational age at laser (weeks)	19·4 (2·6)	19·9 (2·7)
Location of placenta		
Anterior	56 (41%)	62 (46%)
Posterior	81 (59%)	73 (54%)
Quintero stage		
Stage 1	21 (15%)	24 (18%)
Stage 2	50 (37%)	43 (32%)
Stage 3	63 (46%)	62 (46%)
Stage 4	3 (2%)	6 (4%)

Data are mean (SD) or number (%).

Table 1: Demographics and baseline characteristics of women

This sample size calculation differs from the calculation in our original protocol (NTR1245), which was based only on expected reductions in twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome of 15%. The adjustment made to the primary outcome affected our samples size. The Leiden University Research Committee urged us to adjust the primary outcome and therefore the sample size. This advice was part of the original ZonMw grant application, which was awarded during the first phase of the study. The reason for this adaptation was that originally we only included recurrent twin-to-twin transfusion syndrome and twin anaemia polycythaemia sequence as the primary outcome. However, the epidemiological expert panel pointed out that the Solomon technique might also have adverse effects, which should be part of the primary outcome. Since we agreed that this inclusion was of importance and that assessment of the safety of this new treatment modality was paramount, we added mortality and severe neonatal morbidity to the primary outcome. This decision and adjustment was made before any of the data were seen or analysed. The adjustment was approved by the Leiden University Medical Centre Medical Ethics Committee. A data safety monitoring board was appointed at the beginning of the trial and planned to do repeated safety analyses. No interim analyses were planned at the beginning of the trial. During the trial, we planned to report serious adverse events to the data safety monitoring board.

Role of the funding source

The trial was funded by the Netherlands Organization for the Health Research and Development. The sponsor of the study had no role in study design, data collection, data

analysis, data interpretation, or writing of the report. FS, EL, and DO had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 11, 2008, and July 12, 2012, 274 of 393 potentially eligible women were enrolled and were randomly assigned to either the Solomon group (n=139) or the standard

	Solomon group (n=137 women, 274 fetuses)	Standard group (n=135 women, 270 fetuses)	OR (95% CI)
Primary outcome*†	94/274 (34%)	133/270 (49%)	0·54 (0·35–0·82)
1-month postnatal survival			
Overall*	203/274 (74%)	198/270 (73%)	1·04 (0·66–1·63)
At least one surviving neonate	116/137 (85%)	117/135 (87%)	0·85 (0·43–1·68)
Double survival	87/137 (64%)	81/135 (60%)	1·16 (0·71–1.89)
TAPS or recurrent TTTS	6/137 (4%)	29/135 (21%)	0·17 (0·07–0·42)
Recurrent TTTS	2/137 (1%)	9/135 (7%)	0·21 (0·04–0·98)
TAPS	4/137 (3%)	21/135 (16%)	0·16 (0·05–0·49)
Severe neonatal morbidity‡	18/214 (8%)	28/211 (13%)	0·65 (0·31–1·36)
Chronic lung disease	4/214 (2%)	9/211 (4%)	0·48 (0·11–2·03)
Patent ductus arteriosus	6/214 (3%)	15/211 (7%)	0·37 (0·10–1·35)
Necrotising enterocolitis	2/214 (1%)	2/211 (1%)	0·99 (0·14–6·97)
Retinopathy of prematurity§	0/214 (0%)	3/211 (1%)	7·20 (0·69–970·52)
Ischaemic limb injury¶	1/214 (<1%)	1/211 (<1%)	0·99 (0·06–15·72)
Amniotic band syndrome	1/214 (<1%)	3/211 (1%)	0·33 (0·03–3·10)
Severe cerebral injury*‖	8/169 (5%)	10/178 (6%)	0·85 (0·32–2·29)
Intraventricular haemorrhage ≥grade 3§	5/169 (3%)	5/178 (3%)	1·06 (0·27–4·10)
Ventricular dilatation (greater than the 97th percentile)	1/169 (1%)	3/178 (2%)	0·35 (0·04–3·31)
Cystic periventricular leukomalacia ≥grade 2§	0/169 (0%)	1/178 (1%)	2·86 (0·15–419·23)
Porencephalic or parenchymal cysts§	1/169 (1%)	0/178 (0%)	0·31 (0·002–5·94)
Arterial infarction§	1/169 (1%)	0/178 (0%)	0·31 (0·002–5·94)
Other severe cerebral injury§	0/169 (0%)	1/178 (1%)	2·86 (0·15–419·23)

Data are n/N (%) unless otherwise specified. OR=odds ratio. TAPS=twin anaemia polycythaemia sequence. TTTS=twin-to-twin transfusion syndrome. *Measured per fetus using the generalised estimated equation module. †Composite of TAPS and recurrent TTTS, perinatal mortality, or severe neonatal morbidity. ‡Denominator is the number of liveborn neonates. §Measured using the method of Firth.[22] ¶Does not include amniotic band syndrome cases. ‖Denominator is number of neonates who underwent cranial ultrasound.

Table 2: Primary outcome

treatment group (n=135). Because of poor visibility at fetoscopy, four women underwent an alternative, ultrasound-guided procedure (cord occlusion n=2, amniodrainage n=2; figure 1). Two women in the Solomon group were excluded after randomisation because of an incorrect assessment of their eligibility: upon fetoscopy, one twin pregnancy was dichorionic (thus without vascular anastomoses) and the other excluded woman had twin anaemia polycythaemia sequence but not polyhydramnios. Thus, 137 women in the Solomon group and 135 in the standard group were included in the intention-to-treat analyses. The groups were similar with respect to maternal age, gestational age at treatment, placenta localisation, and Quintero stage (table 1).

In the intention-to-treat analysis, the primary outcome was identified in 94 (34%) of 274 fetuses in the Solomon technique group compared with 133 (49%) of 270 fetuses in the standard treatment group (OR 0·54, 95% CI 0·35–0·82; table 2). Table 2 lists the individual parameters within the composite outcome. Survival did not differ significantly between the two groups. Eight women underwent selective feticide via cord occlusion of one of the twins; this occurred either during the initial treatment (Solomon group n=1, standard group n=1), as described earlier, or at a later stage due to severe cerebral injury (Solomon group n=3, standard group n=3).

We found no significant difference between the groups with respect to gestational age at birth (32·4 weeks gestation [SD 3·3] in the Solomon group and 32·3 weeks gestation [3·3] in the standard group; p=0·89) or birth weight (1754g [SD 594] in the Solomon group and 1762g [611] in the standard group; p=0·82). 268 (98%) of 274 women underwent their assigned procedure. None of the women who were assigned to the standard treatment groups were treated with the Solomon technique. None of the aspects of the procedures were significantly different between the two treatment groups, except for total amount of laser energy (table 3). In the Solomon group, the operator

	Solomon group (n=137 women)	Standard group (n=135 women)	p value
Number of anastomoses detected	8 (3)	8 (4)	0·19
Laser complete (surgeon's opinion)	118 (86%)	116 (86%)	0·96
Visibility score	8 (7–10)	9 (8–9)	0·95
Procedure time:			
Skin to skin time (min)	35 (16)	35 (18)	0·99
Fetoscopy time (min)	23 (16)	22 (17)	0·65
Laser time (min)	6 (10)	5 (11)	0·31
Maximum power used (W)	45 (16)	46 (17)	0·59
Total amount of energy used (J)	9275 (9239)	4933 (4418)	<0·0001

Data are mean (SD), number (%), or median (IQR).

Table 3: Procedure details

reported a perceived complete separation of the placental parts in 118 (86%) of 137 women. None of the procedure-related complications differed between groups (table 4).

	Solomon group (n=137 women, 274 fetuses)	Standard group (n=135 women, 270 fetuses)	OR (95% CI)
Iatrogenic monoamnioticity	17 (12%)	14 (10%)	1·22 (0·58–2·60)
Amniondehiscence (membrane separation)	7 (5%)	13 (10%)	0·51 (0·20–1·31)
Bleeding at the introduction side	9 (7%)	7 (5%)	1·29 (0·47–3·56)
Bleeding at the placental surface	8 (6%)	3 (2%)	2·73 (0·71–10·51)
Intrauterine infection*	1 (1%)	0 (0%)	0·34 (0·002–6·35)
Rupture of membranes <24 h	4 (3%)	6 (4%)	0·65 (0·18–2·35)
Premature rupture of membranes	57 (42%)	46 (34%)	1·38 (0·84–2·26)
Intrauterine death of the fetus†	45/274 (16%)	48/270 (18%)	0·90 (0·56–1·49)
Death of fetus before 24 weeks of gestation†	15/274 (6%)	11/270 (4%)	1·34 (0·63–2·87)
Neonatal death within 28 days of birth†‡	11/214 (5%)	13/211 (6%)	0·79 (0·30–2·07)

Data are number (%) or n/N (%) unless otherwise specified. OR=odds ratio. *Measured using the methods of Firth.[22] †Measured per fetus using the generalised estimated equation module. ‡Denominator is the number of liveborn neonates.

Table 4: Procedure-related complications

The placentas of 151 women (Solomon group n=74, standard group n=77) were viable for study using colour dye injection. An example of colour-dye-stained twin-to-twin transfusion syndrome placenta that was treated using the standard technique is shown in figure 2 and one treated using the Solomon technique is shown in figure 3. The remaining placentas were not injected because of maceration after intrauterine fetal death of one or both twins (n=41) or because they were lost (n=45), damaged (n=27), or placed in formalin (n=8). Placentas were mainly lost from women whose fetuses were delivered in referring hospitals. In these hospitals, placental injection studies were not a standard procedure and placentas were sometimes discarded or fixed for routine pathology. In those placentas that were damaged, damage was due to manual removal with disintegration of morphology. Residual anastomoses were present in 14 (19%) of 74 and 23 (30%) of 77 of the placentas in the Solomon and standard treatment groups, respectively (p=0·12).

Outside of the common and well-known complications of twin-to-twin transfusion syndrome and its treatment, no serious adverse events occurred.

Discussion

In this randomised controlled trial, we found that fetoscopic laser coagulation of the entire vascular equator (Solomon technique) significantly reduced the incidence of twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome in monochorionic pregnancies complicated by twin-to-twin transfusion syndrome. The Solomon technique did not seem to be associated with an increase in any identifiable adverse outcome or complication. However, this study was not powered to detect differences in all possible outcome measures or complications.

Ultimate safety and efficacy parameters in our trial were perinatal morbidity and mortality. We included these two variables in the primary outcome to provide evidence that the Solomon

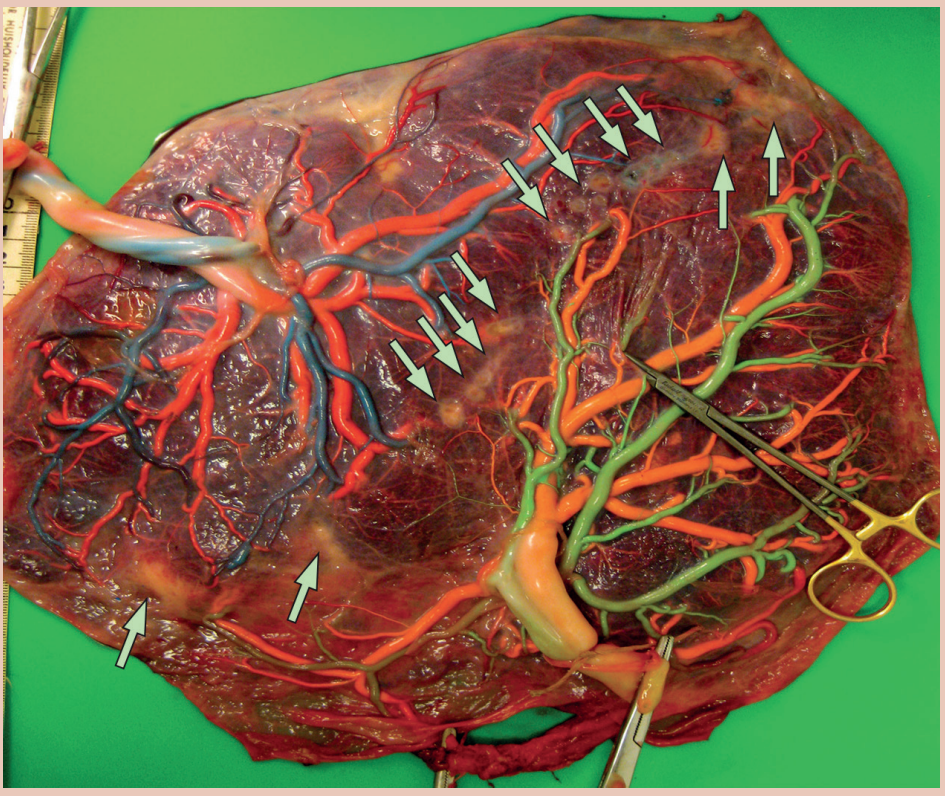

Figure 2. Colour-dye-stained twin-to-twin transfusion syndrome placenta that was treated using the standard technique Blue and green dye was used to stain the arteries, and pink and yellow dye was used to stain the veins. The arrows point to the individual laser spots.

technique is not less safe than the standard procedure. The overall primary outcome and individual comparison of these parameters showed no increased risks related to the Solomon technique. We therefore conclude that the new procedure is at least as safe as the standard procedure. Efficacy was assessed by measuring the recurrence of twin-to-twin transfusion syndrome and twin anaemia polycythaemia sequence, both part of our primary outcome. There was a significant reduction of recurrent twin-to-twin transfusion syndrome and twin anaemia polycythaemia sequence. Future trials by other investigators will hopefully confirm the robustness of these findings.

In the Solomon group, survival of both twins occurred in 64% of cases, with survival of at least one twin in 85% of cases. These values are on par with recent reports by other centres [8,9,23] and are substantially higher than in the early study on fetoscopic laser surgery. [4] Nevertheless, the overall survival rate in twin-to-twin transfusion syndrome is still far from

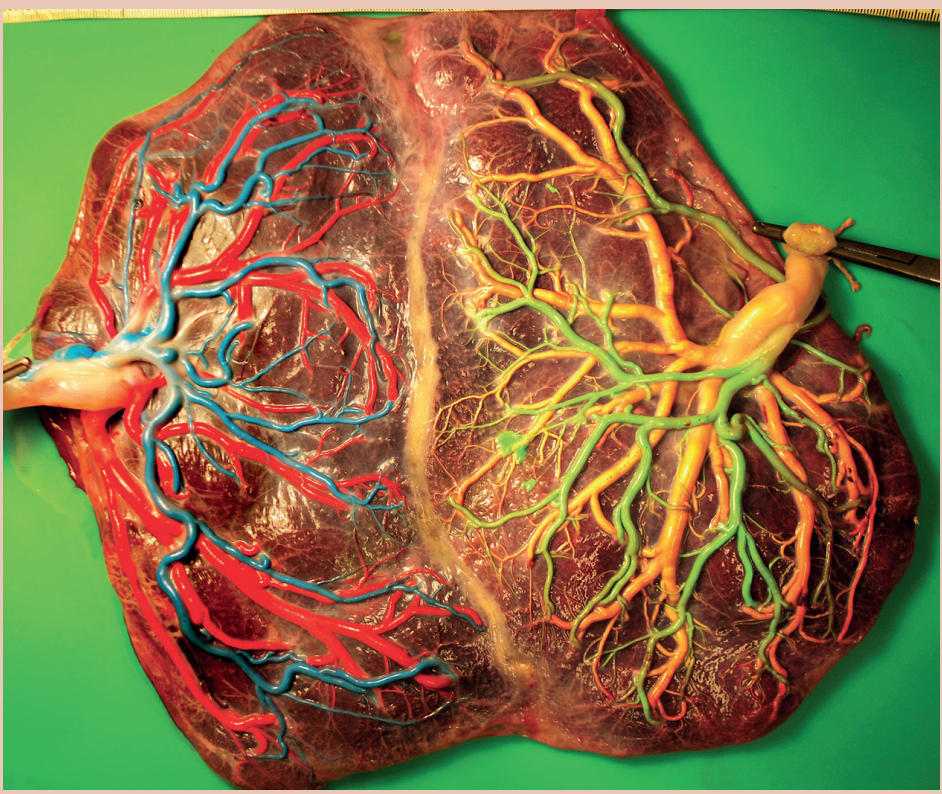

Figure 3. Colour-dye-stained twin-to-twin transfusion syndrome placenta that was treated using the Solomon technique Blue and green dye was used to stain the arteries, and pink and yellow dye was used to stain the veins. After identification and coagulation of each individual anastomosis, the complete vascular equator was coagulated from one placental margin to the other.

optimum, and improved methods for both diagnosis and intervention are urgently needed.

In two recent retrospective studies, the Solomon technique was compared with the standard technique (panel). Ruano and colleagues [24] compared 26 pregnancies treated with the Solomon technique with 76 pregnancies treated with the standard technique and found a substantially higher double survival rate in the Solomon group (84·6% vs 46·1%; p<0·01). Moreover, the Solomon-treated pregnancies had no twin anaemia polycythaemia sequence or recurrent twin-to-twin transfusion syndrome, compared with 7·9% (p=0·33) and 5·3% (p=0·57), respectively, in the standard technique group. Baschat and colleagues [25] compared the outcome of 71 women treated with the Solomon technique with 76 standard-treated women and found a reduction in the incidence of twin anaemia polycythaemia sequence (2·6% vs 4·2%) and recurrent twinto-twin transfusion syndrome (3·9% vs 8·5%), with a higher double-survival rate in the Solomon group (68·4% vs 50·7%; p<0·05).

Strengths of our study are the large number of women and fetuses included, the randomised design with intention-to-treat analysis, and the international, multicentre collaboration, which enhances its generalisability. This design might in part explain the differences with respect to survival rates compared with two recent retrospective cohort studies.[24,25] Survival rates in our two groups were similar, whereas the other studies reported a substantial improvement in survival with the Solomon technique compared with the standard technique. We assume that part of the improvement Ruano and colleagues [24] and Baschat and colleagues [25] noted could be because of increased experience with fetoscopic laser in general and not by the use of the Solomon technique. This type of bias was excluded in our trial by the randomised design.[17]

In this study, in 86% of women in the Solomon technique group, complete coagulation of the entire vascular equator was, according to the operators' judgment, done as planned. Nevertheless, a small percentage of residual anastomoses as well as twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome were still reported. This information should raise awareness by both clinicians and women that close monitoring (including serial MCA-PSV measurements) remains necessary until delivery.

We were unable to show a clear improvement in neonatal morbidity and survival. However, women whose pregnancies are complicated with recurrent twin-to-twin transfusion syndrome and twin anaemia polycythaemia sequence will need frequent visits or admission to the hospital and might need another potentially hazardous fetal intervention, which cause increased amounts of stress and anxiety for both women and doctors. Two factors probably play a part in

Panel: Research in context

Systematic review

We searched PubMed (1985 to Aug 2, 2013), with no language limits set, for the terms "twin to twin transfusion syndrome", "twin anaemia polycythaemia sequence", "fetoscopy", and "laser ablation". Because there was an absence of randomised controlled trials we also looked at prospective and retrospective studies. Two recently published small retrospective studies reported on outcome after use of the Solomon technique compared with the standard technique. Ruano and colleagues24 and Baschat and colleagues25 reported a reduction of twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome with improved double survival rates in women who received the Solomon technique.

Interpretation

Our randomised controlled trial shows a significant reduction of twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome without differences in survival rates.

the absence of statistically significant differences in neonatal morbidity and mortality in this study. First, these women were treated in highly specialised fetal therapy centres where these complications were optimally managed resulting in a low rate of adverse outcomes. Second, some adverse outcomes associated with twin anaemia polycythaemia sequence—such as ischaemic limb injury and brain injury—are too rare to assess properly in a trial of this power. The difference in neonatal outcome between the Solomon group (8%) and the standard group (13%) was not significant, probably because of the small sample size, and we speculate that in larger series and especially in less specialised centres the Solomon technique will lead to true improved neonatal outcome. Additionally, one of the most important issues, which will be addressed in a future study, is long-term neurodevelopmental outcome. The clinical importance of the Solomon technique should also be re-assessed once the results of the longterm follow-up study are available. Because this modified technique reduces the incidence of twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome without any measurable adverse events, we propose that the Solomon technique should be the preferred treatment modality.

As with any assessment of a surgical technique, our study was limited by the fact that the operating teams could not be masked with respect to the treatment group; Nevertheless, the risk of bias in the assessment of the components of our primary outcome measure is probably negligible because the diagnoses of twin anaemia polycythaemia sequence and recurrent twin-to-twin transfusion syndrome were based on standardised ultrasound and doppler velocity measurements according to internationally accepted protocols and images were stored in electronic patient files. Ultrasonographers were often not part of the surgical team or investigator team; however, complete masking of this group was not possible. The neonatologists were often aware of the treatment that was received; however, the risk of bias in ascertaining outcome is limited since the postnatal diagnosis of twin anaemia polycythaemia sequence is based on objective, routine haematological laboratory tests undertaken by

laboratory personnel unaware of the study.

In this study, we reported only short-term neonatal outcome, defined as the presence of chronic lung disease, patent ductus arteriosus, necrotising enterocolitis, retinopathy of prematurity, ischaemic limb injury, amniotic band syndrome, or severe cerebral injury. These diagnoses are mainly clinical diagnoses (not based on specific diagnostic tests) and are routinely recorded by neonatologists in women admitted to neonatal intensive care units. Lastly, the placental injections and subsequent assessment of residual anastomoses (pictures were taken and stored after coloured dye injection) could not be masked because which technique was used is obvious from looking at the placental surface.

Our results suggest that use of the Solomon technique reduces the risk of complications associated with residual anastomoses in monochorionic pregnancies that are treated with laser surgery for twin-to-twin transfusion syndrome. In view of the absence of any adverse effects compared with standard coagulation treatment, we recommend that fetoscopic surgeons consider adopting this strategy for treatment of women with twin-to-twin transfusion syndrome. Additional prospective studies and long-term neurodevelopmental outcome studies will help confirm the benefits of using the Solomon technique for treatment of twin-to-twin transfusion syndrome pregnancies.

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7

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Chapter 8

Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial

Abstract

Objective:

Residual anastomoses after fetoscopic laser surgery for twin-to-twin transfusion syndrome (TTTS) may lead to severe postoperative complications, including recurrent TTTS and twin anemia-polycythemia sequence (TAPS). A novel technique (Solomon technique) using laser coagulation of the entire vascular equator was recently investigated in a randomized controlled trial (Solomon trial) and compared to the Standard selective laser technique. The aim of this secondary analysis was to evaluate the occurrence and characteristics of residual anastomoses in placentas included in the Solomon trial.

Study design:

International multicenter randomized controlled trial in TTTS, randomized 1:1 ratio to either the Solomon laser technique or Standard laser technique. At time of laser, surgeons recorded whether they considered the procedure to be complete. Placental dye injection was performed after birth in the participating centers to evaluate the presence of residual anastomoses.

Results:

A total of 151 placentas were included in the study. The percentage of placentas with residual anastomoses in the Solomon group and Standard group was 19% (14/74) and 34% (26/77), respectively ($P = .04$). The percentage of placentas with residual anastomoses in the subgroup of cases where the procedure was recorded as complete was 8/65 (12%) and 22/69 (32%) in the Solomon group and Standard group, respectively ($P < .01$).

