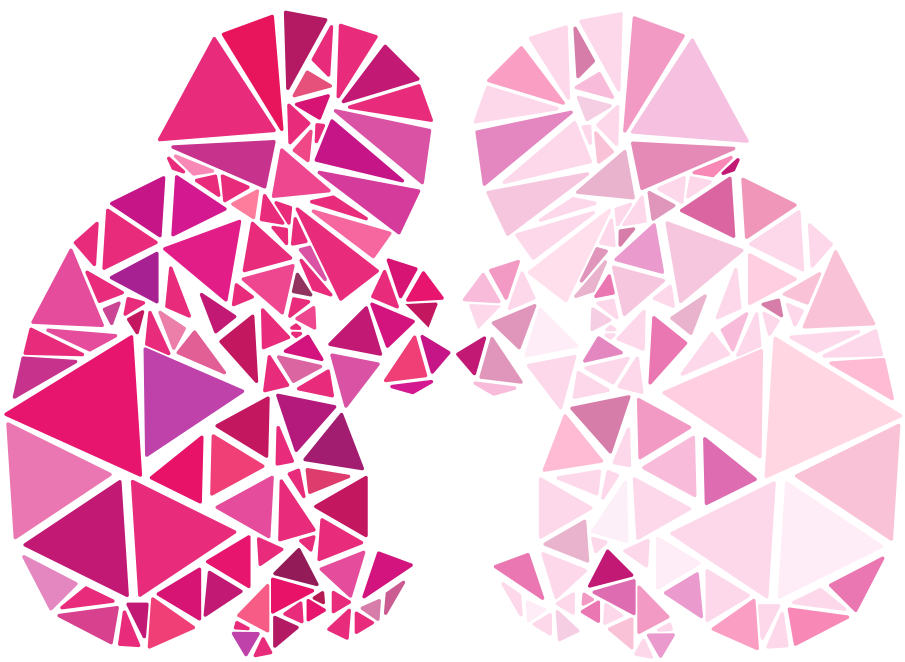
Next steps towards improved care for twin anemia polycythemia sequence

Tollenaar, L.S.A.

NEXT STEPS TOWARDS IMPROVED CARE FOR

twin anemia polycythemia sequence



Lisanne Tollenaar

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The research described in this thesis was performed at the Department of Obstetrics and the Department of Pediatrics of the Leiden University Medical Center, the Netherlands.

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Outline of thesis

PART 1

patient journey

Patient Journey

Pregnant

Dutch couple, Alexandra and Jan Marten are the proud parents of Rosalie and Sarah (fraternal twin girls) and Charlotte and they are expecting again. Their first ultrasound appointment at 8 weeks in the Leiden University Medical Center (LUMC) shows that for the second time, they are having twins! This time, the babies share a placenta (monochorionic) and blood circulation through placental vascular connections between the twins. This type of pregnancy is known to have increased risk of complications, and therefore follow-up care will be continued at the LUMC, a tertiary care center specialized in complicated twin pregnancies and fetal therapy.

Growth problems

As early as 13 weeks' gestation, doctors discover that one of the babies is substantially smaller than the other. A repeat ultrasound at 15 weeks confirms a diagnosis of selective fetal growth restriction (sFGR), with normal blood flow (Gratacos type 1). Their MFM specialist explains the source of the problem: the smaller baby probably is not growing well due to unequal placental sharing. A critically ill, or poorly growing fetus is a dangerous situation for monochorionic twins due to the shared circulation in their placenta. If one fetus dies, the co-twin may suddenly exsanguinate through the vascular anastomoses, with a high risk for subsequent demise or severe brain damage. There are basically two possible scenarios: (1) both babies will continue to grow sufficiently and be delivered between 34-36 weeks, or (2) the smaller twin will stop growing and/or will show signs of decompensation (abnormal blood flow) and intervention would need to be considered to prevent single or dual fetal demise. The news that there is a risk that Alexandra and Jan Marten will lose one or even both babies is devastating. Not too long ago, they lost their daughter Charlotte who was diagnosed prenatally with severe kidney abnormalities and died shortly after she was born.