Conclusion:

The Solomon laser technique reduces the risk of residual anastomoses. However, careful follow-up remains essential also after the Solomon technique, as complete dichorionization is not always achieved.

Introduction

Twin-twin transfusion syndrome (TTTS) occurs in 10% of monochorionic twins and is caused by imbalanced blood flow through placental vascular anastomoses.[1] The best treatment for TTTS is fetoscopic laser coagulation of the anastomoses and is associated with survival rates of both fetuses of 67%. [2] The goal of fetoscopic laser surgery is to coagulate all vascular anastomoses. However, inter-twin vascular connections may remain patent in up to 33% of TTTS cases. [3;4] These residual anastomoses can cause severe post-operative complications such as twin anemia-polycythemia sequence (TAPS) in 13-16% or recurrent TTTS in 7-14% of cases where both babies survive.[5;6]

To minimize the occurrence of residual anastomoses and their complications, we investigated in a randomized controlled trial a modified fetoscopic laser surgery technique called the "Solomon technique". The aim of this technique is to draw a coagulation line along the entire vascular equator to reduce the risk of missing inter-twin vascular anastomoses, in particular the poorly visualized small anastomoses. In the first analysis of the Solomon study, we focused on the perinatal outcome and showed a significant improvement in clinical outcome after the Solomon technique.[6] The aim of this secondary analysis was to perform a detailed analysis of the placentas included in the Solomon study, and to determine the occurrence and characteristics of residual anastomoses. In addition we performed a sub-analysis of all cases in which the surgeon reported that the procedure was technically complete.

Materials and Methods

Study design:

The Solomon trial [6] was an open-label, randomized, controlled trial, performed in five European tertiary referral centres (University Hospital Leuven (Belgium), University Hospital of Strasbourg (France), Birmingham Women's Hospital, University of Birmingham, Birmingham (UK), Buzzi Hospital Milan (Italy), and Leiden University Medical Centre (the Netherlands). The study was approved by the Ethics committee of the Leiden University Medical Centre (MEC P07.261) and each centre's respective Institutional Review Board. The trial was registered with the Dutch trial registry, number NTR 1245. The background of the trial, methods and baseline characteristics have been reported previously.[6] In brief, the trial included 274 patients and were randomly assigned to the Solomon technique or Standard technique. All TTTS cases included in the Solomon trial were eligible for this study, except

	Solomon (n=74)	Standard (n=77)	p value
Gestational age at laser (weeks)	20±2.6	20±2.7	0.57
Location of placenta:			
Anterior	27 (37)	36 (47)	0.20
Posterior	47 (64)	41 (53)	
Quintero stage			
Stage I – n (%)	15 (20)	17 (22)	0.26
Stage II – n (%)	27 (37)	20 (26)	
Stage III – n (%)	31 (42)	35 (46)	
Stage IV – n (%)	1 (1)	5 (7)	
Laser complete (opinion surgeon) n (%)	65 (88)	69 (90)	0.73

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

Table 1: Baseline characteristics

cases treated in Birmingham. These cases (n=25) were excluded from the current study since placentas were not routinely injected. Exclusion criteria were placenta maceration following intrauterine death, placentas with disrupted architecture as a result of the delivery process or when placentas were placed in formalin. In case fetal demise occurred and the time period between fetal demise and birth was within a week the placentas were injected. Placentas were sent and injected in the participating centers of this trial. All other placentas were included in this study and injected with colored dye according to a specific technique reported below.

Placenta injection protocol:

Placental storage and injection was performed according to a previously published report. [4;7] In brief, after birth, placentas were stored in a plastic bowl at a storage temperature of 4 ^{o}C (without being frozen or fixed in formalin) and injected within a week. Before injection the placenta was washed with warm water and amnions were removed for better visualization of the vascular anastomoses. The umbilical vein and at least one artery of each cord were cannulated. Syringes with four different colored dyes were connected to the cannulas and dye was gently injected into the placental vessels. A measuring tape was placed on the placenta and digital high resolution pictures were taken perpendicular to the placental surface. The following placental characteristics were recorded: number, localization, size and type of residual anastomoses. Measurements of the size and localization of the anastomoses were performed using Image J 1.45s equipment and software (Image J, National Institute of Health, USA). The placental injections and subsequent evaluation of residual anastomoses could not be blinded, as it is obvious from observing the placental surface which technique was used. In case of arterio-venous (AV) anastomoses, the caliber of the artery was measured. After measuring the total length of the vascular equator, we calculated its radius, by dividing the

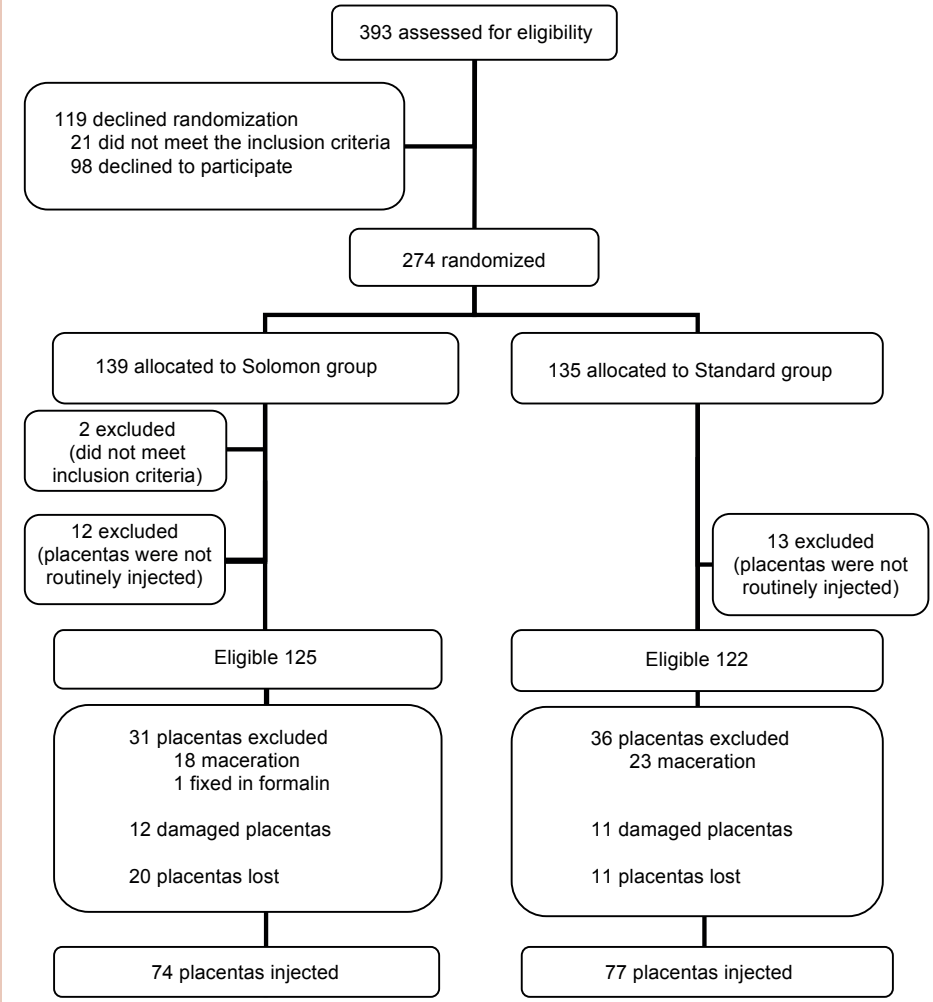

Figure 1. Trial profile

length of the vascular equator in two. The localization of residual anastomoses was recorded as the ratio of their distance and radius (distance/radius) as previously reported.[3] In case residual anastomoses were detected during fetoscopic re-intervention (due to TAPS or recurrent TTTS), these were analyzed in the group with residual anastomoses.

Primary and secondary outcome:

The primary outcome of the Solomon trial was based on the short-term clinical outcome,

and included the presence of at least one of the four following items: TAPS, recurrent TTTS, perinatal mortality or severe neonatal morbidity. One of the secondary outcomes of the Solomon trial was the incidence of residual anastomoses. In this placental study we analyzed the type, size and localization of residual anastomoses after colored dye injection. Comparison between the Solomon group and Standard group was performed in the total group of injected placentas, and in the sub-group of placentas from pregnancies in which the fetoscopic laser surgery was recorded as 'complete' according to the surgeon's opinion directly after the laser intervention. This sub-group analysis was performed to determine the impact of the surgeon's opinion on the final result after fetoscopic laser surgery, and whether this could be useful to direct postoperative management.

We defined TTTS using the Eurofoetus criteria, with a cut-off at a deepest vertical pocket (DVP) of amniotic fluid in the donor ≤2 cm; in addition, we used a cut-off for the DVP in the recipient of ≥8 cm within the first 20 weeks of gestation or ≥10 cm after week 20.[8] The definition of recurrent TTTS was based on the same parameters used to define TTTS. Recurrent TTTS is including cases of reversal of TTTS. The presence or absence of TAPS was identified using previously published criteria.[5] In brief, antenatal TAPS was defined as present when Doppler ultrasound examination revealed an increase in Middle Cerebral Artery - Peak Systolic Velocity (MCA-PSV) of >1.5 Multiples of the Median (MoM) in one fetus that coincided with a decrease in MCA-PSV of <0.8 MoM in the co-twin. Postnatal TAPS was defined as both an inter-twin Hb difference of ≥8 g/dL at birth and at least one of the following: reticulocytosis in the donor with an inter-twin reticulocyte count ratio >1.7

	Total			**Laser complete (surgeon's opinion)**		
	Solomon (n=74)	**Standard (n=77)**	**p value**	**Solomon (n=65)**	**Standard (n=69)**	**p value**
Placentas with residual anastomoses	14 (19)	26 (34)	0.04	8 (12)	22 (32)	<0.01
Overall no. of residual anastomoses per placenta	2 (1-24)	2 (1-8)	0.94	2 (1-4)	2 (1-5)	0.90
Diameter of residual anastomoses (mm)	1.3±1.8	0.8±0.9	0.07	2.1±3.0	0.9±1.0	0.01
Placentas with arterio-venous residual anastomoses	10 (71)	21 (91)	0.11	6 (75)	17 (90)	0.33
Placentas with arterio-arterial residual anastomoses	6 (43)	4 (17)	0.09	3 (38)	2 (11)	0.10
Placentas with veno-venous residual anastomoses	7 (50)	4 (17)	0.04	4 (50)	3 (16)	0.06

Data are median (range), mean ± standard deviation or n (%) unless otherwise specified

Table 2: Prevalence, number and size of residual anastomoses

	Total			Laser complete (surgeon's opinion)		
	Solomon (n=74)	Standard (n=77)	p value	Solomon (n=65)	Standard (n=69)	p value
Gestational age at birth	31±5	32±4	0.48	32±4	32±4	0.68
Twin anemia-polycythemia sequence	3 (4)	17 (22)	<0.01	1 (2)	15 (22)	<0.01
Recurrent TTTS	1 (1)	4 (5)	0.19	0 (0)	4 (6)	0.05
Twin anemia-polycythemia sequence or recurrent TTTS	4 (5)	20 (26)	<0.01	1 (2)	18 (26)	<0.01
Perinatal survival [a]	122/148 (82)	135/154 (88)	0.32	114/130 (88)	124/138 (90)	0.67
Severe neonatal morbidity [a,b]	14/131 (11)	17/144 (11)	0.86	13/121 (11)	14/130 (11)	0.99

Data are mean ± standard deviation or n (%) unless otherwise specified. TTTS = twin-twin transfusion syndrome

[a] Results measured per fetus using the Generalized Estimated Equation module

[b] Severe neonatal morbidity measured per live born neonate

Table 3: Perinatal outcome

and/or the presence of only small (<1 mm in diameter) residual anastomoses seen at the time of postnatal placental injection studies.[9] Severe neonatal morbidity was defined as the presence of at least one of the following: chronic lung disease (defined as oxygen dependency at 36 weeks gestational age), patent ductus arteriosus requiring medical therapy or surgical closure, necrotizing enterocolitis grade 2 or higher, retinopathy of prematurity stage III or higher, ischemic limb injury, amniotic band syndrome or severe cerebral injury. Severe cerebral injury includes at least one of the following: intraventricular haemorrhage grade III or higher, cystic periventricular leukomalacia grade II or higher, ventricular dilatation greater than the 97th percentile, porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome.[10] Neonatal follow-up was done by the treating neonatalogist. Length of follow-up was until term age or at discharge (which ever came first); neonatal outcome in this study only included short term outcome.

Statistical analysis

Continuous variables are reported as the mean ± standard deviation; group differences were compared using the Student's t-test. Proportions were compared using the Chi-square test or the Fisher's exact test, where appropriate. Differences with a p-value <0.05 were considered to be statistically significant. All analyses per fetus or neonate were performed using the Generalized Estimated Equation module to account for the effect that observations between co-twins are not independent. All statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA).

Results

A total of 247 placentas were eligible for this study (Figure 1). We excluded 65 placentas due to maceration following intrauterine fetal death of one or both twins (n=41), damaged (n=23), or placement in formalin (n=1). In cases where the placentas were damaged, this was often due to manual removal with disintegration of morphology. Of the 41 excluded cases were fetal demise occurred, two cases were double demise and 39 cases were single demise. Of the double demise one occurred within a week after laser and the other after a week. Of the single demise 24/39 (62%) occurred within one week after laser and 15/39 (38%) after a week. Data on fetal condition prior to fetal demise was not recorded. Finally, 31 eligible placentas were lost. This mainly occurred when patients delivered in referring hospitals. Two main reasons for delivering at a referral hospital were spontaneous premature delivery in which the patients could not be transferred to the treatment center or in cases with good recovery (normalization of amniotic fluid in both sacs and MCA-PSV Doppler measurements in both twins) in combination with the request of the patient to go back to the referral center (for logistic reasons). In these hospitals placental injection studies were not a standard procedure, and placentas were sometimes discarded or accidentally fixed for routine pathology. Gestational age at birth and the incidence of TAPS or recurrent TTTS was similar in the excluded group due to placental loss compared to the group of placentas included in the study (Table 4).

	Included placentas (n=151)	Lost placentas (n=31)	p value
Gestational age at birth [a,b]	32±3	32±3	0.42
TAPS	20 (13)	2 (7)	0.38
Recurrent TTTS	5 (3)	1 (3)	1.0
TAPS or recurrent TTTS	24 (16)	3 (10)	0.58
Perinatal survival [b]	257/302 (85)	49/62 (79)	0.36
Severe neonatal morbidity [b,c]	31/275 (11)	5/53 (9)	0.68

Data are mean ± standard deviation or n (%) unless otherwise specified
TAPS = twin anemia polycythemia sequence. TTTS = twin-twin transfusion syndrome
[a] Gestational age at birth of liveborn neonates
[b] Results measured per fetus using the Generalized Estimated Equation module
[c] Severe neonatal morbidity measured per live born neonate

Table 4: Perinatal outcome included placentas versus lost placentas

Complete placental dye injection was performed in 151 placentas, 74 (49%) placentas in the Solomon group and 77 (51%) placentas in the Standard group. Of the injected placentas, 19 placentas were after fetal demise, in 9/19 (47%) double fetal demise occurred and placentas were injected, in the remaining 10/19 (53%) placentas could be injected after fetal

demise of the co-twin. The laser procedure was recorded by the surgeons as complete in 65/74 (88%) in the Solomon group, and 69/77 (90%) in the Standard treatment group. There were no differences in baseline characteristics between the two study groups with respect to gestational age at fetoscopy, placenta localization and Quintero stage (Table 1).

A significant reduction of residual anastomoses was seen after using the Solomon technique. Residual anastomoses were detected in 19% (14/74) of placentas in the Solomon group compared to 34% (26/77) in the Standard group (P = .04). In the subgroup of cases in which laser surgery was recorded as complete by the surgeon, an even larger reduction of residual anastomoses was seen, 12% (8/65) in the Solomon group compared to 32% (22/69) in the Standard group (P < .01) (Table 2). In three cases with recurrent TTTS or TAPS requiring re-intervention with laser surgery, residual anastomoses were detected at time of re-intervention during fetoscopy. All three cases were initially treated with the Standard technique and analyzed in the group with residual anastomoses. Placental injection after birth in these three cases showed no residual anastomoses. Two (3%) placentas in the Solomon group had

	TAPS (n=17)	No TAPS (n=18)[b]	p value	Rec TTTS (n=3)	No rec TTTS (n=18)[c]	p value
Diameter of residual anastomoses (mm)	0.4±0.5	1.3±1.07	< 0.01	0.8±1.06	1.4±1.8	0.11
Placentas with residual arterio-venous anastomoses	17 (100)	13 (72)	0.05	2 (67)	13 (72)	1.0
Placentas with residual arterio-arterial anastomoses	1 (6)	7 (39)	0.04	2 (67)	7 (39)	0.55
Placentas with residual veno-venous anastomoses	1 (6)	9 (50)	< 0.01	1 (33)	9 (50)	1.0

Data are mean ± standard deviation or n (%) unless otherwise specified
TAPS= twin anemia-polycythemia sequence, rec TTTS = recurrent twin-twin transfusion syndrome
[a] Sub-analyses performed in subgroup of the 37 placentas with residual anastomoses
[b] In the group "No TAPS" cases with recurrent TTTS were excluded
[c] In the group "No rec TTTS" cases with TAPS were excluded

Table 5: Sub-analysis of the residual anastomoses according to twin anemia-polycythemia or recurrent TTTS[a]

proximate cord insertions (distance < 5cm) and laser surgery was incomplete (though they were stated to be complete by the surgeon) resulting in various large residual anastomoses between the two cords. No placentas with proximate cord insertions were present in the Standard group.

The median number of residual anastomoses was similar in the Solomon and Standard group (see Table 2). In the subgroup of cases in which laser surgery was recorded as complete by

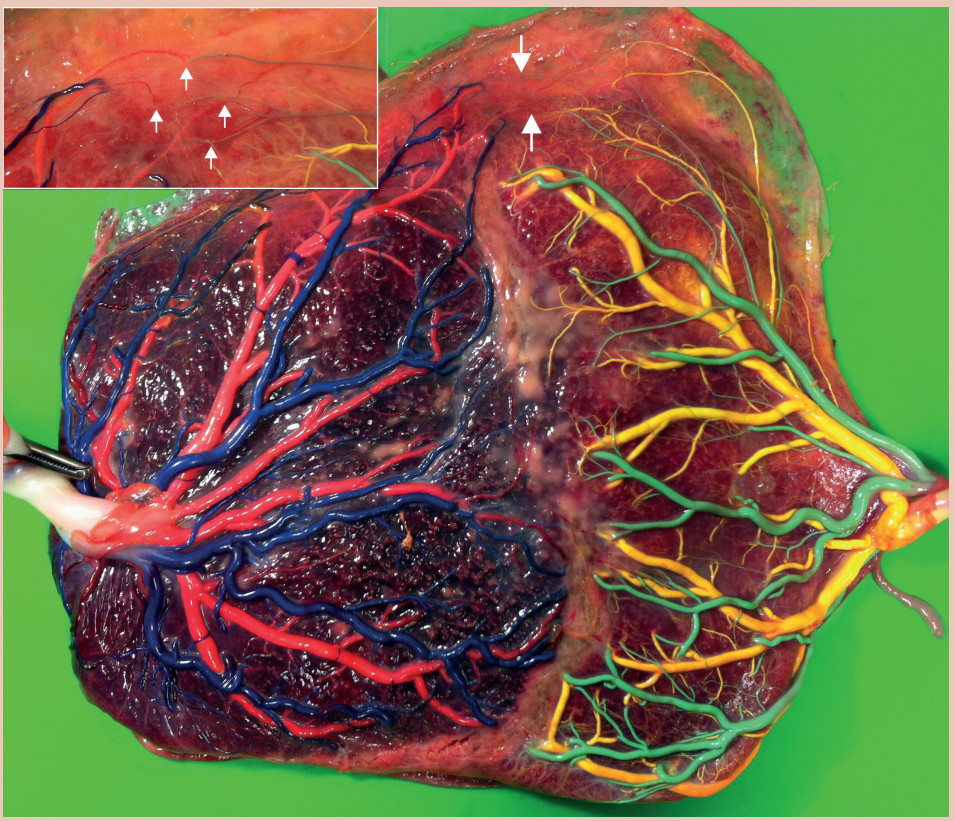

Figure 2. Placenta after dye injection in a TTTS case treated with the Solomon technique. Blue and green dye was used to stain the arteries, and pink and yellow dye was used to stain the veins. This placenta shows a Solomon line along the vascular equator and residual veno-arterial anastomoses at the placental margin (indicated by white arrows). These residual anastomoses caused TAPS.

the surgeon, the mean diameter of residual anastomoses was 2.1 (±3.0) mm in the Solomon group versus 0.9 (±1.0) mm in the Standard group (P = .01). The larger mean diameter of residual anastomoses in the Solomon group was mainly due to the two placentas with proximate cord insertion. After analyzing the data without the two placentas with proximate cord insertions, the mean diameter of residual anastomoses in the Solomon group and in the Standard group was similar, 1.4 (±1.3) mm versus 0.9 (±1.0) mm, respectively (P = .15). The localization of RAs was evenly distributed along the vascular equator. Placentas with residual anastomoses in the Solomon group where the surgeon stated to be complete were characterized by proximate cord insertion (n=2), discontinuous line (n=5) and Solomon line not along the vascular equator (n=1). Figure 2 shows a placenta treated using the Solomon technique with residual anastomoses near the margin of the placenta, causing TAPS.

The risk of recurrent TTTS was 5% (4/77) in the Standard group compared to 1% (1/74)

in the Solomon group (P = .19). A significant reduction of TAPS of 22% (17/77) in the Standard group to 4% (3/74) in the Solomon group was seen (P < .01). A similar reduction in post-operative complications between Standard and Solomon group was detected in the subgroup of cases in which the laser procedure was recorded as complete at time of laser (see Table 3). Overall, the incidence of TAPS and recurrent TTTS in cases with residual anastomoses was 48% (19/40) and 13% (5/40), respectively. The risk of TAPS in the group with residual anastomoses was 21% (3/14) in the Solomon group and 62% (16/26) in the Standard group (P = .02). The risk of recurrent TTTS in the group with residual anastomoses was 7% (1/14) in the Solomon group compared to 15% (4/26) in the Standard group (P = .64). The residual anastomoses in TAPS cases were characterized by a smaller mean diameter 0.4 (±0.5) mm compared to 1.3 (±1.1) mm in cases without TAPS (P < .01). TAPS placentas had less arterio-arterial and veno-venous residual anastomoses compared to cases without TAPS, respectively 6% (1/17) versus 39% (7/18) (P .04.) and 6% (1/17) versus 50% (9/18) (P < .01). There were no differences in size and type of residual anastomoses in the placentas with recurrent TTTS. Details are shown in table 5.

Comment

In this secondary analysis of the Solomon trial, we showed that fetoscopic laser coagulation using the Solomon technique significantly reduces the incidence of residual anastomoses. This is the first randomized trial showing a reduction of residual anastomoses using a new laser technique in TTTS. In three recent retrospective studies, the Solomon technique was compared with the Standard technique, the investigators found a reduction of TAPS or recurrent TTTS in the Solomon group. Unfortunately these investigators did not report placental injection studies.[11-13] Importantly however, even after the Solomon procedure, we still found a clinically important number of residual anastomoses (19% in the overall Solomon group and 12% in the subgroup of placentas which were recorded as complete after the procedure). This highlights the fact that the Solomon procedure does not guarantee a complete dichorionization of the placenta. Therefore, careful follow-up with serial Doppler ultrasound measurements of the middle cerebral artery peak systolic velocity and of amniotic fluid volumes of both twins therefore remains of crucial importance, even after a laser intervention using the Solomon technique.

As shown in this study, the presence of residual anastomoses is associated with a 58% risk of developing TAPS or recurrent TTTS. Interestingly, the risk of TAPS or recurrent TTTS was lower in the Solomon group with residual anastomoses compared to the Standard group

with residual anastomoses (29% versus 73% respectively). This could be explained by a trend towards higher rates of residual arterio-arterial anastomoses in the Solomon group. As previously shown, arterio-arterial anastomoses are known to protect against the development of TTTS or TAPS.[14;15] The relatively high rate of arterio-arterial and veno-venous anastomoses in the Solomon group was partly related to the presence of two placentas with proximate cord insertions in the Solomon group. As shown in a recent study, arterio-arterial and veno-venous anastomoses occur more frequently in monochorionic placentas with proximate cord insertions.[16]

The main reason for residual anastomoses in the Solomon group was the fact that the laser-line along the vascular equator was not continuous. We expected to find the residual anastomoses along the margin of the placenta. However, in the 8 placentas of pregnancies in which the procedure was complete according to the surgeon, residual anastomoses were spread along the vascular equator. A possible explanation could be that the energy used to coagulate the surface of the placenta was not always sufficient. More studies are needed to evaluate the effectiveness of coagulation using different laser energy settings. For the optimal laser effect, a careful balance should be sought between avoiding excessive tissue damage due to too much laser energy, and insufficient tissue damage (and possible residual anastomoses) due to reduced laser energy.

We found that the diameter of residual anastomoses in the Solomon group was increased compared to residual anastomoses in the Standard group. However, after exclusion of two of the placentas with residual anastomoses in the Solomon group with proximate cord insertions and large residual anastomoses no significant difference in diameter was found. As shown in a recent study16 monochorionic placentas with proximate cord insertions treated with laser surgery have often an increased rate of residual anastomoses due to technical and surgical problems related to the identification of the vascular equator.[16;17]

The risk of TAPS or recurrent TTTS was significantly higher in the Standard group and was directly related to the presence of residual anastomoses. The incidence of residual anastomoses of 34% in the Standard group was similar to previous published reports by our own group and by Lewi et al (range 27-33%). [3;4;18] Other authors [19;20] reported a lower incidence of residual anastomoses in their series after using the Standard laser technique (4-6%). This difference might be related to operator-experience, although we believe that differences in placental injection technique are more likely. In the studies with lower incidence of residual anastomoses, placental injection was performed with air instead of colored dye, which hampers visualization of small missed anastomoses. As shown in this

and other studies, residual anastomoses in TAPS cases are very small (diameter <1.0mm) and may only be recorded reliably after accurate dye injection.[21-23]

One of the limitations of this study is the fact that we were unable to inject all placentas. Lost or damaged placentas mainly came from patients delivered at referring hospitals where placental injection studies were not a standard procedure. A selection bias based on the lost placentas is not likely since the outcome of these pregnancies did not differ from the included placenta group.

In conclusion, the Solomon technique reduces the incidence of residual anastomoses. Nevertheless, since the risk of residual anastomoses after using the Solomon technique is still existent, careful antenatal follow-up with Doppler ultrasound remains necessary.

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Chapter 9

Intrauterine transfusion combined with partial exchange transfusion for twin anemia polycythemia sequence: modelling a novel technique

Abstract

Introduction:

Twin anemia-polycythemia sequence (TAPS) is a newly described disease in monochorionic twin pregnancies, characterized by large inter-twin hemoglobin differences. Optimal management for TAPS is not clear. One of the possible treatment modalities is intrauterine blood transfusion (IUT) in the donor with or without combination of partial exchange transfusion (PET) in the recipient.

Methods:

We applied a computational model simulation to illustrate the mechanism of IUT with and without PET in TAPS occurring after laser surgery for twin-twin transfusion syndrome (TTTS). Model simulations were performed with the representative anastomotic pattern as observed during laser intervention, and after placental dye injection.

Results:

The model was tested against different cases where IUT was combined with PET for the treatment of post-laser TAPS. Model simulations using the observed anastomotic pattern showed a significant reduction of hyperviscosity in the recipient after IUT/PET compared to IUT without PET.

Discussion:

In this model simulation we show that the addition of PET to IUT reduces the severity of polycythemia in the recipient. PET may thus be important to prevent complications of hyperviscosity.

Conclusion:

This model simulation shows the beneficial effect of PET for the recipient in TAPS cases treated with IUT.

Introduction

Monochorionic twin pregnancies can be complicated by the twin anemia-polycythemia sequence (TAPS), which is a chronic form of feto-fetal transfusion. TAPS is characterized by large inter-twin hemoglobin (Hb) differences but without signs of the oligo-polyhydramnios sequence. TAPS placentas are characterized by the presence of only few, miniscule vascular anastomoses [1]. The incidence of TAPS varies between 1-5% in spontaneous TAPS [2-5] and up to 16% in post-laser TAPS [6;7]. TAPS can be diagnosed antenatally or postnatally. "Prognosis of TAPS can vary from two healthy neonates with transient hematological problems to severe neonatal morbidity, such as limb necrosis, severe cerebral injury or perinatal death." Antenatal management options include expectant management, induction of labor, intrauterine blood transfusion (IUT) with or without combination of partial exchange transfusion (PET), selective feticide or (repeat) fetoscopic laser surgery [8-11]. Treatment with IUT at least temporarily improves the condition of the donor twin, however, the transfer of transfused red cells to the already polycythemic recipient may worsen its hyperviscosity and increases the risk for associated complications such as limb necrosis and severe cerebral injury [6;12]. The PET procedure implies replacement of the polycythemic fetal blood with saline solution and leads to a reduction of hyperviscosity. In this study we tested a model simulation against different cases of IUT in combination with PET. With this model we illustrate the mechanism of IUT with PET compared to IUT without PET.

Methods

We selected different post-laser TAPS cases treated with IUT in combination with PET with full details of vascular anastomotic pattern during laser treatment and from postnatal placental injection studies[13]. Details on IUT and PET were recorded, including the amount of blood transfused and exchanged. Simulations of the effect of IUT and PET were performed in our computational model of monochorionic twin pregnancies [11].

Mathematical model simulation

The model as used for the simulations was based on the previous TTTS models with nonpulsating circulations [14;15] as was previously applied to predict development of hydrops in the TTTS recipient [16] and the presence of a discordant hematocrit in the presence of normal amniotic fluid volumes after incomplete laser therapy of vascular anastomoses [11]. In brief, the model applies 13 coupled differential equations for each twin

to describe changes in volumes of fetal arterial and venous blood, volumes of interstitial, intracellular, and amniotic fluid, colloid osmotic pressures of fetal blood and interstitial fluid, osmolality of fetal blood and amniotic fluid, the concentration of vasoconstrictive peptides in the fetal blood, blood hematocrit, arterial wall elastin content, arterial wall thickness, as well as measures of brain and placental vascular resistances. The differential equations of the nonpulsatile model are programmed in Delphi 5.0 (Borland Interprise Corp., Cupertino, CA) and are numerically solved for 12 to 36 weeks with a time step of approximately 0.6 sec. Input variables for computation of anastomotic flow include the type of anastomosis, i.e. arterio-venous (AV), veno-arterial (VA), arterio-arterial (AA) or veno-venous (VV) and the anastomosis resistances. Following the application of Ohm's law of each of the resistances, multiple parallel placental anastomoses of identical type can be computationally represented by a single corresponding replacement resistance. Laser intervention of a set of anastomoses combined with amnioreduction is simulated as cessation of fetofetal transfusion of blood and constituents through the anastomoses and the normalization of the recipient amniotic fluid volume. IUT as well as PET are simulated by instantaneous increase or replacement of blood volume and its constituents respectively with blood of normal properties.

Results

Vascular anastomotic pattern at time of laser for TTTS, amount of blood transfused or exchanged were available in four post-laser TAPS cases and are reported in Table 1. Figure 1 shows the middle cerebral artery – peak systolic velocity (MCA-PSV) measurements of case 1. Placenta injection with color dye of case 1 showed the presence of 1 miniscule AV anastomosis (diameter 0.2mm) from the anemic twin to the polycythemic twin (figure 2).

Model simulations were performed using as input variables the anastomotic pattern as observed during laser intervention and after placental injection analysis. Hence, in the model, prior to laser intervention, large bidirectional arteriovenous flow was simulated whereas after laser intervention only one small uncompensated arteriovenous anastomosis from ex-recipient to ex-donor was simulated. Placental sharing between the twins was set as equal and all interventions were modelled at identical gestational ages and blood volumes used for IUT and PET as performed clinically. A model simulation for case 1 and 2 showing computed hematocrit as a function of gestational age is shown in Figure 3. Model simulations were performed for IUT with and without PET of the TAPS recipient for all the reported post-laser TAPS cases. Model simulations were comparable for these four cases, model simulation for case 1 and 2 are shown in Figure 3. The model simulation shows the difference in hematocrit

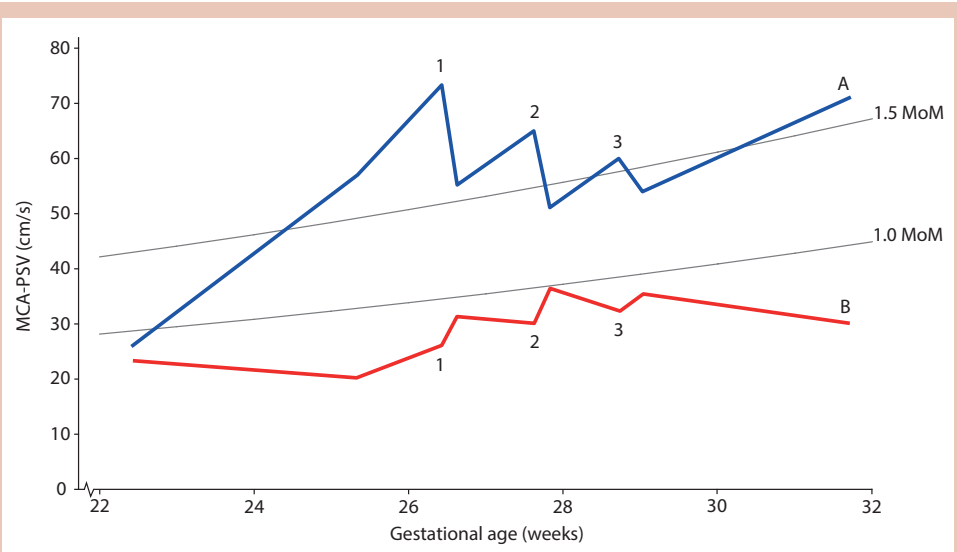

Figure 1. Doppler measurements of Middle Cerebral Artery – Peak Systolic Velocity (MCA-PSV) in cm/sec and gestational age in weeks. In blue the MCA-PSV measurements of the ex-TTTS recipient and post-laser TAPS donor (A). In red the MCA-PSV measurements of the ex-TTTS donor and post-laser TAPS recipient (B). The numbers indicate the time points of intrauterine blood transfusion and partial exchange transfusion.

in the recipient with and without the combination of PET. Figure 4 shows for case 1 the blood flow for TTTS recipient (TAPS donor) to TTTS donor (TAPS recipient) in ml per day. Net blood flow before laser resulted in TTTS, and after laser the net blood flow through the miniscule remaining AV anastomosis resulted in post-laser TAPS.

Discussion

We showed theoretically that including PET with IUT for the recipient in TAPS has a strong beneficial effect compared to IUT without PET, as illustrated in a model simulation. This is the first study that shows the importance of PET, confirming previous case-reports which suggested a possible advantage of PET [6;9]. The advantage of model simulation is that with the same patient characteristics hematocrit levels can be given for two treatment options (IUT with and without combination of PET).

Our model simulation shows that the addition of PET to IUT reduces the hyperviscosity in the TAPS recipient. Also shown in this model is that the risk of severe polycythemia and hyperviscosity increases with repeated IUTs when performed without PET. Hyperviscosity is associated with complications such as ischemic limb necrosis and severe cerebral injury.

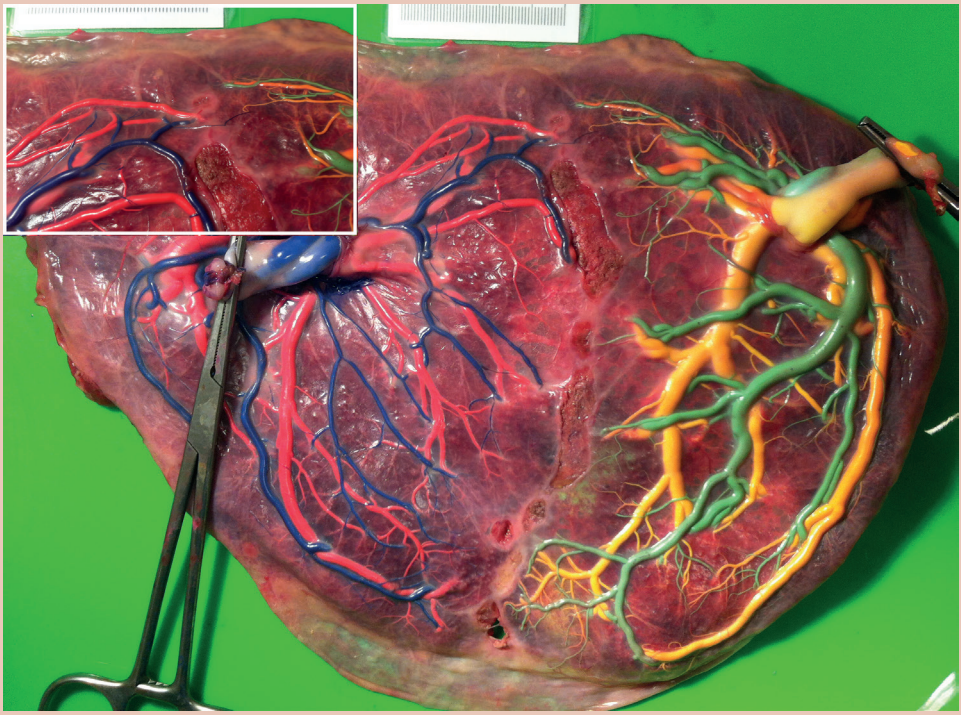

Figure 2. Post-laser TAPS placenta after color dye injection. Blue and green dye was used to stain the arteries and yellow and pink was used to stain the veins. The placenta share of the TAPS donor is on the left side of the picture and the placenta share of the TAPS recipient is on the right side. At the top and in the enlargement one residual arterio-venous anastomoses in seen which caused post-laser TAPS.

Our model also shows the differences that occur in the development of TTTS compared with TAPS. Net blood flow from donor to recipient is much higher in TTTS compared to the small amount of blood transfused from donor to recipient in TAPS.

Our findings thus strongly support the use of IUT combined with PET in the management of TAPS. However, IUT with or without PET is a symptomatic treatment for TAPS because it does not solve the underlying problem, which are small (residual) anastomoses. In a recent publication by our group, results suggested that laser treatment for TAPS may improve survival and neonatal outcome by prolonging the pregnancy [10]. Prolonging the pregnancy is of paramount importance for neonatal outcome. If laser surgery is not feasible, IUT in combination with PET might be a good alternative to prolong the pregnancy while temporarily improving the condition of both twins.

A recent long-term follow-up study on post-laser TAPS cases showed that low gestational age at birth and low birth weight are important risk factors for cognitive delay [17]. A subgroup

analysis on antenatally detected post-laser TAPS cases showed that the lowest median cognitive scores were in the IUT-group (without PET). Whether the lower scores were due to

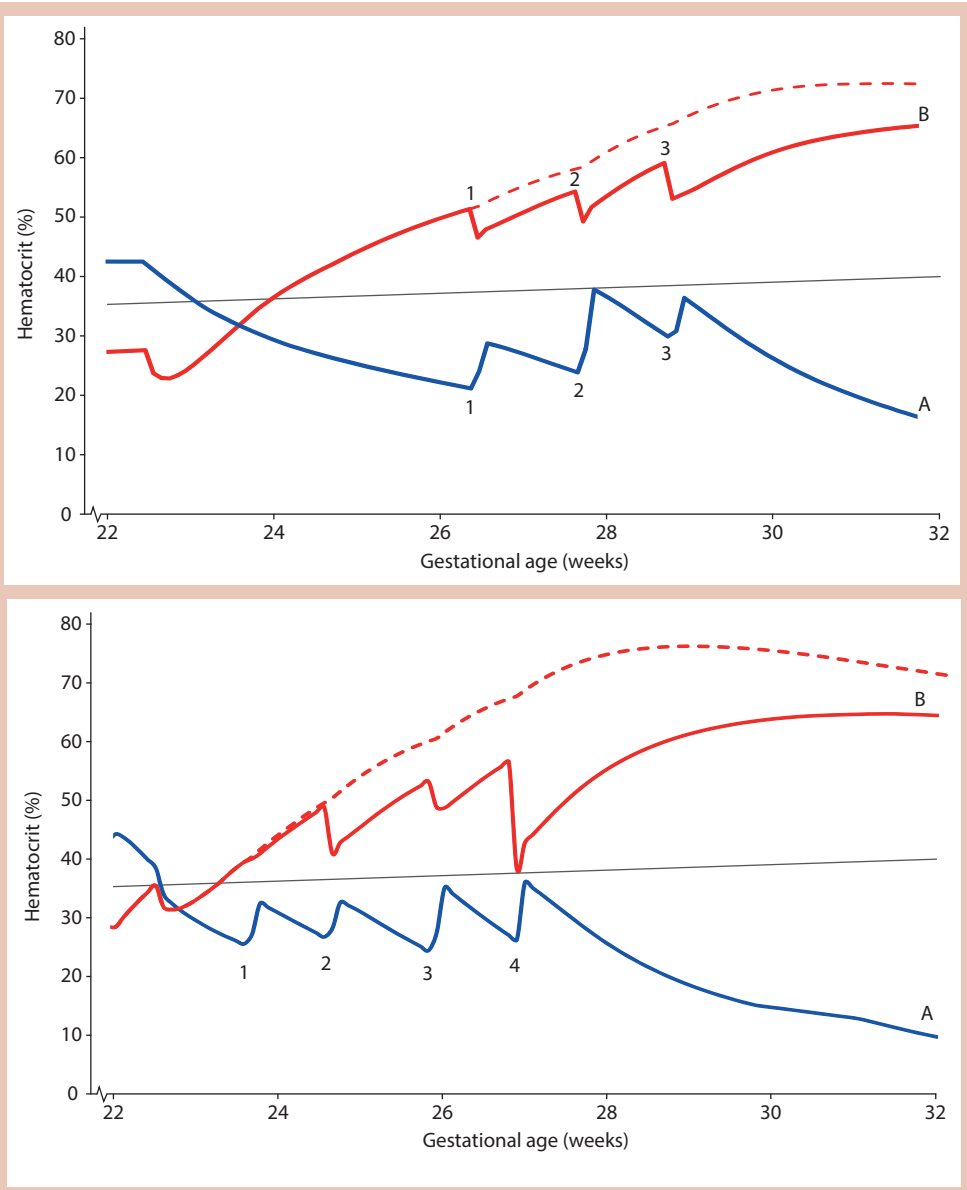

Figure 3. Model simulation showing the computed hematocrit (%) as a function of gestational age (weeks). In blue the percentage of hematocrit in the ex-TTTS recipient and post-laser TAPS donor (A). In red the percentage of hematocrit in the ex-TTTS donor and post-laser TAPS recipient. The red dotted line indicates the model outcome in case of no simulated PET. The first shows the model for case 1, and the second shows the model for case 2.

GA (weeks)	anastomotic pattern during laser for TTTS	Intervention	twin A Hb before	twin A Hb after	Birthweight twin A	Amount of blood ex-changed (ml)	twin B Hb before	twin B Hb after	Birthweight twin B	Amount of blood transfused (ml)
Case 1										
22+2 (laser for TTTS)	2 AV, 4 VA									
26+3		1st IUT & PET	18.2	15.0		32 out + 32 in	5.0	8.0		23 iv + 20 ip
27+5		2nd IUT & PET	17.4	13.9		38 out + 40 in	6.6	10.9		47 iv + 30 ip
28+6		3rd IUT & PET	16.3	14.6		26 out + 20 in	10.9	13.1		27 iv + 10 ip
31+5 (at birth)			23.4		1445 gram	reticulocyte count 28‰	9.3		1715 gram	reticulocyte count 115‰
Case 2										
21+5 (laser for TTTS)	4 AV, 3 VA									
23+4		1st IUT & PET	17.9	n/a		6ml out, 5ml in	1.9	7.5		N/A
24+4		2nd IUT & PET	19.3	11.2		50ml	2.6	8.6		12ml
25+6		3rd IUT & PET	18.6	17.6		30ml out, 25ml in	3.7	10.7		N/A
26+6		4th IUT + PET	20.6	13.1		94ml out, 100ml in	3.5	11.5		50ml iv +30 IP
27+5 (at birth)			21.5		980 gram	reticulocyte count 39‰	9.3		1235 gram	reticulocyte count 143‰
Case 3										
25+1 (laser for TTTS)	4 AV, 4 VA									
27+2		1st IUT + PET	18.9	15.0		30ml out, 30ml in	4.3	9.3		30ml IV + 25ml IP
29+3		2nd IUT + PET	-	-		-	7.0	9.3		20ml IV + 30ml IP
32+3 (at birth)			23.0		1664 gram	reticulocyte count 43‰	12.8		1615 gram	reticulocyte count 94‰
Case 4										
15+3 (laser for TTTS)	2 AV, 3 VA									
28+2		1st IUT + PET	18.2	14.7		55ml out, 55ml in	5.0	13.8		65ml IV + 50ml IP
29+1		2nd IUT + PET	17.8	14.6		57ml out, 45ml in	9.6	13.6		45ml IV + 50ml IP
31+1 (at birth)			26.1		1520 gram	reticulocyte count 29‰	12.0		1759 gram	reticulocyte count 108‰

GA: gestational age; AV: arterio-venous anastomosis; VA: veno-arterial; IUT: intrauterine transfusion, PET: partial exchange transfusion, Hb: hemoglobin (g/dL); iv: intravenous; ip: intraperitoneal

Table 1: Hematological measurements and management information.

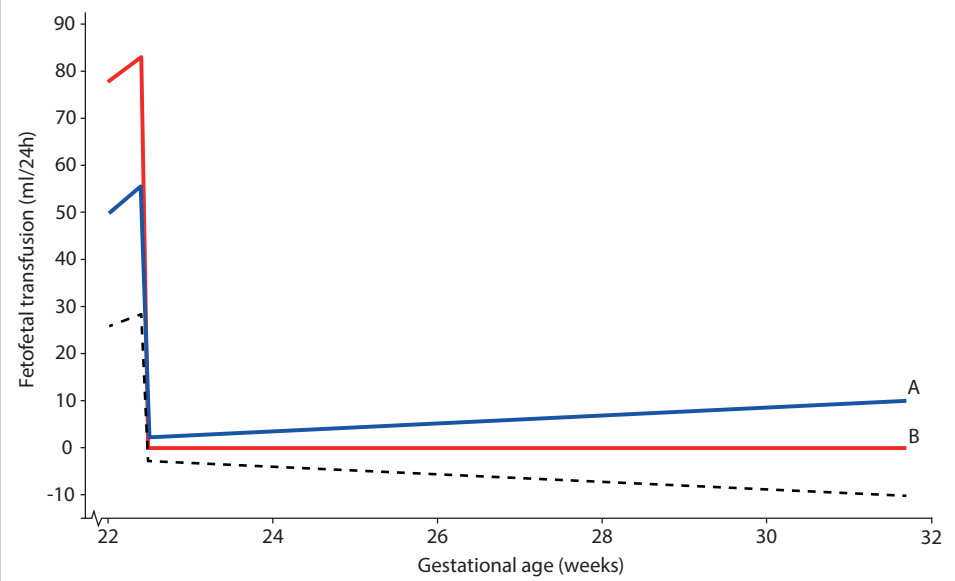

Figure 4. Model simulation showing the computed blood flow in ml/day based on case 1. In blue the blood flow from ex-TTTS recipient and post-laser TAPS donor (A). In red the blood flow from ex-TTTS donor and post-laser TAPS recipient (B). The dotted line shows the net blood flow between the twins, before laser resulting in TTTS and after laser resulting in post-laser TAPS.

the lower gestational age or due to severe anemia and polycythemia in this subgroup could not be established.

In conclusion, when IUT is considered as treatment option for TAPS, our model simulations strongly suggest to add PET, to reduce the risk of severe polycythemia and the possible complications due to hyperviscosity. More clinical, prospective studies are needed to confirm our theoretical findings.

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Chapter 10

Laser surgery as a management option for twin anemia polycythemia sequence

Abstract

Objective:

To evaluate the effectiveness of laser treatment for antenatally detected twin anemia–polycythemia sequence (TAPS) compared with intrauterine transfusion or expectant management.

Methods:

All monochorionic twin pregnancies with TAPS managed between 2005 and 2013 in two European fetal therapy centers were evaluated. The outcomes of TAPS cases treated primarily with laser surgery were compared with the outcomes of cases managed expectantly or treated with intrauterine transfusion.

Results:

In this retrospective study 52 cases of TAPS were detected antenatally and were managed with either laser surgery (n = 8; 15%) or intrauterine blood transfusion (n = 17; 33%) or expectantly (n = 27; 52%). Perinatal survival in the laser group was 94% (15/16) vs 85% (29/34) in the intrauterine-transfusion group and 83% (45/54) in the expectant-management group (P = 0.30). The rates of severe neonatal morbidity in liveborn neonates in the laser, intrauterine-transfusion and expectant-management groups were 7% (1/15), 38% (12/32) and 24% (12/50), respectively (P = 0.17). There was a significant reduction in respiratory distress syndrome in cases treated by laser. No severe postnatal hematological complications were detected in the laser group compared with 72% (23/32) in the intrauterine-transfusion group and 52% (26/50) in the expectant-management group (P < 0.01). Median time between diagnosis and birth was 11 weeks in the laser group compared to 5 weeks after intrauterine transfusion and 8 weeks after expectant management (P < 0.01). After injection of colored dye no residual anastomoses were found in the laser group.

Conclusions:

Laser surgery for TAPS appears to improve perinatal outcome by prolonging pregnancy and reducing respiratory distress syndrome. Larger, adequately controlled studies are needed to reach firm conclusions on the optimal management of TAPS.

Introduction

Twin anemia–polycythemia sequence (TAPS) is a newly described form of fetofetal transfusion in monochorionic twins. The pathogenesis of TAPS is based on the unique placental angioarchitecture resulting in chronic intertwin blood transfusion through a few minuscule placental anastomoses leading to large intertwin hemoglobin differences, without signs of twin oligo–polyhydramnios sequence (TOPS)[1]. TAPS may occur spontaneously or after laser treatment for twin–twin transfusion syndrome (TTTS) (post-laser form). The incidence of TAPS varies between 1% and 5% when it occurs spontaneously[2–5] and between 1% and 16% when it occurs post laser[6]. 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 velocity in the recipient twin, suggestive of polycythemia, without concomitant signs of TOPS[1]. Perinatal mortality and morbidity rates in TAPS are not well known, and outcome may vary from two healthy neonates to severe neonatal morbidity or neonatal death[1,6,7].

The optimal management for TAPS is not clear and options include intrauterine blood transfusion of the donor (with or without partial exchange transfusion in the recipient), expectant management and selective laser surgery in TAPS feticide. The rationale for intrauterine treatment is to prevent or treat severe fetal anemia or polycythemia. Recently, several TAPS cases treated with fetoscopic laser surgery have been reported[1,5,8–10]. Laser coagulation of the vascular anastomoses is the only curative treatment for TAPS[1,11–13]. The aim of this study was to evaluate the effectiveness of laser treatment in antenatally detected TAPS cases, compared to treatment by intrauterine transfusion or expectant management.

Methods

All monochorionic twin pregnancies with TAPS detected antenatally in two European fetal therapy centers, the Leiden University Medical Center (The Netherlands) and the Center Medico-Chirurgical Obstetrical in Strasbourg (France), between 2005 and 2013, were included in this retrospective study, which was approved by the institutional review board at the Leiden University Medical Center.

TAPS was identified using previously published criteria[1]. 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

Antenatal TAPS stage	Findings at Doppler ultrasound examination
Stage 1	Middle cerebral artery peak systolic velocity > 1.5 MoM in the donor and < 1.0 MoM in the recipient, without other signs of fetal compromise
Stage 2	Middle cerebral artery peak systolic velocity > 1.7 MoM in the donor and < 0.8 MoM in the recipient, without other signs of fetal compromise
Stage 3	As Stage 1 or 2 with cardiac compromise of donor, defined as critically abnormal flow
Stage 4	Hydrops of donor
Stage 5	Single or double intrauterine fetal death

MoM, multiples of the median.

Table 1: Twin anemia–polycythemia sequence (TAPS) antenatal staging criteria

Postnatal TAPS stage	Intertwin hemoglobin difference at birth (g/dL)
Stage 1	> 8.0
Stage 2	> 11.0
Stage 3	> 14.0
Stage 4	> 17.0
Stage 5	> 20.0

Table 2: Twin anemia–polycythemia sequence (TAPS) postnatal staging criteria

that coincided with a decreased velocity of < 1.0 MoM in the cotwin, in the absence of TOPS. Antenatal and postnatal TAPS stages were based on previously published staging criteria (Tables 1 and 2)[1].

Intrauterine treatment was offered in TAPS Stages 3 and 4. Cardiac compromise (TAPS Stage 3) was defined as critically abnormal Doppler flows, which consisted of absent or reversed flow in the umbilical artery, absent or reversed flow in the ductus venosus or a pulsatile waveform in the umbilical vein. In TAPS Stage 1 or 2, intrauterine treatment was offered in cases in which TAPS was quickly progressing (within days) or other signs of severe anemia not meeting the criteria for Stage 3 (such as increasing heart size or prehydropic signs) were present. Intrauterine intervention consisted of laser coagulation of the vascular anastomoses or treatment with intrauterine blood transfusion (intravascular, intraperitoneal or a combination) in the donor twin, combined with a partial exchange transfusion in the recipient in cases of suspected severe polycythemia. Expectant management consisted of serial ultrasound scans or planned delivery. In cases in which TAPS was managed by delivering the twins, the preferred mode of delivery was determined individually, taking into account several factors, including position of the first twin, estimated fetal weights, ability to monitor both fetuses, obstetric history, patient's preference and future reproductive plans of the parents. If cases were managed with serial ultrasound from diagnosis onwards and at a later stage labor was induced or Cesarean section was planned, they were scored as serial ultrasound.

To evaluate the change of attitude of the operators towards this condition over time, we

compared the management between the first period (2005–2009) and the second period (2010–2013) of the study.

Details of the study population were retrieved from the medical records, including perinatal and neonatal outcomes. Variables studied were: antenatal treatment, gestational age at diagnosis, at treatment and at birth, perinatal survival, hematological complications, severe neonatal morbidity and placental injection studies. Hematological complications were defined as the donor requiring blood transfusion and/or the recipient requiring a partial exchange transfusion on the first postnatal day. Severe neonatal morbidity was defined as the presence of at least one of the following: respiratory distress syndrome (RDS) 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 included at least one of the following: intraventricular hemorrhage ≥ Grade III, cystic periventricular leukomalacia ≥ Grade II, ventricular dilatation > 2 SD above the mean, porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome[14].

Placentae were routinely injected with colored dye as previously described to record the presence, size and location of vascular anastomoses[15]. We determined the position of the anastomoses along the vascular equator in relation to the margin of the placenta, as previously reported[16]. A few cases included in this study have been reported in detail in previous publications of our group[1,13,17].

The primary outcome was adverse perinatal outcome, defined as the presence of one of the following: intrauterine fetal death (IUFD), neonatal death or severe neonatal morbidity. Secondary outcomes were hematological complications after birth and gestational age at birth. Outcomes were analyzed and compared between the three groups (laser surgery, intrauterine transfusion and expectant management). TAPS cases treated with cord coagulation and termination of pregnancy were excluded from the analysis.

We performed a systematic search of the literature on TAPS management, in which publications from January 2005 to December 2013 reporting data on interventions and perinatal outcome in TAPS pregnancies were reviewed. An electronic literature search using MEDLINE, EMBASE, ISI Web of Science and the Cochrane database was performed to find all relevant articles reporting perinatal outcome and fetal interventions in TAPS pregnancies, using the following keywords: ‘Twin Anemia Polycythemia Sequence’ OR ‘Twin Anemia Polycythemia Syndrome’ AND ‘Fetal Therapies’ OR ‘Laser Therapy’ OR ‘laser’ OR ‘Intra

Uterine Transfusion' OR 'intrauterine intervention' OR 'expectant management'. No language restrictions were applied. We included only original articles. Articles were included irrespective of their primary objective.

Continuous variables were reported as median (range) or mean ± SD; group differences were compared using the Mann–Whitney U-test or independent Student's t-test. Proportions were compared using the chi-square test or Fisher's exact test, where appropriate. The type of TAPS (spontaneous or post-laser) and gestational age at diagnosis were studied by multivariate analysis, where possible. All analyses per fetus or neonate were performed using the Generalized Estimated Equation module to account for the fact that observations between cotwins are not independent. All statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA), and $P < 0.05$ was considered to be statistically significant.

Results

During the study period, 55 patients were diagnosed antenatally with TAPS, of which 41 cases were from the fetal therapy center of Leiden University Medical Center and 14 cases were from the fetal therapy center of Strasbourg. Thirty-seven cases (67%) occurred after laser surgery for TTTS and 18 (33%) occurred spontaneously. Spontaneous TAPS was detected in 2% (18/784) of uncomplicated monochorionic twin pregnancies evaluated in both centers. Post-laser TAPS was detected in 7% (37/504) of TTTS cases treated with laser surgery in both centers. TTTS Quintero stage (in post-laser TAPS) and TAPS stage at diagnosis and treatment are reported in Table 3. Umbilical cord ligation was performed in two cases and in one case termination of pregnancy was carried out; these three cases were excluded from the analysis. Overall, TAPS cases were managed with laser surgery in 15% (8/52), intrauterine blood transfusion in 33% (17/52) and expectantly in 52% (27/52). TAPS management in the first period (2005–2009) consisted of expectant management in 68% (15/22), intrauterine transfusion in 18% (4/22) and laser surgery in 14% (3/22). In the second period (2010–2013) TAPS cases were managed expectantly in 40% (12/30), with intrauterine blood transfusion in 43% (13/30) and laser surgery in 17% (5/30) ($P = 0.10$).

Gestational age at diagnosis of TAPS was significantly lower in the laser group than in the intrauterine-transfusion and expectant-management groups, with a median of 19 vs 26 and 25 weeks' gestation, respectively ($P = 0.02$). There were more cases of spontaneous TAPS in the laser group (63% (5/8)) than in the intrauterine-transfusion group (24% (4/17)) and the expectant-management group (30% (8/27)), though the difference was not statistically

significant (P = 0.14). TTTS Quintero stage (in post-laser TAPS) and TAPS stage at time of diagnosis and treatment were equally distributed between treatment groups (Table 3).

The incidence of adverse perinatal outcome was 13% (2/16) in the laser therapy group compared with 41% (14/34) in the intrauterine-transfusion group and 30% (16/54) in the expectant-management group (P = 0.11). Significantly more postnatal hematological complications were detected in the intrauterine-transfusion group (72% (23/32)) and in the expectant-management group (52% (26/50)) compared with in the laser group, in which no hematological complications occurred (P < 0.01). Rates of preterm prelabor rupture of membranes (PPROM) in the intrauterine-transfusion and expectant-management groups were 6% (1/17) and 15% (4/27), respectively. The rate of PPROM in the laser group was

	Expectant management (n=27)	Intrauterine transfusion (n=17)	Laser therapy (n=8)	p value
GA at diagnosis (weeks)	25 (17–34)	26 (19–29)	19 (17–24)	0.02
Spontaneous TAPS	8 (30)	4 (24)	5 (63)	0.14
GA at diagnosis of spontaneous TAPS (wk)	25 (17–32)	28 (27–29)	19 (17–23)	0.05
Post-laser TAPS	19 (70)	13 (76)	3 (37)	0.14
GA at diagnosis of post-laser TAPS (wk)	25 (17–34)	25 (19–28)	24 (19–24)	0.65
Posterior placental location TTTS	15 (56)	10 (59)	2 (25)	0.31
Quintero stage in post-laser TAPS cases				0.09
Stage I	—	—	1/3 (33)	
Stage II	8/19 (42)	5/13 (38)	2/3 (67)	
Stage III	8/19 (42)	8/13 (62)	—	
Stage IV	3/19 (16)	—	—	
TAPS stage at diagnosis				0.42
Stage 1	13 (48)	6 (35)	2 (25)	
Stage 2	8 (30)	5 (29)	3 (38)	
Stage 3	1 (4)	4 (24)	2 (25)	
Stage 4	5 (18)	2 (12)	1 (12)	
TAPS stage at treatment				1.0
Stage 1		1 (6)	—	
Stage 2		6 (35)	3 (38)	
Stage 3		5 (29)	3 (38)	
Stage 4		5 (29)	2 (25)	

Values are given as n (%), n/N (%) or median (range). GA, gestational age; TTTS, twin–twin transfusion syndrome.

Table 3: Baseline characteristics of the study population of monochorionic twin pregnancies with twin anemia–polycythemia sequence (TAPS), according to management

significantly higher, at 75% (6/8) (P < 0.01). Median gestational age at PPROM in the laser group was 32 weeks, compared to 26 weeks in the one case of PPROM in the intrauterine-transfusion group, and a median gestational age of 30 weeks in the expectant-management group (P = 0.04). There was no significant difference in gestational age at birth between the groups. Median time between diagnosis and birth was 11 weeks in the laser group compared to 5 weeks after intrauterine transfusion and 8 weeks after expectant management (P < 0.01) (Table 4).

Laser surgery was performed in eight cases of TAPS (five cases of spontaneous TAPS and three post laser), of which two cases were in combination with intrauterine transfusion. In the first case, intrauterine transfusion was performed in the same session, just after the laser procedure, at 25 weeks' gestation. In the second case, intrauterine transfusion was first performed at 24 weeks and laser treatment was performed 1 week later owing to rapidly recurring signs of severe fetal anemia. In the other six cases, laser treatment was performed between 17 and 24 weeks' gestation.

	Expectant management (n=27)	Intrauterine transfusion (n=17)	Laser therapy (n=8)	p value
GA at treatment (weeks)		27 (22–32)	20 (17–25)	0.06
PPROM	4/27 (15)	1/17 (6)	6/8 (75)	< 0.01
GA at PPROM	30 (25–34)	26	32 (32–34)	0.04
Intrauterine fetal death*	4/54 (7)	2/34 (6)	1/16 (6)	0.69
GA at birth (weeks)	33 (23–41)	31 (24–34)	32 (30–36)	0.07
GA at birth < 30 weeks	7 (26)	7 (41)	—	0.10
Neonatal death*	5/50 (10)	3/32 (9)	—	0.16
Time between diagnosis and birth (weeks)	8 (0–21)	5 (1–14)	11 (8–18)	< 0.01
Intertwin hemoglobin difference at birth (g/dL)	13.1 ± 4.4	14.9 ± 2.9	3.1 ± 2.1	< 0.01
Hematological complications*	26/50 (52)	23/32 (72)	—	< 0.01
Severe neonatal morbidity*	12/50 (24)	12/32 (38)	1/15 (7)	0.17
Respiratory distress syndrome*	10/50 (20)	12/32 (38)	—	0.01
Patent ductus arteriosus*	1/50 (2)	1/32 (3)	—	1.0
Necrotizing enterocolitis*	3/50 (6)	—	—	0.60
Severe cerebral injury*	4/50 (8)	1/32 (3)	1/15 (7)	0.87
Perinatal survival*	45/54 (83)	29/34 (85)	15/16 (94)	0.30
Adverse perinatal outcome*†	16/54 (30)	14/34 (41)	2/16 (13)	0.11

Data are given as n (%), n/N (%), median (range) or mean ± SD. *Measured per fetus or neonate using the Generalized Estimated Equation module. †Adverse perinatal outcome defined as intrauterine fetal death, neonatal death or severe neonatal morbidity. GA, gestational age; PPROM, preterm prelabor rupture of membranes.

Table 4: Outcome of twin anemia–polycythemia sequence according to management

Intrauterine transfusion consisted of intravenous transfusion (n=5), intraperitoneal transfusion (n=4), a combination of intravenous and intraperitoneal transfusion (n=4) and a combination of intrauterine transfusion for the donor and partial exchange transfusion for the recipient twin (n=4). In 11 cases (65%) intrauterine transfusion was performed only once, and in two cases (12%) intrauterine transfusion was performed twice, with an interval of 13 and 21 days, respectively. Intrauterine transfusion combined with partial exchange transfusion was performed twice in two cases (12%) and in the other two cases three and four times, respectively, with the interval between the procedures varying from 6 to 13 days. Intertwin hemoglobin differences at birth are shown in Table 4, and Table 5 shows intertwin hemoglobin difference at birth and postnatal hematological complications for the different types of intrauterine transfusion. In the only case of TAPS Stage 1 treated with intrauterine transfusion, the donor showed additional signs of severe anemia (increased heart size and prehydropic signs (a small amount of fluid around the bowels without clear signs of ascites)). In another case, intrauterine transfusion was performed at 26 weeks' gestation (TAPS Stage 2) and 2 weeks later severe cerebral injury (infarction of the middle cerebral artery) was detected in one of the fetuses (an ex-recipient that became the new TAPS donor after laser treatment). Cord coagulation was performed at 33 weeks' gestation after extensive counseling.

Patients in the expectant-management group were observed closely with serial ultrasound evaluation including middle cerebral artery peak systolic velocity Doppler measurements (n=20) or managed with induction of labor (n=7). Of the 13 cases that had TAPS Stage 1, eight cases remained stable Stage 1, two cases progressed to Stage 2, one case progressed to Stage 4 and Cesarean section was performed and two cases progressed to Stage 5. Of these last two cases progressing to Stage 5, one was a spontaneous TAPS diagnosed at 20 weeks resulting in double IUFD at 22 weeks. In the other case, TAPS was diagnosed at 25 weeks (post-laser TAPS), progressing slowly towards Stage 3 at 31 weeks and Stage 4 at 32 weeks, and the following day fetal demise of the donor occurred. At 33 weeks a Cesarean section was performed and the recipient was born without severe neonatal morbidity. In the only case of TAPS Stage

	IUT iv (n = 4)*	IUT ip (n = 4)	IUT iv/ip (n = 4)	IUT/PET (n = 4)	p value
Intertwin Hb difference (g/dL)	15.4 ± 3.1	16.0 ± 0.7†	16.1 ± 3.6	12.7 ± 1.8	0.36
Hematological complications	8/8 (100)	3/6 (50)‡	8/8 (100)	4/8 (50)	0.01

Data are given as mean ± SD or n/N (%). *n = 4 (one case excluded since no postnatal TAPS). †n = 2 (in two cases intrauterine fetal death and no intertwin Hb difference). ‡n = 6 (hematological complications per liveborn neonate). ip, intraperitoneal; IUT, intrauterine transfusion; iv, intravenous; PET, partial exchange transfusion.

Table 5: Intertwin hemoglobin (Hb) difference and hematological complications after intrauterine transfusion for treatment of twin anemia–polycythemia sequence (TAPS)

3 in the expectant-management group, the patient delivered spontaneously at 29 weeks' gestation. Management in the five cases of TAPS Stage 4 was as follows: in one case a secondary Cesarean section (scored as induction of labor) was performed, in two cases TAPS Stage 4 improved to Stage 2 (labor was induced or Cesarean section was performed at 33 and 35 weeks, respectively), in one case Stage 4 improved to Stage 3 and the patient delivered spontaneously at 29 weeks and in one case TAPS Stage 4 progressed to Stage 5 with IUFD of the donor; since there were no signs of fetal distress in the recipient in the last case, the pregnancy was allowed to progress to 41 weeks.

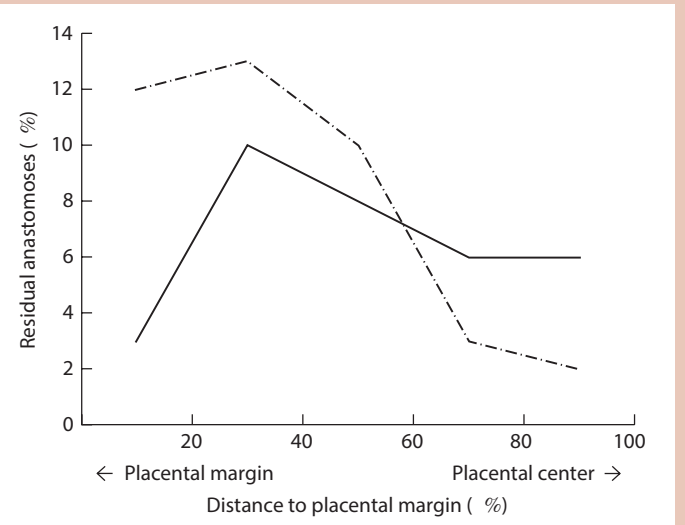

Figure 1. Distance of residual anastomoses in relation to margin of the placenta in cases of spontaneous (——) and post-laser treatment (- - -) for twin anemia–polycythemia sequence (TAPS).

Complete evaluation of the placenta, with colored dye injection, was available in 49/52 (94%) of cases. One placenta was excluded owing to extensive maceration after fetal death and two placentae could not be injected owing to damage after their manual removal. All TAPS placentae treated with laser (n=8) were injected and no residual anastomoses were detected. In the subgroup of TAPS placentae not treated with laser (n=41), the mean number of anastomoses per placenta in spontaneous TAPS cases (n=13) and post-laser TAPS cases (n=31) was 3.7 ± 3.1 and 1.7 ± 1.4, respectively (P = 0.005). All anastomoses (73/73) were very small (diameter < 1 mm). Mean diameter was 0.19 ± 0.1 mm in spontaneous TAPS and 0.21 ± 0.1 mm in post-laser TAPS cases (P = 0.44). In the post-laser TAPS cases (residual) anastomoses were detected more frequently near the margin. Location of the anastomoses in both subgroups is shown in Figure 1, and a typical post-laser TAPS placenta is shown in Figure 2. A combination of the four search strategies revealed a total of 44 references during our systematic review of the literature. A manual search revealed no additional studies for consideration. In total, after removal of duplicates, 26 relevant published reports were

screened. None of these reports published series on the management of TAPS.

Discussion

This is the largest study to date evaluating the management and outcome after different treatments of antenatally detected TAPS. We found that TAPS is associated with high rates of perinatal mortality and morbidity. Laser surgery is more likely to prolong the pregnancy and may improve survival and neonatal outcome, compared with treatment by intrauterine transfusion or expectant management. From this retrospective cohort, we cannot firmly conclude that the improved outcome after laser treatment reflects a true benefit of this modality. Undoubtedly, selection bias plays a role. It is conceivable that laser treatment may have been performed preferably in the more favorable TAPS cases. Indeed, we found that laser surgery was performed more often in spontaneous TAPS cases. Theoretically, post-laser TAPS cases may be more at risk of adverse perinatal outcome owing to the preceding laser treatment for TTTS. However, TAPS stages and TTTS Quintero stages were similar in both groups.

Although the difference in survival did not reach statistical significance, there were no neonatal deaths in the laser group versus 10% (5/50) in the expectant-management group and 9% (3/32) in the intrauterine-transfusion group. The survival rate of 94% after laser therapy for TAPS was higher than the survival rates after laser therapy for TTTS, varying from 65% to 80%[17–20]. Survival rates in TAPS treated with laser in this study were high, suggesting that laser surgery may be a safe and effective treatment for TAPS. More research, preferably involving large multicenter randomized controlled trials, is required to determine the optimal management of TAPS. This study also shows that the median interval between diagnosis and gestational age at birth increases after laser therapy. Prolonging the pregnancy is known to be of paramount importance for neonatal outcome. Low gestational age at birth is independently associated with increased risk for severe neonatal morbidity[21,22], severe cerebral lesions[23] and impaired neurodevelopmental outcome[24]. In the laser group all neonates were born after 30 weeks' gestation, which may explain the low rate of severe neonatal morbidity (7%). The most important reduction in severe neonatal morbidity in this study was related to a reduction in rates of RDS. In the laser group, none of the neonates had RDS compared with 10/50 (20%) in the expectant-management group and 12/32 (38%) in the intra-uterine-transfusion group. The criteria for RDS in this study were restricted to the most severe form of respiratory failure (requiring mechanical ventilation and surfactant). Severe RDS is associated with an increased risk of chronic lung disease and a concomitant increase in the rate

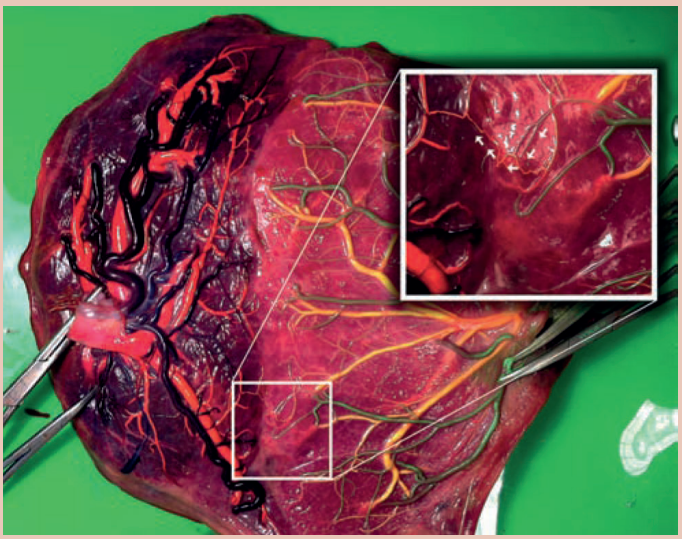

Figure 2: Placenta in a case of post-laser twin anemia–polycythemia sequence showing the characteristic difference in color between the two placental shares and the typical few tiny anastomoses. Blue and green dye was used to stain the arteries, and pink and yellow dye was used to stain the veins. Small arrows show transfusion direction of the tiny anastomoses (inset).

of adverse long-term outcome[21,22].

An additional argument for considering laser treatment as the first treatment option is that laser treatment is the only curative treatment for TAPS. However, laser therapy in TAPS can be technically more challenging than in TTTS because of the absence of polyhydramnios and anhydramnios, which makes it more difficult to visualize the vascular equator. Other challenging problems in cases of previous laser treatment are the risk of dehiscence of the amnion and the presence of cloudy amniotic fluid. Since the anastomoses in TAPS are very small, they can be hard to detect and therefore easily missed, especially at the margin in post-laser TAPS.

Differences in management in TAPS cases could be due to differences in gestational age at presentation. In the present study gestational age at diagnosis in the laser group was significantly lower at 19 (range, 17–24) weeks vs 26 (range, 19–29) weeks in the intrauterine-transfusion group and 25 (range, 17–34) in the expectant-management group ($P = 0.02$). Fetal surgeons probably felt that laser treatment was more justified in cases in which TAPS presented early and well before delivery. Another explanation could be that these early cases were considered more favorable and more feasible for laser treatment because of the smaller size of the uteri and fetuses. The disadvantage of choosing intrauterine transfusion as the first management option in TAPS cases presenting early in gestation is that a relatively large number of transfusions are needed to reach a reasonable gestational age for survival until induced (preterm) delivery.

Our data also show a change in approach to the management of TAPS towards more intrauterine interventions in recent years. In the second time period (2010–2013) most TAPS cases were managed with intrauterine intervention, whereas in the first period most TAPS cases were managed expectantly. This change in approach probably came about owing to increasing knowledge and awareness of the diagnosis and outcome of TAPS, including the possible negative effects such as fetal demise or severe cerebral injury.

When laser treatment is considered infeasible, intrauterine transfusion (intravascular and/or intraperitoneal) might be a valuable alternative treatment. Intrauterine transfusion may temporarily improve the condition of the donor, allowing prolongation of the pregnancy. A negative effect of intrauterine transfusion is that it may worsen the polycythemia in the recipient twin and possibly lead to severe complications such as limb necrosis or severe cerebral injury[6,7]. Our results show a lower hemoglobin difference at birth when intrauterine transfusion is combined with partial exchange transfusion, although this difference was not statistically significant. We suggest combining intrauterine transfusion with partial exchange transfusion in cases of severe polycythemia in the recipient twin. Lastly, expectant management can still be considered a valuable treatment option, for example in presentations after 30 weeks' gestation with a stable fetal condition. In addition, one should bear in mind that, as shown in a previous case, TAPS can also resolve spontaneously without intervention[11]. However, the odds of spontaneous recovery should probably be regarded as low. Placental injection studies showed no residual anastomoses in TAPS cases treated with laser and only the typical small (residual) anastomoses on the placental surface after expectant management and intrauterine transfusion. In post-laser TAPS cases residual anastomoses were mainly located at the placental margin, whereas anastomoses in spontaneous cases were more equally spread along the vascular equator, confirming previous reports[16,25].

Our data should be interpreted with caution because of the retrospective nature of the study, the relatively small number of cases in each subgroup and varying gestational ages at diagnosis and intervention. More studies (ideally using a randomized controlled design, including long-term follow-up) are needed to determine optimal management and to evaluate the possible benefits of laser therapy for the treatment of TAPS. In the meantime, a prospective evaluation of the management of TAPS cases worldwide may further improve our understanding of this complex and rare disease. To facilitate this evaluation, we recently set up a web-based TAPS registry: www.TAPSregistry.org.

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Chapter 11

Hypoalbuminemia in donors with twin anemia polycythemia sequence: a matched case-control study

Abstract

Objective:

To determine the differences in albumin levels between donors and recipients with twin anemia-polycythemia sequence (TAPS).

Methods:

We included all consecutive monochorionic twins with TAPS with double survivors. Each twin pair was matched for gestational age at birth with 2 control monochorionic twin pairs unaffected by TAPS or twintwin transfusion syndrome. We measured levels of albumin, total protein, and hemoglobin on the first day of life in donors and recipients (TAPS group) and the control group.

Results:

A total of 25 TAPS twin pairs and 50 control twin pairs were included in the study. The median gestational age at birth was 32 weeks in both groups. In the TAPS group, median levels (IQR) of albumin in donor twins were significantly lower than in recipient twins, i.e. 28.0 g/l (24.0–32.0) versus 32.0 g/l (30.0– 34.5) ($p = 0.008$). Median levels (IQR) of total protein in donor twins were also lower than in recipients, i.e. 44.0 g/l (36.5– 49.0) versus 49.0 g/l (46.5–51.0), respectively ($p = 0.004$). The median (IQR) intertwin albumin difference was significantly higher in the TAPS group than in the control group, i.e. 4.0 g/l (2.5–10.5) versus 2.0 g/l (1.0–4.0) ($p = 0.003$). The rate of hypoalbuminemia (<20 g/l) and hypoproteinemia (<40 g/l) in donor twins with TAPS was 20% (5/25) and 32% (8/25).

Conclusions:

In addition to lower hemoglobin levels, donor twins with TAPS also have significantly lower albumin and total protein levels compared to recipient twins.

Introduction

Vascular anastomoses are almost invariably present in all monochorioric twin placentas and may lead to several complications including twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) [1]. TTTS occurs in 9% of monochorionic twins and is characterized by the development of twin oligopolyhydramnios sequence (TOPS) [2]. In contrast, TAPS is characterized by large intertwin hemoglobin differences without signs of TOPS [3]. TAPS can be detected in 2–13% of TTTS pregnancies treated with laser surgery (postlaser TAPS) [4, 5] and can also occur spontaneously in about 3–5% of monochorionic twin pregnancies (spontaneous TAPS) [6]. TAPS placentas are characterized by the presence of only few minuscule arteriovenous placental anastomoses allowing slow but chronic intertwin blood transfusion, resulting in chronic anemia (with reticulocytosis) in donors and polycythemia in recipients [3].

Knowledge on biochemical differences between donors and recipients in TAPS is sparse but important. Hypoalbuminemia in neonates is an independent risk factor for mortality and morbidity [7, 8] and has been associated with various adverse clinical conditions like necrotizing enterocolitis, respiratory distress syndrome, intracranial hemorrhage, sepsis, chronic lung disease, and edema [9– 11]. In analogy to hypoproteinemia in donors with TTTS, we hypothesize that similar differences may be detected in TAPS twins.

The aim of this study was to determine the differences in albumin and total protein between donor and recipient twins with TAPS compared to a control group of uncomplicated monochorionic twins matched for gestational age at birth.

Methods

We conducted a retrospective analysis of consecutive monochorionic twin pairs with TAPS (TAPS group) and uncomplicated monochorionic twins (control group) delivered at the Leiden University Medical Center (The Netherlands) between August 2003 and August 2012. The Leiden University Medical Center is a tertiary care center managing all types of complications of monochorionic pregnancies and a national referral center for fetoscopic laser treatment for TTTS.

For the purpose of this study, TAPS was diagnosed using the following proposed postnatal criteria: an intertwin Hb difference >8.0 g/dl and at least one of the following: reticulocyte

count ratio >1.7 or placenta with only small (diameter <1 mm) vascular anastomoses [3]. Each twin pair with TAPS was compared with 2 uncomplicated monochorionic twin pairs unaffected by TAPS or TTTS and matched for gestational age at birth (±1 week). We excluded all twin pregnancies with single or double intrauterine death, TAPS cases treated with fetoscopic laser surgery, and cases with incomplete placental injection study. At birth, levels of hemoglobin, reticulocyte count, albumin, and total protein were measured routinely in all twins. Blood samplings were primarily obtained from umbilical cord. If umbilical cord blood was not available, venous blood samplings were obtained on day 1. We defined hypoalbuminemia at birth as an albumin level <20 g/l and hypoproteinemia as a total protein level <40 g/l [12–14].

	TAPS group (n=50)[b]	Control group (n=100)[b]
Caesarian delivery	28 (56%)	44 (44%)
Females	24 (48%)	52 (52%)
Gestational age at birth, weeks	32 (29–34)	32 (29–33)
Birth weight difference, % [a]	10.4 (4.5–18.5)	10.4 (4.3–24.7)
Small for gestational age	2 (4%)	4 (4%)

Values are presented as numbers (%) or medians (IQR).
[a] Calculated as: [(birth weight of the larger twin – birth weight of the smaller twin)/ birth weight of the larger twin] ×100.
[b] Refers to the number of fetuses or neonates.

Table 1: Baseline characteristics in the twins with TAPS (TAPS group) and monochorionic twins uncomplicated by TAPS or TTTS (control group)

We recorded the following perinatal variables: gestational age at birth, birth weight, birth weight discordance, individual placental territory, and placental territory discordance. Birth weight discordance was assessed and calculated as follows: [(birth weight of the larger twin – birth weight of the smaller twin)/birth weight of the larger twin] × 100. The definition of small for gestational age was a birth weight <2 SD according to the growth charts for the Dutch population [15]. The percentage of individual placental territory was determined by dividing each individual placental territory by the sum of both territories. The following neonatal data were collected: respiratory distress syndrome, necrotizing enterocolitis, patent ductus arteriosus, sepsis, mortality, and cerebral injury. Cerebral injury was defined as the presence of at least 1 of the following findings: cystic periventricular leukomalacia ≥grade 2, intraventricular hemorrhage ≥grade 3, ventricular dilatation, arterial or venous infarct, or other severe cerebral dilatation detected with cranial ultrasound. Sepsis was defined as a clinically ill neonate with positive bacterial culture. Neonatal mortality was defined as death within 28 days after birth.

Primary outcome was the level of albumin and total protein at birth which was compared between donors and recipients in the TAPS group and between the smaller one (lowest birth

weight) and the larger one in the control group. We also calculated the intertwin difference in levels of albumin, total protein, and hemoglobin and compared the results between the TAPS group and the control group.

Statistics

Data are reported as medians and IQR. Results of continuous variables within twin pairs were analyzed using a related-samples Wilcoxon signed rank test (when not normally distributed). Similarly, a Mann-Whitney test was used to compare continuous variables between the TAPS group and the control group. For analyses of paired nominal variables, the McNemar test was used. For statistical analyses, two-sided tests were used and $p < 0.05$ was considered statistically significant. Analysis was performed using SPSS version 17 (SPSS, Inc., Chicago, Ill., USA).

Results

During the 10-year study period, 216 monochorionic twins were born at our center. TAPS was diagnosed in 32 (15%) twins. Seven eligible twin pairs (22%) in the TAPS group were excluded because of incomplete biochemical data at birth. Of the remaining 25 TAPS twin pairs, 6 (24%) twins were spontaneous TAPS cases and 19 (76%) were postlaser TAPS cases. In the 19 postlaser TAPS twins the mean gestational age at laser treatment for TTTS was 21 weeks (IQR 18–25). Each TAPS twin pair (n = 25) was matched with 2 uncomplicated monochorionic twins (n = 50). The baseline characteristics in the TAPS and control groups are listed in table 1.

In the TAPS group, median albumin levels at birth in donors were significantly lower compared

	TAPS group			Control group		
	donors (n=25)	recipients (n=25)	p value	smaller twins (n=50)	larger twins (n=50)	p value
Hemoglobin, g/dl	9.5 (7.7–11.2)	22.6 (20.6–24.5)	0.000	16.3 (14.7–18.5)	16.5 (15.3–18.1)	0.882
Albumin, g/l	28.0 (24.0–32.0)	32.0 (30.0–34.5)	0.008	31.0 (29.0–33.0)	31.0 (28.8–35.0)	0.692
Albumin <25 g/l	6 (24%)	0 (0%)	0.031	1 (2%)	5 (10%)	0.375
Albumin <20 g/l	5 (20%)	0 (0%)	0.063	1 (2%)	0 (0%)	1,000
Total protein, g/l	44.0 (36.5–49.0)	49.0 (46.5–51.0)	0.004	46.0 (43.0–1.5)	47.0 (42.3–53.0)	0.656
Total protein <40 g/l	8 (32%)	1 (4%)	0.039	4 (8%)	7 (14%)	0.453

Values are presented as medians (IQR) or numbers (%).

Table 2: Hematological and biochemical differences at birth between donors and recipients (TAPS group) and smaller and larger twins (control group)

	TAPS group	Control group	p value
Intertwin hemoglobin difference, g/dl	12.2 (9.7–15.3)	1.2 (0.3–3.7)	0.000
Intertwin albumin difference, g/l	4.0 (2.5–10.5)	2.0 (1.0–4.0)	0.003
Intertwin total protein difference, g/l	7.0 (3.0–14.0)	3.0 (2.0–6.8)	0.012

Data are presented as medians (IQR).

Table 3: Intertwin hematological and biochemical differences at birth between the TAPS group and the control group

to those of the recipient twins, i.e. 28.0 versus 32.0 g/l (p=0.008) (table 2). Hypoalbuminemia (albumin level <20 g/l) was detected in 5 donors (20%). Skin edema was present in 2 of the donors with hypoalbuminemia. The first infant had an albumin level of 16 g/l (and required several albumin infusions) while her cotwin sister had an albumin level of 27 g/l. In the second case the donor had an albumin level of 11 g/l, and received several albumin infusions, while his cotwin brother had an albumin level of 33 g/l. On day 8 the infant died of severe respiratory and circulatory failure and persistent pulmonary hypertension of the newborn. In another donor with hypoalbuminemia (albumin of 13 g/l), fetal hydrops at birth was diagnosed. His cotwin brother had an albumin of 34 g/l. Albumin infusions were given in combination with diuretics to treat the hypoalbuminemia. The donor died due to multiorgan failure. In the 2 other infants with hypoalbuminemia no fetal hydrops was present and treatment with albumin transfusions was not administered.

	TAPS group			Control group		
	donors (n=25)	recipients (n=25)	p value	smaller twins (n=50)	larger twins (n=50)	p value
Birth weight, g	1,459 (1,117–1,815)	1,695 (1,208–1,893)	0.016	1,435 (1,141–1,733)	1,671 (1,385–2,013)	0.000
Individual placental territory, %	53.6 (47.2–64.0)	46.5 (36.0–52.8)	0.107	42.4 (36.0–51.7)	57.6 (48.4–64.0)	0.007
RDS	5 (20%)	6 (24%)	1,000	15 (30%)	20 (40%)	0.180
NEC	0 (0%)	0 (0%)	1,000	5 (10%)	4 (8%)	1,000
PDA	2 (8%)	0 (0%)	0.500	2 (4%)	2 (4%)	1,000
Cerebral injury [a]	1 (4%)	1 (4%)	1,000	3 (6%)	2 (4%)	1,000
Sepsis [b]	4 (16%)	4 (16%)	1,000	6 (12%)	6 (12%)	1,000
Mortality	2 (8%)	1 (4%)	1,000	1 (2%)	3 (6%)	0.500

Data are presented as medians (IQR) or numbers (%). RDS = Respiratory distress syndrome; NEC = necrotizing enterocolitis; PDA = patent ductus arteriosus.

[a] Defined as any of the following: cystic periventricular leukomalacia grade 2 or higher, intraventricular hemorrhage grade 3–4, ventricular dilatation, arterial or venous infarct, or other severe cerebral dilatation.

[b] Defined as blood culture-proven clinical sepsis.

Table 4: Clinical outcome and placental characteristics in the TAPS group and the control group

All of the recipients in the TAPS group had an albumin level >25 g/l. One infant in the control group had a albumin level <20 g/l.

Median levels of total protein in donors were lower than in recipients, i.e. 44.0 g/l versus 49.0 g/l ($p = 0.004$). Hypoproteinemia was detected in 32% (8/25) of the donor twins and in 4% (1/25) of the recipient twins ($p = 0.039$). Intertwin hematological and biochemical differences at birth between the TAPS group and control group are shown in table 3.

Clinical and placental characteristics in twins in the TAPS group and control group are presented in table 4. We found no differences in neonatal mortality and morbidity between donors and recipients in the TAPS group. Donors in the TAPS group had a lower birth weight than recipients, but larger individual placental territories, whereas the lower birth weight in the smaller twins in the control group was correlated with a smaller placental share.

Discussion

TAPS was first described in 2007 [16] and data on the pathophysiology of TAPS is mostly limited to reports on hematological features of TAPS [17]. This is the first study evaluating differences in biochemical variables in TAPS twins and showing that donor twins with TAPS have significantly lower levels of albumin and total protein levels at birth compared to recipients, in addition to lower hemoglobin levels. In a previous study, we and others found similar differences in albumin and total protein between donors and recipients with TTTS [18, 19]. The intertwin albumin difference in the TTTS group in our previous study was slightly larger (5.0 g/l) compared to the TAPS group in the current study (4 g/l) [20].

Our data may suggest that placental vascular anastomoses in TAPS, such as in TTTS, allow intertwin transfusion not only of hemoglobin but also of albumin and total protein [16]. Another explanation could be that the lower albumin and total protein levels in donors may not only be due to loss of proteins into the circulation of the recipients but could also be due to a reduced production of albumin in donors.

An additional finding of this study concerns the correlation between birth weight and individual placental share. The smaller twins in the control group have a significantly lower individual placental territory, confirming the well-known association between placental function and birth weight [1]. In contrast, although donors in the TAPS group have larger placental shares than recipients, we found a trend in lower birth weights compared to recipients. This

paradoxical finding was previously described by Lewi et al. [21]. It is temping to speculate that the lower levels of albumin and total protein may play a role in the reduced growth of donors despite the larger placental share.

Albumin, which makes up 50% of the normal intravascular protein mass and is responsible for 75–80% of the plasma colloid pressure, has several important physiological properties. Albumin is involved in protein binding and transport of many endogenous and exogenous substances (e.g. drugs, bilirubin) and acts as a free radical scavenger. Furthermore, albumin inhibits platelet function and has antithrombotic effects and finally affects vascular permeability [9, 22]. Serum albumin concentrations increase with gestational age. Possible explanations for this could be increased synthesis by the fetal liver [23] or the greater placental transfer of albumin towards term [24]. Serum levels of albumin may fall during periods of stress, trauma, or sepsis [25] despite its long half-life of 17–19 days [22].

Although hypoalbuminemia is associated with an increased risk of neonatal morbidity and mortality [7, 8], we found no differences in morbidity or mortality between donors and recipients. However, our study was not designed to detect such differences.

The data in this study should be interpreted with care due to the known methodological limitations associated with retrospective study designs.

In conclusion, our data add to the understanding of the pathophysiologic characteristics of TAPS. Our findings confirm that placental vascular anastomoses in TAPS may allow transport not only of hemoglobin from donors to recipients but also of albumin and total protein.

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Chapter 12

Severe cerebral injury in a recipient with twin anemia polycythemia sequence

Abstract

Twin anemia–polycythemia sequence (TAPS) results from slow intertwin blood transfusion through minuscule placental vascular anastomoses and is characterized by large intertwin hemoglobin differences in the absence of amniotic fluid discordance. The optimal management of TAPS is not clear. We report a case of TAPS detected antenatally by Doppler ultrasound examination at 15 + 6 weeks' gestation. After counseling, the parents opted for expectant management. Regular Doppler measurements were performed and these remained fairly stable. An emergency Cesarean section was performed at 34 + 5 weeks following signs of fetal distress. The donor twin was severely anemic while the recipient twin had severe polycythemia–hyperviscosity syndrome. On day 1, the recipient developed respiratory insufficiency and subclinical status epilepticus. Magnetic resonance imaging showed a total loss of gray–white matter differentiation as a sign of severe diffuse cerebral ischemia and bilateral intra- and extra-axial hemorrhages. There was almost complete lack of arterial and venous cerebral blood flow. On day 3 intensive care treatment was withdrawn in view of the severity of the brain injury. This case report demonstrates that TAPS may lead to severe cerebral injury and fatal outcome in the recipient twin, and highlights the importance of antenatal Doppler ultrasound monitoring and choice of management.

Case Report

A 28-year-old primigravida was referred to our institution at 15 + 6 weeks' gestation owing to suspected twin–twin transfusion syndrome (TTTS). Serial ultrasound examination showed normal amniotic fluid volume in both sacs and normal bladder filling in both fetuses, without signs of TTTS. Routine measurements of middle cerebral artery peak systolic velocity (MCA-PSV) with Doppler ultrasound were performed on a weekly or biweekly basis. MCA-PSV in Twin A (recipient) was low (< 0.8 multiples of the median (MoM)), suggesting the presence of polycythemia, whereas MCA-PSV in Twin B (donor) was often increased (> 1.5 MoM), suggesting the presence of anemia (Figure 1), indicative of twin anemia–polycythemia sequence (TAPS). After counseling, the parents opted for a conservative management approach.

At 30 weeks' gestation, the patient was referred back to her own obstetrician. At 34+5 weeks, a Cesarean section was performed owing to signs of fetal distress in the recipient. The first-born twin (recipient) was plethoric and weighed 2065 g and the second-born twin

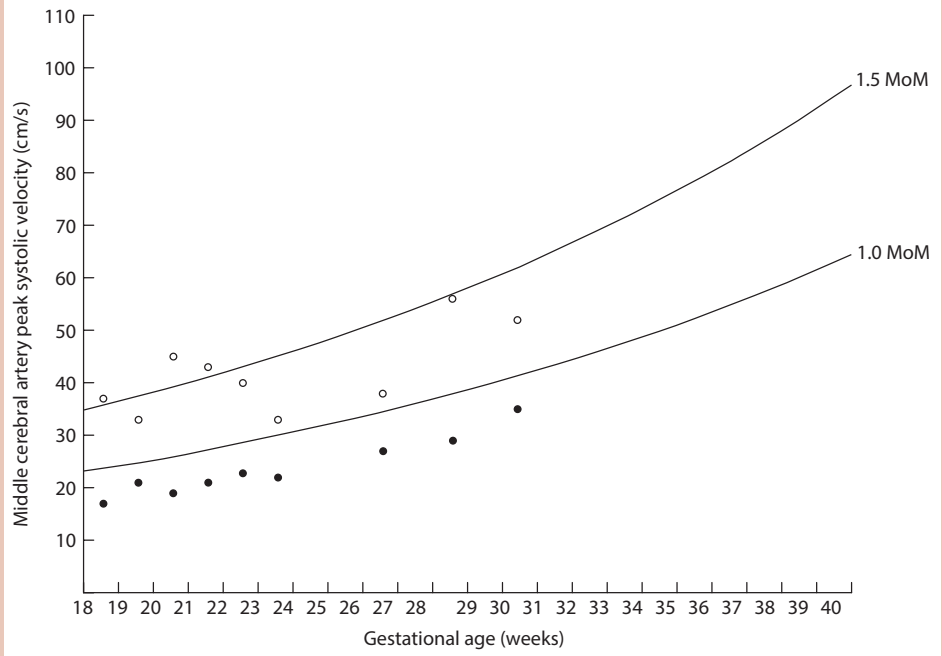

Figure 1. Middle cerebral artery peak systolic velocity in a case of twin anemia–polycythemia sequence plotted against gestational age, showing decreased velocities in recipient twin (●) and increased velocities in donor twin (○).

(donor) was pale and weighed 1805 g. Umbilical cord pH in the first and second twins was 7.21 and 7.20, respectively and Apgar scores (at 1 and 5 min) were 8/9 and 9/9, respectively. Hemoglobin levels and reticulocyte counts in recipient and donor were 25.4 and 4.3 g/dL (difference, 21.1 g/dL) and 12.3 and 66.4% (reticulocyte count ratio of 5.4), respectively, fulfilling the postnatal criteria for severe TAPS stage 5 [1].

A partial exchange transfusion was performed in the recipient twin, who also had thrombocytopenia at birth (platelet count, 98 × 10^9 /L). Soon after the partial exchange transfusion, the recipient developed respiratory insufficiency with apnea and was transferred to the neonatal intensive care unit for mechanical ventilation. The infant subsequently developed disseminated intravascular coagulation (DIC) and worsening thrombocytopenia, for which he received several fresh frozen plasma and platelet transfusions. Soon after admission, subclinical status epilepticus was detected using two-channel amplitude integrated electroencephalography (aEEG). Following a loading dose of phenobarbital, the background pattern changed from a discontinuous normal voltage to an iso-electric trace without subsequent recovery during admission (Figure 2). Cranial ultrasonography performed on day 1 showed

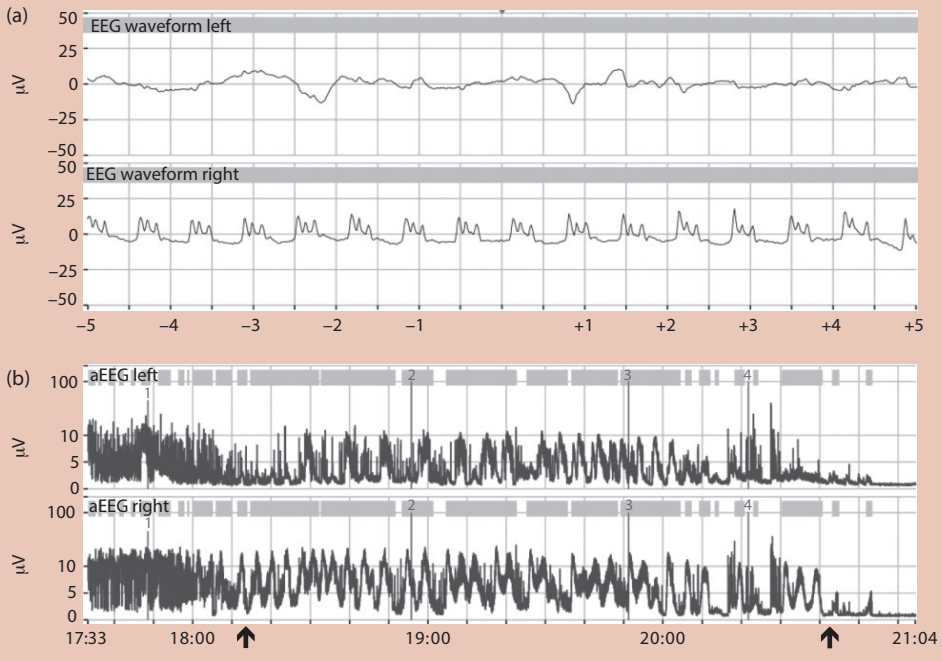

Figure 2. Electroencephalography (EEG) (a) and amplitude-integrated EEG (aEEG) (b) findings in recipient twin in a case of twin anemia–polycythemia sequence. aEEG starts with discontinuous normal voltage background pattern that evolves into status epilepticus (left arrow) and then into an iso-electric recording (right arrow) following administration of phenobarbital.

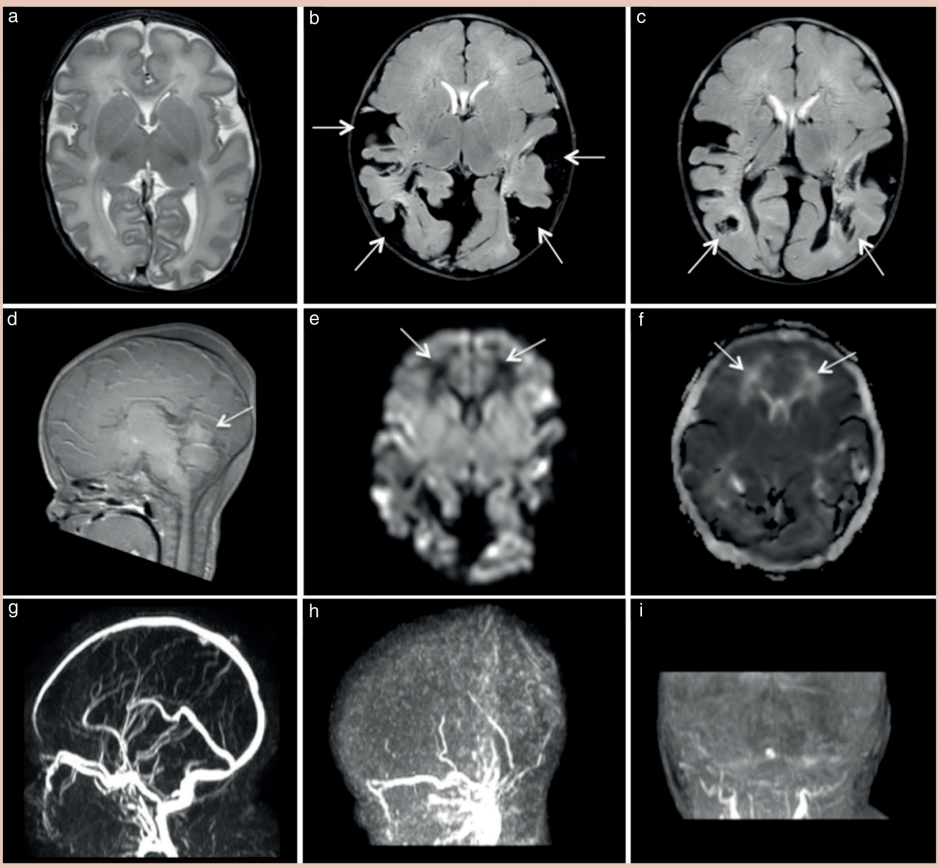

Figure 3. Magnetic resonance imaging in a case of twin anemia–polycythemia sequence. (a–c) Axial T2-weighted images of healthy donor twin (a) and recipient twin (b, c). Bilateral extra- and intracerebral hemorrhage filling all sulci and fissures and compressing both hemispheres can be seen in recipient (b, arrows), as well as a complete loss of gray–white matter differentiation compared with donor twin. Parenchymal venous infarction can be seen in the occipitotempoparietal regions (c, arrows). (d) Sagittal T1-weighted image of recipient twin showing supratentorial blood (arrow) in posterior fossa, compressing cerebellum. (e) Diffusion-weighted image of recipient twin showing diffusely increased signal intensity over almost the whole slice, compatible with loss of gray–white matter differentiation on T1- and T2-weighted images, with only a small amount of relatively normal signal tissue (arrows). (f) Same slice as in (e) of apparent diffusion coefficient map with decreased intensity in same areas (arrows). Magnetic resonance venography in a normal neonatal brain (g) and in recipient twin (h), showing clear loss of venous and arterial cerebral blood flow in recipient twin, as is also seen on magnetic resonance angiography (i).

severely increased echogenicity, most marked in the temporal lobes. Magnetic resonance imaging (MRI) performed on day 3 showed intra- and extra-axial hemorrhages filling all sulci and fissures, compressing both hemispheres, and severe diffuse ischemia with complete loss of gray–white matter differentiation (Figure 3). Furthermore, there was loss of arterial and venous cerebral blood flow on both MRI and Doppler ultrasonography. Diffusion-weighted imaging

showed an increased signal of the cortical and subcortical white matter and cerebellum, indicating diffuse ischemia of almost the entire brain (Figure 3). In view of the MRI and aEEG findings, the decision was made to redirect care and the infant died on day 3. Permission for autopsy was not obtained.

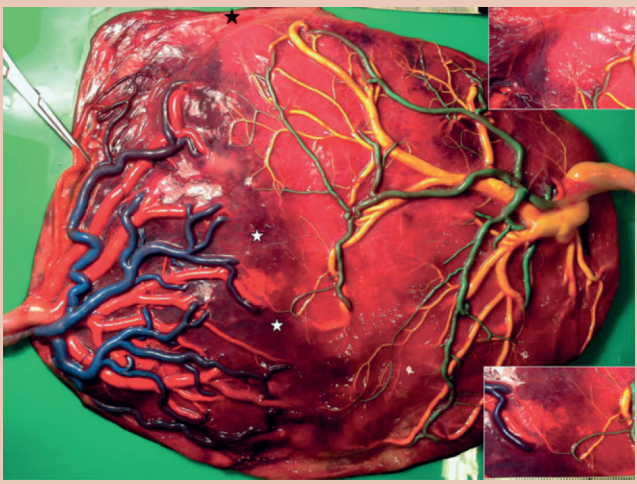

Figure 4. Photograph of placenta after dye injection (blue or green for arteries and orange or yellow for veins) in a case of twin anemia–polycythemia sequence. Placental shares of donor and recipient are on right and left sides of picture, respectively ✪. Arteriovenous (AV) anastomoses from donor to recipient (inset, bottom right)✪. AV anastomosis from recipient to donor (inset, top right).

Macroscopic examination of the placenta revealed a plethoric appearance of the recipient's share of the placenta and a pale appearance of the donor's share. Injection with colored dye showed two minuscule unidirectional arteriovenous (AV) anastomoses (diameter 0.3 and 0.2 mm, respectively) from donor to recipient and one AV anastomosis (diameter 0.2 mm) from recipient to donor (Figure 4).

Discussion

We report on a recipient twin with spontaneous TAPS who suffered massive cerebral injury due to severe polycythemia–hyperviscosity syndrome. This is the first report showing that spontaneous TAPS may lead to extensive cerebral damage, emphasizing the need for increased awareness of the importance of timely detection of the condition and for further research into possible treatment options.

TAPS is a form of chronic fetofetal transfusion, characterized by large intertwin hemoglobin differences, without signs of twin oligo–polyhydramnios sequence (TOPS) [2] . TAPS may occur spontaneously or after laser surgery for TTTS (post-laser form). The spontaneous form complicates approximately 3–5% of monochorionic pregnancies [1,3], whereas the post-laser form occurs in 2–13% of TTTS cases [4,5]. The pathogenesis of TAPS is based

on the presence of few, minuscule AV anastomoses (diameter < 1 mm) [6] allowing slow transfusion of blood from donor to recipient and leading gradually to discordant hemoglobin levels [1].

Diagnosis can be made antenatally with Doppler ultrasound, showing an increased MCA-PSV (> 1.5 MoM) in the donor (suggestive of fetal anemia) and a decreased MCA-PSV (< 0.8 MoM) in the recipient (suggestive of polycythemia), in the absence of TOPS. In the current case, the MCA-PSV in the recipient was mostly < 0.8 MoM, but Doppler values in the donor were often (but not always) > 1.5 MoM. However, the hemoglobin level at birth in the donor (4.3 g/dL) was lower than expected. The discrepancy between the observed hemoglobin level and the findings on prenatal Doppler ultrasound could be due to several factors, including imprecise Doppler measurements or worsening of the MCA-PSV measurements from 30 weeks' gestation (it is of note that MCA-PSV measurements were not performed at 32 weeks or prior to delivery at 34 weeks). Importantly, the sensitivity, specificity and predictive value of MCA-PSV measurements have not yet been evaluated in a TAPS cohort.

Postnatal diagnosis of TAPS is based on the presence of (chronic) anemia in the donor (including reticulocytosis) and polycythemia in the recipient, in association with typical placental angioarchitecture as identified by injection of colored dye. According to a recently proposed classification system, the present case fulfilled the postnatal criteria for the most severe stage of TAPS (Stage 5) [1].

Perinatal morbidity and mortality rates in TAPS are not well known and vary according to the severity of TAPS, reflecting the heterogeneous nature of this disease. Outcome may range from dual intrauterine demise to the birth of two healthy neonates without major morbidity aside from a large discordance in hemoglobin levels [1].

The optimal management of TAPS is not clear, and includes expectant management [7], induction of labor, intrauterine blood transfusion (IUT (intravenous and/or intraperitoneal)) [8,9], selective feticide and fetoscopic laser surgery [1,10,11]. In the present case, treatment with IUT for the donor twin with fetal anemia would probably have resulted in a further increase of polycythemia–hyperviscosity in the recipient and thus worsened its condition. We hypothesize that an alternative approach in TAPS management would be to combine IUT with intrauterine partial exchange transfusion in the recipient twin to reduce hyperviscosity. Fetoscopic laser surgery, to coagulate the anastomoses, is a valid alternative and could have prevented the fatal outcome. However, laser surgery is technically challenging owing to the absence of polyhydramnios and the presence of only minuscule anastomoses. Moreover,

laser surgery is associated with procedure-related complications such as premature rupture of the membranes, disruption of the intertwin dividing membrane and fetal demise. Whether induction of labor before 32 weeks' gestation would have prevented the fatal outcome is not clear, as the optimal timing of delivery in TAPS remains to be determined. Induction of labor should always be weighed against the disadvantages of prematurity, and the vast majority of TAPS cases delivered after 32 weeks' gestation have favorable outcomes [2,10,12]. In addition, the exact timing of the onset of the cerebral lesions in this case was not clear. Cerebral lesions on cranial ultrasound and neurologic symptoms were already present soon after birth (day 1), suggesting that cerebral injury was most probably of antenatal rather than postnatal origin.

Knowledge of the neonatal and pediatric morbidity in TAPS cases is limited and based mostly on case reports and small series. Neonatal morbidity appears to be mainly limited to hematological problems at birth. Donors may be severely anemic and require blood transfusion, whereas recipients may be severely polycythemic and require partial exchange transfusion. Robyr et al. [4] reported a case of post-laser TAPS treated with several IUTs in which the recipient developed skin necrosis of the leg (hemoglobin level at birth, 28 g/dL).

The incidence of cerebral injury and neurodevelopmental impairment in TAPS has not been well studied, and to the best of our knowledge this is the first case report showing that recipient twins may also suffer severe cerebral injury. The exact cause is of this is not clear. The infant had extensive intra- and extra-axial hemorrhages with a bilateral intraventricular hemorrhage and hemorrhagic lesions in the occipitotemporal and parietal periventricular white matter. This appearance is not dissimilar to what has been described as 'the white brain' in the full-term infant with hypoxia–ischemia [13]. However, the infant reported on here had good Apgar scores and only deteriorated a few hours following the partial exchange transfusion. This may have been due to extensive subarachnoid and parenchymal hemorrhages with hemorrhagic diathesis and intravascular coagulation with thrombocytopenia. The timing of these hemorrhages is not clear and may have been antenatal or immediately postnatal, worsened by suboptimal coagulation with subsequent DIC. DIC might have been caused by consumption of coagulation factors due to severe polycythemia–hyperviscosity syndrome. Polycythemia may have led to vascular sludging, obstructing the sinuses and the inflow and outflow of the brain, thus leading to cerebral sinovenous thrombosis. Sinovenous thrombosis is known to be associated with intraventricular hemorrhage and parenchymal infarction [14].

However, although magnetic resonance venography suggested the presence of cerebral sinus venous thrombosis with loss of flow across the superior sagittal sinus and straight sinus, it

is more likely that in this patient the lack of flow was due to increased intracranial pressure caused by the substantial amount of subarachnoid blood, which led to compression of the sinuses.

In conclusion, spontaneous TAPS can lead to severe cerebral injury in the recipient twin when managed conservatively. Increasing awareness and careful monitoring extending to the third trimester are crucial in order to improve the detection and treatment of future TAPS cases. More information on short- and longterm morbidity is needed, ideally through international cooperation between fetal centers using a web-based registry.

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Chapter 13

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

Abstract

Objective:

To evaluate the long-term neurodevelopmental outcome in children who developed twin anemia-polycythemia sequence (TAPS) after fetoscopic 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 surgery between 2004 and 2011 and complicated by post-laser TAPS. Primary outcome was neurodevelopmental impairment, a composite outcome including any of the following: cerebral palsy, bilateral deafness, blindness, severe motor and/or cognitive developmental delay (>2 SD below the mean). 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 neurodevelopmental impairment was 4/47 (9%), occurring in one donor (1/20; 5%) and three recipients (3/27; 11%) (P=0.63). Mild-to-moderate cognitive delay, i.e. scores below 85, was detected in 8/47 (17%) children. Risk factors for low cognitive scores were low gestational age at birth (P=0.02) and low birth weight (P<0.01). The 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 were found in almost one in five children surviving post-laser TAPS. Better treatment and, ideally, prevention of this complication after laser treatment for TTTS is urgently needed.

Introduction

Twin anemia–polycythemia sequence (TAPS) is a chronic form of fetofetal transfusion in monochorionic twins through small anastomoses at the placental surface[1]. TAPS is characterized by a large intertwin hemoglobin difference without signs of twin oligo–polyhydramnios sequence (TOPS). TAPS may occur spontaneously (spontaneous TAPS) or after twin–twin transfusion syndrome (TTTS) treated with fetoscopic laser surgery (post-laser TAPS). The incidence varies between 1% and 5% in spontaneous TAPS and 1% and 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 of the donor twin, suggestive of fetal anemia, and decreased velocity in the recipient twin, suggestive of polycythemia, without concomitant signs of TOPS. Postnatal diagnosis is based on intertwin hemoglobin 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 of 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 Dutch national referral center for fetal therapy, 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 to participate.

TAPS was identified using previously published criteria[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 cotwin, in the absence of TOPS. Diagnosis of postnatal TAPS was based on an intertwin hemoglobin difference of ≥ 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 was classified 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 treatment or cord coagulation), gestational age at birth, birth weight and 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 ≥ Grade III[12], cystic periventricular leukomalacia ≥ Grade II[13], ventricular dilatation ≥ 97th percentile[14], porencephalic cysts or arterial or venous infarction detected on cerebral imaging.

A follow-up visit was performed at a minimum age of 24 months and included a neurological 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 age 3 years or older were tested with the Wechsler Preschool and Primary Scale of Intelligence third edition (WPPSI-III)[17]. These three tests provide cognitive scores that follow a normal distribution with a mean of 100 and an SD of 15. BSID-II and BSID-III also provide motor development scores. When each separate score was below 70, i.e. > 2 SD below the mean, this was indicative of severe delay in either cognitive or motor development. Scores below 85, i.e. > 1 SD below the mean, were indicative of mild-to-moderate delay. Cerebral palsy 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, including at least one of the following: cerebral palsy, cognitive development score of less than 70 (> 2 SD below the mean), motor development score of less than 70 (> 2 SD below the mean), bilateral blindness or bilateral deafness requiring amplification. The

primary aim of our study was to assess the incidence of neurodevelopmental impairment in post-laser TAPS cases and to compare outcomes between donors and recipients. The secondary outcome was an estimation of risk factors associated with lower cognitive scores including gestational age at birth, birth weight, gestational age at diagnosis of TAPS, TAPS management in the antenatally detected TAPS cases and severe neonatal morbidity (including severe cerebral injury).

Data are reported as mean ± SD or as median (range), as appropriate. Statistical analysis was performed using Student's t-test and the Mann–Whitney U-test for continuous variables. The 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. Analysis was carried out using the Generalized Estimated Equation module to account for the effect that observations within twin pairs are not independent. All statistical data were analyzed using SPSS version 20.0 (IBM, Armonk, NY, USA), and $P<0.05$ was considered as indicating statistical significance.

Charachteristic	Value
TAPS donor	20 (43)
Gestational age at laser (weeks)	21 (15–27)
Quintero stage	II (I–IV)
Antenatally detected TAPS stage (n = 28)	2 (1–5)
Stage 1	4 (14)
Stage 2	11 (39)
Stage 3	4 (14)
Stage 4	7 (25)
Stage 5	2 (7)
Postnatally detected TAPS stage (n = 19)	2 (1–4)
Stage 1	8 (42)
Stage 2	9 (47)
Stage 3	—
Stage 4	2 (11)
Stage 5	—
Gestational age at birth (weeks)	32 (26–41)
Birth weight (g)	1635 (750–3667)
Sex female	22 (47)
Severe cerebral injury*	2/46 (4)
Severe neonatal morbidity†	18 (38)

Data are presented as median (range) or n (%). *46 children underwent cranial ultrasound. †Severe neonatal morbidity defined as any of the following characteristics: respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis ≥ Stage II or severe cerebral injury.

Table 1: Baseline characteristics of post-laser twin–anemia polycythemia sequence (TAPS) survivors assessed at follow-up (n = 47)

Results

A total of 306 monochorionic twin pregnancies were treated at our center with fetoscopic laser surgery for TTTS between 2004 and 2011.

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

TAPS was detected antenatally in 28/47 cases (60%) and postnatally in the remaining 19/47 cases (40%). Median gestational age at birth in TAPS cases detected antenatally and postnatally was 32 (range, 26–37) and 32.5 (range, 26–41) completed weeks, respectively (P=0.62). Of the 28 antenatally detected post-laser TAPS cases, 17 were managed expectantly, eight underwent intrauterine transfusion, two were treated with further laser surgical intervention and in one case cord coagulation of the cotwin was performed. Intrauterine treatment was offered in all cases of TAPS Stages 3 and 4. In TAPS Stage 1 or 2, intrauterine treatment was offered only in cases in which TAPS was rapidly progressing (within days), or when the fetus showed other signs of severe anemia not meeting the criteria for Stage 3, such as increasing heart size or prehydropic signs. When treatment was performed, laser surgery was the first choice if this appeared technically feasible. Laser surgery in TAPS can be more challenging owing to the absence of TOPS. Intrauterine transfusion was chosen when laser treatment was not considered to be feasible. Cord coagulation was performed in one case in which we observed severe cerebral injury in the ex-TTTS recipient (the new TAPS donor)[19]. Median gestational age at birth of the cases treated in utero (intrauterine transfusion, laser or cord

	Overall (n = 47)	TAPS donor (n = 20)	TAPS recipient (n = 27)	p value
Cerebral palsy	1 (2)	1 (5)	—	0.43
Cognitive score	95.3 ± 12.5	94.5 ± 11.3	95.8 ± 13.4	0.74
Cognitive score < –2 SD	2 (4)	—	2 (7)	0.50
Cognitive score < –1 SD	8 (17)	3 (15)	5 (19)	1.0
Motor score*	93.9 ± 12.4	93.2 ± 7.8	94.4 ± 15.3	0.81
Motor score < –2 SD*	1/26 (4)	—	1/15 (7)	1.0
Motor score < –1 SD*	5/26 (19)	1/11 (9)	4/15 (27)	0.36
Bilateral blindness/deafness	—	—	—	—
Neurodevelopmental impairment†	4 (9)	1 (5)	3 (11)	0.63

Data are expressed as n (%) or mean ± SD. *Number of children with assessment of motor development with Bayley scales, 11 donors and 15 recipients. †Neurodevelopmental impairment included any of the following: cerebral palsy, cognitive development > 2 SD below the mean, motor development > 2 SD below the mean, bilateral deafness or blindness.

Table 2: Long-term outcomes of 47 children with post-laser twin anemia–polycythemia sequence (TAPS)

coagulation) was 29 (range, 26–33) weeks compared with 33 (range, 27–41) weeks in the cases treated expectantly (P=0.07).

Of the 47 children available for follow-up, neonatal cranial ultrasound was performed in 46 cases (98%). The remaining child was born at term in a referral hospital at which cranial ultrasound was not part of the standard procedure. Two children were diagnosed with severe cerebral injury. In one case, the TAPS donor (a former TTTS recipient) was diagnosed with cystic periventricular leukomalacia Grade III. In the other case, a TAPS recipient (a former TTTS recipient), cerebral imaging showed venous infarction and intraventricular hemorrhage Grade II.

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

The overall incidence of neurodevelopmental impairment in the studied cohort was 4/47 (9%), occurring in one donor (1/20, 5%) and three recipients (3/27, 11%) (P=0.63). Cerebral palsy was diagnosed in one case (2%). Severe cognitive delay was detected in two children (4%) and severe motor delay in one child (2%). Mild-to-moderate cognitive delay was detected in 8/47 (17%) and mild-to-moderate motor delay in five (19%) children in whom it could be assessed. Long-term outcomes are reported in Table 2. Patient characteristics of the four children with neurodevelopmental impairment are presented in Table 3.

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

We performed univariate analysis of potential risk factors for cognitive outcome in the whole cohort. Risk factors for low cognitive scores were 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),

#	TAPS donor/ recipient	Highest TAPS stage	Treatment of TAPS	GA at birth (wk)	Birth weight (g)	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 Stage I	Cognitive score <–2SD
2	Recipient (former TTTS donor)	2	IUT	29	1009	RDS	No abnormalities	Cognitive score <–2SD
3	Recipient (former TTTS donor)	3	Cord coagulation co-twin	28	955	RDS	No abnormalities	Motor score <–2SD
4	Donor (former TTTS recipient)	2	IUT followed by re-laser surgery intervention	32	1635	No	cPVL Stage III	CP: quadriplegia

CP, cerebral palsy; cPVL, cystic periventricular leukomalacia; GA, gestational age; IUT, intrauterine transfusion; IVH, intraventricular hemorrhage; RDS, respiratory distress syndrome; TTTS, twin–twin transfusion syndrome.

Table 3: Patient characteristics of the four post-laser twin anemia–polycythemia sequence (TAPS) survivors with neurodevelopmental impairment

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).

Discussion

This is the first study evaluating long-term neurodevelopmental outcome in TTTS survivors who developed TAPS after laser surgery. Neurodevelopmental impairment was detected in 9%, with no differences 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 neurodevelopmental impairment reported in case series of TTTS treated with laser (range 6% to 18%) [9–11]. Unfortunately owing 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 cases treated with laser therapy. 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 with uncomplicated TTTS cases.

The incidence of cerebral palsy of 2% in our series is similar to that in previously published TTTS follow-up studies, which range from 3% to 12%[9–11]. In the general population, cerebral palsy occurs in approximately 6% of infants born at 28–31 weeks' gestation, 0.7% of those born at 32–36 weeks and 0.1% of term infants[20]. Severe cognitive delay (4%)

and severe motor delay (2%) were 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 rate of 2.3% in the general population.

Cerebral injury and neurologic impairment in TAPS survivors can theoretically be due to several factors, including 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 at birth 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 and impaired neurodevelopmental outcome[21,22]. In a subgroup analysis on antenatally detected/managed TAPS cases, we found that the TAPS subgroup treated with intrauterine transfusion had the lowest median cognitive score (82.5) compared with the other subgroups (Table 4). A possible explanation for the low cognitive scores could be that these cases were born at a lower gestational age of 29 weeks (interquartile range, 27.5–33.0) owing to induced labor or planned Cesarean section for severe anemia or polycythemia. Intrauterine transfusion may temporarily improve the condition of the donor, allowing prolongation of the pregnancy. However, intrauterine transfusion may also worsen polycythemia in the recipient twin and lead to possible severe complications such as severe cerebral injury[8]. Additionally intrauterine transfusion is a palliative treatment, not a curative treatment for TAPS.

Treatment antenatal TAPS	n	Antenatal TAPS stage	GA at birth (weeks)	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)

Data are expressed as n or median (range). GA, gestational age.

Table 4: Cognitive scores in 28 post-laser twin anemia–polycythemia sequence (TAPS) survivors diagnosed antenatally

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 three children with severe developmental delay, two were tested with BSID-III and one with BSID-II. Children aged 3 years or older were tested with WPPSI. With advanced age a more reliable view of capacities can be obtained. Also, two children had already been tested elsewhere with the Snijders Oomen Non-Verbal Intelligence Scale because of failure of previous WPPSI assessment.

The most important limitation of this study was the relatively small sample size. Thus, although this is the largest study to date reporting on neurodevelopmental outcomes in post-laser TAPS, our data should be interpreted with caution.

Since post-laser TAPS is caused by small residual anastomoses that might have been missed at initial laser treatment for TTTS, it is of great importance to reduce the number of these residual anastomoses. A recently published randomized controlled trial showed a significant reduction in 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, in which 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 neurodevelopmental impairment and 17% incidence of at least mild-to-moderate cognitive delay, with no difference between donors and recipients. Risk factors for a lower cognitive score are lower gestational age at birth and lower birth weight. Antenatal TAPS management consisting of intrauterine transfusion was a risk factor for lower cognitive scores. Larger studies are needed to investigate reliably 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 the utmost importance to increase sample size.

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Summary and General discussion

Summary and general discussion

Summary

This thesis consists of a series of studies on a newly described complication in monochorionic twins, which we named twin anemia polycythemia sequence (TAPS). A review of the literature is given in part II. We studied the pathogenesis by placental injection studies in part III. In part IV diagnostic criteria were studied for antenatal and postnatal TAPS. We studied antenatal management and outcome including a randomized trial on the prevention of post-laser TAPS in part V. In part VI, studies on postnatal management and outcome including long-term neurodevelopmental follow-up are described.

Review

Chapter 1 opens with a review of the literature, and further comprises of our description of the TAPS staging system for antenatal and postnatal detected TAPS. The aim of this staging system is to reflect the increasing severity of anemia and polycythemia. The antenatal staging system is based on Doppler ultrasound measurements and signs of cardiac compromise due to severe anemia. Postnatal staging is based on the intertwin hemoglobin (Hb) difference, the larger the differences the higher the stage.

Pathogenesis

The pathogenesis of TAPS is based on its unique placenta angioarchitecture. TAPS placentas are characterized by the presence of only a few and very small (<1mm) arterio-venous (AV) anastomoses. TAPS placentas differ from uncomplicated monochorionic twin placentas. The latter are characterized by more and larger anastomoses and have AA anastomoses in up to 90% [1]. In TAPS placentas, AA-anastomoses are very rare. Studies described in Chapter 2 show that after fetoscopic laser for twin-twin transfusion syndrome (TTTS), most residual anastomoses are small and localized near the margin of the placenta. Almost half of the cases with residual anastomoses resulted in TAPS. Studies comparing placentas of spontaneous TAPS and post-laser TAPS cases in chapter 3 showed the presence of more anastomoses in spontaneous TAPS as compared with post-laser TAPS placentas. In both groups, most anastomoses were localized near the margins of the placenta. In contrast to a previous study by our group [1] we found AA anastomoses in both groups, although all AA-anastomoses in TAPS were very small (<1mm). In chapter 4 we report on our study on

AA-anastomoses in spontaneous TAPS cases compared with uncomplicated monochorionic twins. In TAPS cases, AA anastomoses were very small (all <1mm), much smaller than AA anastomoses in uncomplicated monochorionic twins. The placenta findings in these studies help us to understand the pathophysiology of TAPS. The presence of only a few and small anastomoses suggests that the transfusion process is slow and a hemodynamic compensation takes place resulting in an unchanged amount of amniotic fluid for both twins but with large Hb difference.

Diagnosis

Antenatal diagnosis

TAPS can be diagnosed antenatally or postnatally. Antenatal diagnosis is based on Doppler ultrasound abnormalities showing an increased peak systolic velocity in the middle cerebral artery (MCA-PSV) in the donor twin, >1.5 multiples of the mean (MoM), suggestive of fetal anemia. In the recipient twin decreased velocities in the MCA-PSV <1.0 MoM are seen, without signs of the oligo-polyhydramnios sequence as in TTTS, as reported in chapter one. MCA-PSV measurement, a non-invasive test, has become the standard test for the prediction of fetal anemia in singletons in a variety of fetal diseases [2]. In chapter 5 we showed the diagnostic accuracy of MCA-PSV measurements to predict abnormal fetal hemoglobin levels in TAPS. We found high sensitivities and specificities of MCA-PSV both for anemia and for polycythemia, confirming the clinical usefulness of this noninvasive test. Our results also strengthen our previous findings and proposal to use 1.0 MoM as cut-off level for the TAPS recipient, as described in chapter one. With the use of the previously suggested cut-off level of 0.8 MoM, a significant number of cases of severe polycythemia would be missed [3].

Postnatal diagnosis

In chapter 6 we proposed to use a fixed cut-off level of Hb difference ≥ 8g/dL to define TAPS based on a case-control study where we compared hematologic values in TAPS compared with uncomplicated monochorionic twins. We found that all TAPS cases had an intertwin difference of at least 8.0 g/dL. In addition to the Hb difference, an essential part of the postnatal definition is an increased reticulocyte count measured in the TAPS donor resulting from chronic anemia. An intertwin reticulocyte count >1.7 was pathognomonic for TAPS. An additional criterion for postnatal TAPS is the presence of small (residual) anastomoses at the placental surface. These two criteria enable us to distinguish between chronic feto-fetal transfusion as it occurs in TAPS, and the acute form of transfusion as in acute TTTS [4]. Acute TTTS is also characterized by the large Hb differences but is based on large amount

of feto-fetal transfusion through large anastomoses, whereas TAPS is a chronic form of transfusion through small anastomoses.

Antenatal management and outcome

Solomon trial; prevention of post-laser TAPS

For the prevention of post-laser TAPS we compared in chapter 7, in a randomized controlled trial, two laser techniques for the treatment of TTTS. Post-laser TAPS is caused by residual anastomoses at the placental surface after fetoscopic laser surgery for TTTS. In order to reduce the number of residual anastomoses, a new technique named the Solomon technique was introduced. With the standard technique all visible anastomoses are coagulated. With the Solomon technique, after identifying and coagulating the individual anastomoses, a line is drawn with the laser from one placenta margin to the other. In this randomized trial we showed a significant reduction of the primary outcome, which was a composite outcome of TAPS, recurrent TTTS, perinatal mortality or severe neonatal morbidity, of 49% in the standard group versus 34% in the Solomon group. A significant reduction of post-laser TAPS of 16% in the standard group to 3% in the Solomon group was seen. In addition, a reduction of recurrent TTTS was seen of 7% in the standard group to 1% in the Solomon group. With the Solomon technique we showed an important improvement for the prevention of post-laser TAPS and recurrent TTTS. The Solomon technique did not appear to be associated with an increase in any identifiable adverse outcome or complication.

Secondary analysis Solomon trial, residual anastomoses

In chapter 8 we performed a secondary analysis of the Solomon trial. All placentas that were injected with color dye were analyzed. After using the standard technique, residual anastomoses were seen in 34% of the placentas, which is in line with a previous published incidence of residual anastomoses (32%) in chapter two. Using the Solomon technique an almost 50% reduction to 19% was seen. In a subgroup analysis where the surgeon stated that the procedure had been complete, a reduction to 12% was seen. The presence of residual anastomoses was associated with a 58% risk of developing TAPS or recurrent TTTS, a similar risk as was described in chapter two. The main reason for residual anastomoses in the Solomon group was the fact that the laser-line along the vascular equator was not complete. In conclusion, the Solomon technique reduces the incidence of residual anastomoses and their clinical complications such as TAPS and recurrent TTTS, but does not guarantee a complete dichorionization of the placenta. Therefore, careful follow-up with serial Doppler

ultrasound measurements of the middle cerebral artery peak systolic velocity and of amniotic fluid volumes of both twins remains of crucial importance, even after a laser intervention using the Solomon technique.

Management antenatal detected TAPS

The optimal management for TAPS is not clear. Options include fetoscopic laser surgery, intrauterine blood transfusions (IUT) in the donor, with or without combination of partial exchange transfusion (PET) in the recipient, expectant management or selective feticide. The rationale for intrauterine treatment is to prevent or treat severe fetal anemia or polycythemia. In chapter 9 we applied a computational model simulation to illustrate the mechanism of IUT with and without PET in TAPS occurring after laser surgery for TTTS. Model simulations were performed with the representative anastomotic pattern as observed during laser intervention, and after placental dye injection. In this model simulation we showed that the addition of PET to IUT reduces the severity of polycythemia in the recipient. PET may thus be important to prevent complications of hyperviscosity, such as limb necrosis and severe cerebral injury. However, IUT with or without PET remains a symptomatic treatment for TAPS, it does not solve the underlying problem, which are the small (residual) anastomoses. Laser coagulation of the vascular anastomoses is the only causal treatment for TAPS.

In chapter 10 we compared fetoscopic laser therapy with intrauterine transfusion and expectant management for the treatment of antenatal detected TAPS. The results of this retrospective cohort suggest that laser treatment for TAPS may improve survival and neonatal outcome by prolonging the pregnancy, compared to treatment with intrauterine transfusion or expectant management. Prolonging the pregnancy is known to be of paramount importance for neonatal outcome. Low gestational age at birth is independently associated with increased risk for severe neonatal morbidity [5;6], severe cerebral lesions [7] and impaired neurodevelopmental outcome [8]. We cannot firmly conclude that the improved outcome after laser reflects a true benefit of laser treatment. To determine the optimal management and to evaluate possible benefits of laser surgery for TAPS, adequately powered, prospective studies are needed.

Postnatal management and outcome

In chapter 11 we evaluated differences in biochemical variables in TAPS and showed that donor twins have significantly lower levels of albumin and total protein levels at birth compared to recipients, in addition to lower hemoglobin levels. Lower albumin levels and total protein levels in donors compared to recipients is also seen in TTTS [9-11]. The findings of this study suggest that placental vascular anastomoses in TAPS allow intertwin transfusion

not only of hemoglobin but also of albumin and other proteins. Another explanation could be that the production of albumin in donors is reduced. An additional finding in this study was that in the control group (uncomplicated monochorionic twins) the smaller twin had a significantly lower placental share. In contrast the donors in the TAPS group had lower birth weight but larger placental shares. This finding was also reported by Lewi et al. [12]. Lower levels of albumin and total protein may play a role in the reduced growth of the donors despite the larger placental share.

In chapter 12 we report a case of spontaneous TAPS with severe cerebral injury resulting in neonatal death due to severe polycythemia-hyperviscosity syndrome in the recipient twin. This case-report shows that complications of TAPS are not only limited to hematological complications and may be severe. The risk of severe polycythemia-hyperviscosity syndrome is substantial, and our findings support our computed model (chapter 9) where we suggest adding partial exchange transfusion to reduce the severity of polycythemia and hyperviscosity. Chapter 13 is the first study evaluating long-term neurodevelopmental outcome in post-laser TAPS. Neurodevelopmental impairment (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%) [13-15]. 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 [7] and impaired neurodevelopmental outcome [8]. In a subgroup analysis on antenatally detected and managed TAPS cases, we found that the TAPS subgroup treated with IUT had a lower median cognitive score 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 due to induced labor or planned caesarean for severe anemia or polycythemia. Larger studies are needed to reliably investigate long-term neurodevelopmental outcome and evaluate risk factors for adverse outcome.

In summary, in this thesis we describe that TAPS is a form of chronic feto-fetal transfusion based on a small amount of blood transfusion through very small anastomoses. For the antenatal diagnosis of TAPS, MCA-PSV Doppler measurements appear to be an accurate predictor for anemia and polycythemia. If antenatally detected, fetoscopic laser therapy might improve pregnancy outcome by treating the cause, and thereby prolonging the pregnancy. Another very important finding for the prevention of post-laser TAPS is that by using the Solomon technique for the treatment of TTTS the incidence of post-laser TAPS is

reduced. If laser is not feasible and IUT is the preferred treatment option, we suggest adding PET to prevent severe polycythemia and possible complications as limb necrosis and severe cerebral injury. Long-term neurodevelopmental outcome in post-laser TAPS is 9% and seems comparable with long-term outcome after treated TTTS.

In conclusion, with this thesis we increased our knowledge on the pathophysiology, the diagnostic tools, management options, short and long-term outcome. However management of TAPS remains a challenging problem and more research is necessary to improve treatment and outcome.

Discussion

Pathogenesis

Based on the placental injection studies we showed that TAPS has its own unique placental angio-architecture. Interestingly TAPS donors tend to have a bigger placental share but a lower birth weight compared to TAPS recipients. Future research should focus on the placental territory and birth weight ratio in TAPS pregnancies. Another interesting study on placentas would be to analyze the type, size and localization of residual anastomoses after using the Solomon technique. After using the Solomon technique we showed a reduction of residual anastomoses, however in most cases with residual anastomoses it was due to a discontinuous laser line. For the optimal laser effect, a careful balance should be sought between avoiding excessive tissue damage due to too much laser energy, and insufficient tissue damage (and possible residual anastomoses) due to reduced laser energy. Future research should be aimed at optimizing the laser energy use, for which numerous in vitro and in vivo experiments are likely needed. Some are currently carried out in our Medical Delta collaboration with the Technical University Delft and the Erasmus Medical Center, Rotterdam.

Diagnosis

In this thesis we showed that MCA-PSV Doppler is a powerful tool for the prediction of fetal anemia and polycythemia in selected pregnancies with an increased risk for TAPS. Whether MCA-PSV Doppler is also a good predictor of anemia and polycythemia in uncomplicated monochorionic twins needs to be established. For postnatal diagnosis, fixed Hb concentration cut-of levels have been abandoned and an intertwin Hb difference is used. Whether delta MoM of MCA-PSV would be a more accurate tool in predicting TAPS compared to fixed cut-off levels, and whether the prenatal staging system predicts the stage of postnatal

TAPS should be studied in future. In addition to these studies, MCA-PSV values in all monochorionic twins close to delivery could be compared with postnatal Hb levels to test the accuracy of MCA-PSV measurement in relation to Hb levels. Apart from abnormal Doppler measurements, distinct differences in placenta echodensity and thickness (an increased and hydropic placental part of the TAPS donor and a normal part of the TAPS recipient) [16;17], and a starry sky aspect of the liver in the TAPS recipient suggestive for polycythemia have been reported in TAPS [18]. Future studies should address the sensitivity, specificity and reproducibility of these additional ultrasound findings.

Prenatal management and outcome

Recent retrospective studies [19-21] on the Solomon technique showed a reduction of TAPS and recurrent TTTS and an improvement in survival. However our study was not powered to detect such differences. The effect of the Solomon technique on survival, and the effect of post-laser TAPS and recurrent TTTS on neonatal morbidity need to be studied prospectively in sufficiently large cohorts.

We compared the different treatment options for TAPS in a retrospective cohort, and found that laser seemed to improve pregnancy outcome. We cannot yet firmly conclude that the improved outcome reflects a true benefit of laser treatment. More studies (ideally using a randomized controlled design, including long term follow-up) are needed to determine the optimal management and to evaluate the possible benefits of laser therapy for the treatment of TAPS. In the meantime, a prospective evaluation of the management in TAPS cases worldwide may further improve our understanding in this complex and rare disease. To facilitate this evaluation, we recently set up a web-based TAPS registry: www.TAPSregistry.org.

Postnatal management and outcome

Future long-term follow-up studies are of utmost important to increase our knowledge on the neurodevelopmental outcome of TAPS pregnancies. By comparing the reported neurodevelopmental impairment of 9% with a control group of treated TTTS cases the influence of post-laser TAPS on treated TTTS twins will be studied. In addition, evaluation of long-term neurodevelopment in survivors after spontaneous TAPS and in relation to the different treatment modalities for TAPS requires further study.

Since disease-free survival is the most important outcome in fetal therapy we will also assess the long-term neurodevelopmental outcome in all surviving twins at the age of 2 in the Solomon trial.

Nederlandse samenvatting

Er bestaan twee soorten tweelingen: een- en twee-eiige. De kans op een twee-eiige tweelingzwangerschap is ongeveer 1,5%. Bij twee-eiige tweelingen heeft elke foetus altijd een eigen placenta (moederkoek) en een eigen vruchtzak, met een amnionvlies en een chorionvlies. Dit heet een "dichoriale" tweeling. De kans op een eeneiige tweelingzwangerschap is ongeveer 0,5%. Van de eeneiige tweelingen heeft 1/3 ook elk hun eigen placenta en vruchtzak (en zijn dus dichoriaal). Tweederde van de eeneiige tweelingen delen echter samen één placenta, maar zitten wel elk in een eigen vruchtzak. De vruchtzakken hebben alleen een eigen amnionvlies. Er is maar één chorionvlies. Dit wordt een "monochoriale, diamniotische" tweeling genoemd. In 1-2% van de monochoriale tweelingzwangerschappen bevinden de kinderen zich in dezelfde vruchtzak. Dit wordt een "monoamniotische" tweeling genoemd. Monochoriale tweelingzwangerschappen hebben een verhoogde kans op complicaties en sterfte doordat de bloedsomlopen van de kinderen met elkaar zijn verbonden op het placentaoppervlak. Door deze vaatverbindingen stroomt bloed van de ene foetus naar de andere. Bij 9 van de 10 monochoriale tweelingen stroomt even veel bloed van een foetus naar de ander als andersom. Dit veroorzaakt geen problemen. Als de bloedstroom tussen beide foetussen echter niet in balans is, ontstaan er complicaties zoals het tweeling transfusie syndroom (TTS) en de tweeling anemie polycythemie sequentie (TAPS).

Tweeling Transfusie Syndroom (TTS)

Door de vaatverbindingen stroomt bloed van de ene foetus (de "donor") naar de andere foetus (de " recipiënt" of "ontvanger") en de donor krijgt hiervoor maar weinig bloed terug. Bij de donor ontstaat hierdoor een tekort aan bloed, waardoor hij eerst minder en later helemaal niet meer plast en daardoor uiteindelijk geen vruchtwater meer heeft. Het vlies van zijn vruchtzak zit dan strak om hem heen en het vlies is op de echo nauwelijks nog te zien. De ontvanger krijgt juist te veel bloed en gaat steeds meer plassen. Hij krijgt daardoor te veel vruchtwater in zijn vruchtzak. Hierdoor groeit de buik van de moeder hard. Bij heel veel vruchtwater kunnen weeën ontstaan, of kunnen de vliezen breken. De foetussen hebben een grote kans dat ze veel te vroeg geboren worden. Als TTS niet behandeld wordt dan is de sterfte 73-100%. De beste behandeling voor TTS is foetoscopische laser behandeling waarbij de bloedvatverbindingen tussen de foetussen worden dicht gebrand [22].

Tweeling Anemie Polycythemie Sequentie (TAPS)

Als er slechts enkele zeer kleine bloedvatverbindingen (<1mm) zijn, kunnen er wel rode

bloedcellen stromen van donor naar recipiënt, maar ontstaat er geen vruchtwaterverschil zoals bij TTS. De donor krijgt bloedarmoede (anemie) en de ontvanger krijgt te dik bloed (polycythemie). Ernstige anemie kan leiden tot hartfalen, het vasthouden van vocht of tot sterfte. Polycythemie kan slechte doorbloeding van huid, vingers of tenen, hersenen of andere organen veroorzaken. Bij de geboorte is de donor bleek, en de ontvanger donkerrood. TAPS kan zowel voor als na de geboorte ontdekt worden.

Dit proefschrift beschrijft de pathogenese, diagnostiek, behandeling, preventie, korte- en langetermijnuitkomsten van TAPS.

Review

Hoofdstuk 1 begint met een review van de literatuur en de door ons ontwikkelde stadiëring van TAPS voor zowel een antenataal (voor de geboorte) als postnataal (na de geboorte) gediagnosticeerde TAPS. Bij toename van anemie en polycythemie neemt ook het stadium toe. De antenatale stadiëring is gebaseerd op echo Doppler-bevindingen (techniek om bloedstroom patronen en snelheden in bloedvaten te meten) en op tekenen van cardiale decompensatie (hartfalen) als gevolg van de anemie. De postnatale stadiering is gebaseerd op het verschil in hemoglobine (rode bloedcellen) tussen beide kinderen; hoe groter het verschil hoe hoger het stadium.

Pathogenese

Om meer te weten te komen over het ontstaan van het ziektebeeld TAPS, werden placenta's onderzocht van monochoriale tweelingen met en zonder TAPS. Er zijn verschillende soorten vaatverbindingen op het placenta-oppervlak. Een arterie (slagader) van het ene foetus die verbonden is met een vene (ader) van de andere foetus, wordt een AV-anastomose (vaatverbinding) genoemd. Een vaatverbinding van een arterie naar een arterie is een AA-anastomose. AA-anastomosen in ongecompliceerde monochoriale tweelingen zorgen voor een balans tussen de twee bloedstromen van de foetussen. AA-anastomosen werden in meer dan 90% van de placenta's van ongecompliceerde monochoriale tweelingen gezien. Bij TAPS-placenta's zijn AA-anastomosen zeldzaam. Verder hebben TAPS-placenta's minder vaatverbindingen dan de placenta's zonder TAPS en zijn de aanwezige vaatverbindingen zeer klein (<1mm).

"Spontane TAPS" ontstaat in 3-5% van de monochoriale tweelingen doordat alleen enkele heel kleine bloedvatverbindingen op het placenta-oppervlak aanwezig zijn, met een onbalans in de verdeling van AV- en VA-anastomosen. Door de geringe maar continu doorgaande

transfusie van de donor naar de recipiënt krijgt de donor bloedarmoede, en de recipiënt dik bloed. TAPS kan ook ontstaan na laserbehandeling voor TTS (tot in 16% van de behandelde TTS-tweelingen). In dit geval zijn er bij de behandeling enkele kleine bloedvatverbindingen niet dicht gelaserd waardoor bloedarmoede bij de ene foetus en te dik bloed bij de andere foetus ontstaat. Dit wordt "post-laser TAPS" genoemd.

In hoofdstuk 2 wordt beschreven dat na fetoscopische laserbehandeling voor TTS, in 32% van de placenta's nog rest-anastomosen aanwezig zijn. De meeste rest-anastomosen zijn klein en bevinden zich aan de rand van de placenta. Wanneer rest-anastomosen aanwezig zijn, ontstaat in de helft van de gevallen TAPS.

In hoofdstuk 3 worden spontane TAPS-placenta's met post-laser TAPS-placenta's vergeleken. Spontane TAPS-placenta's hebben meer anastomosen in vergelijking met post-laser TAPS-placenta's. Bij beide groepen zijn de anastomosen gelokaliseerd aan de rand van de placenta. In tegenstelling tot eerder onderzoek [1] zijn in beide groepen bij enkele placenta's AA-anastomosen aanwezig, maar slechts met een zeer geringe (<1mm) diameter.

AA-anastomosen van spontane TAPS-tweelingen worden in hoofdstuk 4 vergeleken met AA-anastomosen van ongecompliceerde monochoriale tweelingen. In tegenstelling tot de AA-anastomosen van ongecompliceerde monochoriale tweelingen zijn alle AA-anastomosen van spontane TAPS tweelingen zeer klein (<1mm).

Diagnose

Antenatale diagnose

TAPS kan zowel antenataal als postnataal worden gediagnosticeerd. De prenatale diagnose is gebaseerd op echoscopische bloedstroomsnelheidsmetingen met de Doppler-techniek. Een verhoogde Vmax (maximale bloedstroomsnelheid) in de arteria cerebri media (ACM) van boven de 1,5 MoM (Multiples of the Mean) wijst op foetale anemie. Een ACM-Vmax kleiner dan 1,0 MoM op foetale polycythemie. De ACM-Vmax is een niet-invasieve test voor het voorspellen van foetale anemie bij zwangerschappen met verschillende aandoeningen, bijvoorbeeld Rhesus immunisatie. In hoofdstuk 5 laten we zien dat ACM-Vmax metingen in TAPS ook een goede voorspeller van anemie en polycythemie zijn. We vonden een hoge sensitiviteit en specificiteit van ACM Vmax-metingen voor zowel anemie als voor polycythemie. De resultaten van deze studie laten ook zien dat met een afkapwaarde van 1,0 MoM voor polycythemie minder gevallen van ernstige polycythemie gemist worden ten opzichte van een eerder voorgestelde afkapwaarde van 0,8 MoM [3].

Postnatale diagnose

In hoofdstuk 6 beschrijven we de definitie van de postnatale diagnose TAPS. Voorgesteld wordt om als maat een "verschil in concentratie" van hemoglobine (de stof die zuurstof bindt in de rode bloedcellen) van ≥ 8 g/dL (=5 mmol/l) te gebruiken. Om postnatale TAPS, dat veroorzaakt wordt door een chronische transfusie, te onderscheiden van een acute transfusie (waarbij ook een wit en een rood kind worden geboren), moet aan één van de twee volgende criteria voldaan worden. Het eerste criterium is een verhoogd aantal reticulocyten (jonge rode bloedcellen) van de donor, met een ratio groter dan 1,7 vergeleken met het aantal reticulocyten van de recipiënt. Het tweede is de aanwezigheid van kleine (rest-) anastomosen op het placenta oppervlak. Acute TTS wordt veroorzaakt door acute transfusie door grote anastomosen in tegenstelling tot chronische transfusie door kleine anastomosen zoals in TAPS [4].

Prenatale behandeling en uitkomst

Solomon studie: preventie van post-laser TAPS

Voor de preventie van post-laser TAPS vergelijken we in hoofdstuk 7, in een internationale gerandomiseerde multicentrum studie, twee lasertechnieken voor de behandeling van TTS. Bij de standaardtechniek worden alleen alle zichtbare anastomosen gecoaguleerd. Bij de Solomon-techniek wordt, na identificatie en coagulatie van de individuele anastomosen, een lijn getrokken met de laser van de ene placentarand naar de andere, waarbij eventuele (nog) niet zichtbare kleine anastomosen ook worden gecoaguleerd. In deze gerandomiseerde studie tonen we een significante reductie van de primaire uitkomst. De primaire uitkomst is een samengestelde uitkomst van TAPS, recidief TTS, perinatale sterfte of ernstige neonatale morbiditeit. De Solomon-techniek laat een reductie van de primaire uitkomst van 49% naar 34% zien. Ook vinden we een significante reductie van post-laser TAPS van 16% in de standaardgroep naar 3% met gebruik van de Solomon-techniek. Recidief TTS daalt van 7% in de standaardgroep, naar 1% in de Solomon groep. Met de introductie van de Solomon-techniek wordt een belangrijke verbetering voor de preventie van post-laser TAPS en recidief TTS gezien. De Solomon-techniek lijkt geen nadelen te hebben. Daarom wordt geadviseerd, en dit wordt inmiddels internationaal aanvaard, om de Solomon-lasertechniek te gebruiken voor de behandeling van TTS.

Secundaire analyse: rest-anastomosen in de Solomon studie

In hoofdstuk 8 wordt de secundaire analyse van de Solomon studie beschreven. Alle placenta's zijn opgespoten met kleurverf voor analyse van eventuele rest-anastomosen.

Na gebruik van de standaardtechniek wordt in 34% van de placenta's rest-anastomosen gezien, dit is vergelijkbaar met de eerder gepubliceerde incidentie van rest-anastomosen van 32% (hoofdstuk 2). Met de Solomon techniek wordt een reductie van bijna 50% naar 19% aangetoond. In een analyse van de subgroep waarbij de chirurg direct na de ingreep verklaart dat de procedure compleet is geweest, wordt een reductie tot 12% gezien. De aanwezigheid van rest-anastomosen wordt geassocieerd met een risico op TAPS of recidief TTS van 58%. De belangrijkste reden voor rest-anastomosen in de Solomon groep is dat de laser-lijn langs de vasculaire equator niet volledig was. Kortom, de Solomon-techniek vermindert de incidentie van de rest-anastomosen en de daarbij behorende complicaties zoals TAPS en recidief TTS. Omdat de kans op rest-anastomosen en eventuele complicaties aanwezig blijft, is het van cruciaal belang dat minimaal tweewekelijkse echocontrole met Doppler metingen plaatsvindt.

Behandeling van TAPS

De beste behandeling voor TAPS is nog niet bekend. Behandelopties zijn onder andere foetoscopische laserbehandeling, intra-uteriene bloedtransfusie (IUT) aan de donor, met of zonder combinatie van partiële wisseltransfusie (PWT) aan de recipiënt, een afwachtend beleid of selectieve reductie (dichtmaken van een navelstreng, waarbij die foetus overlijdt, met als doel de kansen voor de ander te vergroten). De reden voor intra-uteriene behandeling van TAPS is het behandelen of voorkomen van sterfte of blijvende schade door ernstige foetale anemie of polycythemie.

Een simulatiemodel voor het ontstaan van post-laser TAPS wordt beschreven in hoofdstuk 9. Het wiskundig computermodel simuleert het ontstaan van post-laser TAPS door een kleine rest-anastomose (na laserbehandeling voor TTS). In het model laten we het effect zien van het toevoegen van een partiële wisseltransfusie aan de recipiënt, naast een bloedtransfusie aan de donor. Door toevoeging van een PWT aan een IUT wordt de ernst van polycythemie in de recipiënt verminderd. Ernstige polycythemie kan lijden tot complicaties zoals ledemaat-necrose of ernstige hersenschade. Met de behandeling van IUT met of zonder PWT wordt niet het onderliggende probleem (de kleine (rest-) anastomosen) behandeld, maar worden de symptomen bestreden. Laser coagulatie van de vasculaire anastomosen is de enige behandeling die de oorzaak (rest- anastomosen) van TAPS wegneemt.

Laserbehandeling voor TAPS wordt vergeleken met intra-uteriene transfusie en een afwachtend beleid in hoofdstuk 10. In vergelijking met intra-uteriene transfusie of een afwachtend beleid, kan met laserbehandeling voor TAPS het interval tussen behandeling

en geboorte verlengd worden. In eerder onderzoek wordt aangetoond dat een lage zwangerschapsduur bij de geboorte onafhankelijk geassocieerd is met een verhoogd risico op ernstige neonatale morbiditeit [5, 6], ernstige cerebrale schade [7] en een gestoorde neurologische ontwikkeling [8]. Om de beste behandeling voor TAPS te bepalen en de mogelijke voordelen van laserbehandeling te evalueren, zijn prospectieve (internationale) studies nodig.

Postnatale behandeling en uitkomst

In hoofdstuk 11 evalueren we verschillende biochemische variabelen in TAPS en wordt aangetoond dat TAPS-donoren significant lagere waarden van albumine en totaal eiwit hebben bij geboorte ten opzichte van recipiënten. Lagere albumine en totaal eiwitwaarden in donoren vergeleken met recipiënten worden ook gezien in TTS [9-11]. Deze bevindingen suggereren dat door de anastomosen in TAPS niet alleen transfusie van rode bloedcellen/hemoglobine plaatsvindt maar ook van albumine en andere eiwitten. Een andere verklaring is dat de productie van albumine in donoren is verminderd. Tevens wordt in dit onderzoek gevonden dat TAPS-donoren een lager geboortegewicht hebben met een groter placentadeel vergeleken met TAPS-recipiënten. In een ongecompliceerde monochoriale tweeling heeft het kleine kind juist een significant kleiner placentadeel. Lagere waarden van albumine en totaal eiwit kunnen een rol spelen bij de verminderde groei van de donoren, ondanks het grotere placentadeel. Deze interessante hypothese verdient nader onderzoek.

In hoofdstuk 12 beschrijven we een casus van spontane TAPS , waarbij ernstige hersenschade met neonatale sterfte ontstaat als gevolg van een polycythemie-hyperviscositeitsyndroom in de recipiënt. Deze casus toont aan dat complicaties van de TAPS zeer ernstig kunnen zijn en zich niet alleen beperken tot hematologische complicaties.

De eerste studie naar neurologische langetermijnontwikkeling in post-laser TAPS kinderen wordt beschreven in hoofdstuk 13. Ernstige neurologische ontwikkelingsachterstand wordt gedetecteerd in 9%, daarbij wordt geen verschil gezien tussen donoren en recipiënten. Het voorkomen van neurologische ontwikkelingsachterstand bij post-laser TAPS kinderen (op de leeftijd van 2 jaar) lijkt vergelijkbaar met het voorkomen bij post-laser TTS kinderen (variërend 6% tot 18%) [13-15]. In een univariate risico-analyse van cognitieve scores vinden we dat lage zwangerschapsduur bij geboorte en laag geboortegewicht belangrijke risicofactoren zijn voor neurologische ontwikkelingsachterstand. Van lage zwangerschapsduur bij de geboorte en een laag geboortegewicht is bekend dat ze onafhankelijk van elkaar zijn geassocieerd met een verhoogd risico op ernstige cerebrale (hersen) schade [7] en neurologische ontwikkelingsachterstand [8]. In een subgroepanalyse waarbij TAPS

voor de geboorte ontdekt en behandeld is, wordt in de gevallen die behandeld zijn met IUT, een lagere gemiddelde cognitieve ontwikkeling gezien in vergelijking met de andere subgroepen (laserbehandeling, afwachtend beleid en selectieve reductie). Een mogelijke verklaring voor de lage cognitieve ontwikkeling kan zijn dat deze kinderen bij een lagere zwangerschapsduur worden geboren, doordat de behandelaar beslist om de kinderen geboren te laten worden vanwege de ernst van de anemie en polycythemie. Studies met grote aantallen zijn nodig om risicofactoren voor cognitieve en lange termijn neurologische ontwikkelingsachterstand betrouwbaar te onderzoeken.

Samenvattend, in dit proefschrift wordt beschreven dat TAPS een vorm van chronische foeto-foetale transfusie door zeer kleine anastomosen is. Voor de prenatale diagnose van TAPS blijken ACM Vmax-metingen een goede voorspeller van anemie en polycythemie. Als TAPS voor de geboorte ontdekt wordt, kan laserbehandeling de uitkomst van de zwangerschap verbeteren door de oorzaak te behandelen en de tijd tussen behandeling en geboorte te verlengen. Een andere belangrijke bevinding in dit proefschrift is dat de Solomon techniek voor de behandeling van TTS, post-laser TAPS reduceert. Wanneer laserbehandeling technisch niet haalbaar is en IUT de behandeloptie is, raden we het toevoegen van PWT aan om ernstige polycythemie (en eventuele complicaties) te voorkomen. Neurologische ontwikkelingsachterstand op 2 jarige leeftijd komt even vaak voor bij post-laser TAPS kinderen als bij post-laser TTS kinderen.

Dit proefschrift beschrijft de pathofysiologie, diagnostiek, behandelopties, korte- en langetermijnuitkomsten van TAPS. Behandeling van TAPS blijft een uitdagend probleem en meer onderzoek is nodig om de behandeling en de uitkomsten van TAPS te verbeteren.

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Appendices

Publications
Curriculum Vitae
Dankwoord
List of abbreviations

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Curriculum Vitae

Femke Slaghekke werd op 27 april 1981 thuis geboren in het Twentse Bentelo. Na haar lagereschooltijd in Bentelo, behaalde ze haar eindexamen Atheneum aan het Twickel College in Hengelo in 1999. In datzelfde jaar begon zij aan de studie Farmacie aan de Universiteit Utrecht. In 2000, na toelating via de decentrale selectie, ging zij geneeskunde studeren aan de Universiteit Leiden. Na het behalen van haar doctoraal heeft ze negen maanden onderzoek gedaan naar pre-eclampsie in Adelaide, Australië onder leiding van professor G. Dekker. Na het behalen van haar artsenbul begon ze als arts prenatale geneeskunde op de afdeling Verloskunde en Foetale Therapie in het Leids Universitair Medisch Centrum (LUMC). Naast haar werkzaamheden als arts-echoscopist zette zij de Solomon studie op onder leiding van professor D. Oepkes. Hiervoor ontving ze een AGIKO Stipendium beurs van ZonMw, waardoor het mogelijk was om haar opleiding tot gynaecoloog af te wisselen met fulltime onderzoek.

In 2010 en 2011 werkte zij als AIOS gynaecologie in het Haga Ziekenhuis onder leiding van perifeer opleider B.W.J. Hellebrekers. In 2012 vervolgde zij haar opleiding tot gynaecoloog in het LUMC onder leiding van academisch opleider professor J.J.M. van Lith. Eind 2012 tot en met mei 2014 heeft ze zich fulltime bezig gehouden met onderzoek en bezocht ze meerdere internationale congressen waar ze de resultaten van de Solomon studie presenteerde. Meerdere malen vielen haar presentaties in de prijzen. Vanaf juni 2014 continueerde zij haar opleiding tot gynaecoloog in het LUMC.

Begin 2004 leerde zij Pieter-Kees de Groot kennen in een Leidse horecagelegenheid. Ze trouwden in 2008 op Twentse grond waar per toeval in 2012 dochter Fleur, iets te vroeg, werd geboren.

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List of abbreviations

AA-anastomosis	Arterio-arterial anastomosis
ACM Vmax	Arteria Cerebri Media Vmax (maximale bloedstroomsnelheid)
AV-anastomosis	Arterio-venous anastomosis
BSID-II	Bayley Scales of Infant and Toddler Development - second edition
BSID-III	Bayley Scales of Infant and Toddler Development - third edition
DVP	Deepest Vertical Pocket
GA	Gestational Age
Hb	Hemoglobin
IUT	Intra Uterine Transfusion
IUFD	Intra Uterine Fetal Demise
MC	MonoChorionic
MCA-PSV	Middle Cerebral Artery - Peak Systolic Velocity
MoM	Multiples of the Mean
NND	NeoNatal Death
PET	Partial Exchange Transfusion
PPROM	Preterm Premature Rupture Of Membranes
RA	Residual Anastomosis
sIUIGR	Selective Intra Uterine Growth Restriction
SOLOMON	Selective Or Laser Of the entire equator in MONochorionic twins
TAPS	Twin Anemia Polycythemia Sequence
TOP	Termination Of Pregnancy
TOPS	Twin Oligo Polyhydramnios Sequence
TTTS	Twin-Twin Transfusion Syndrome
TTS	Tweeling-Transfusie syndroom
VA-anastomosis	Veno-arterial anastomosis
VV-anastomosis	Veno-venous anastomosis
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence - third edition