Stressful weeks

During the following ultrasound examinations at 17, 19 and 21 weeks of gestation, the parents receive good news: although the smaller baby remains small, it shows adequate interval growth. In addition, the blood flow remains normal,

showing that the babies are doing fine. Unfortunately, at 23 weeks things seem to change for the worse, as the smaller baby seems to have almost stopped growing since the last check-up. There is also a large difference in middle cerebral artery peak systolic velocity (MCA-PSV) – blood flow in the brain that is used to diagnose fetal anemia – that is seen, with a decreased measure for the bigger baby (0.8 multiples of the median (MoM)) and an increased measure for the smaller baby (1.4 MoM), but as the latter value does not cross the 1.5 MoM line that indicates fetal anemia, this observation is not clinically relevant now. It is also possible that the increased MCA-PSV in the smaller baby could be related to its growth restriction.

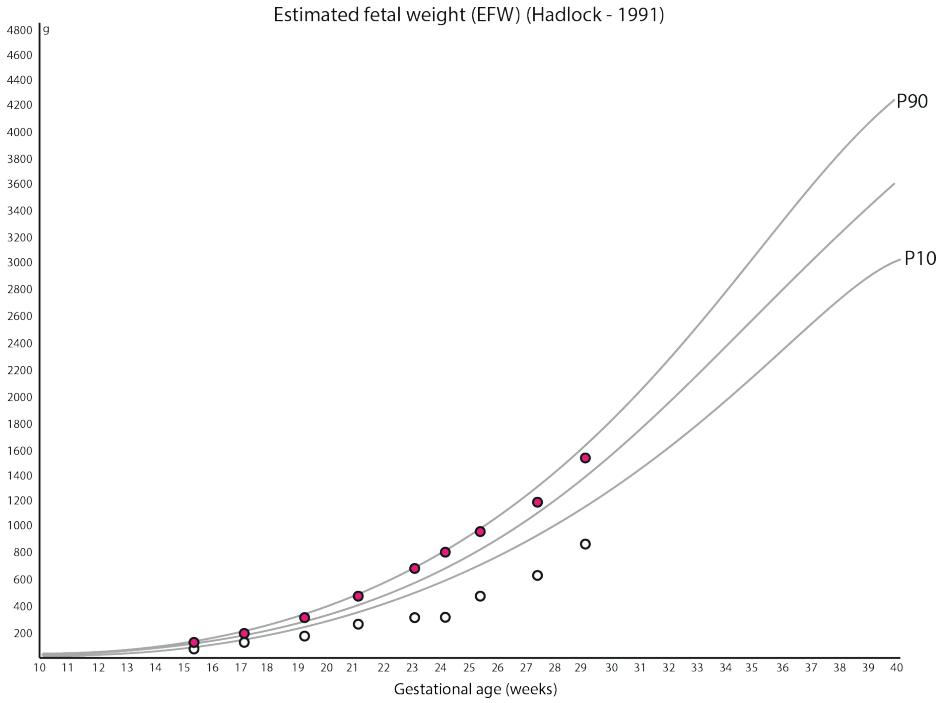


Figure 1. Estimated fetal weight for the twins (red dots: bigger baby; white dots: smaller baby)

The parents are counseled again in regard to the different treatment scenarios and "learn words they don't want to learn", such as *cord coagulation*, a procedure during which the umbilical cord of their smaller baby is clamped causing it to die, thereby precluding the damage of acute exsanguination in the co-twin. After discussing all possibilities with the parents, they elect expectant management for the interim. The parents are instructed to be conscious of decreased fetal movements and to visit our out-patient clinic again at 24 weeks.

A week later the smaller baby is still not growing well. His prognosis is unsure and the situation seems very precarious.

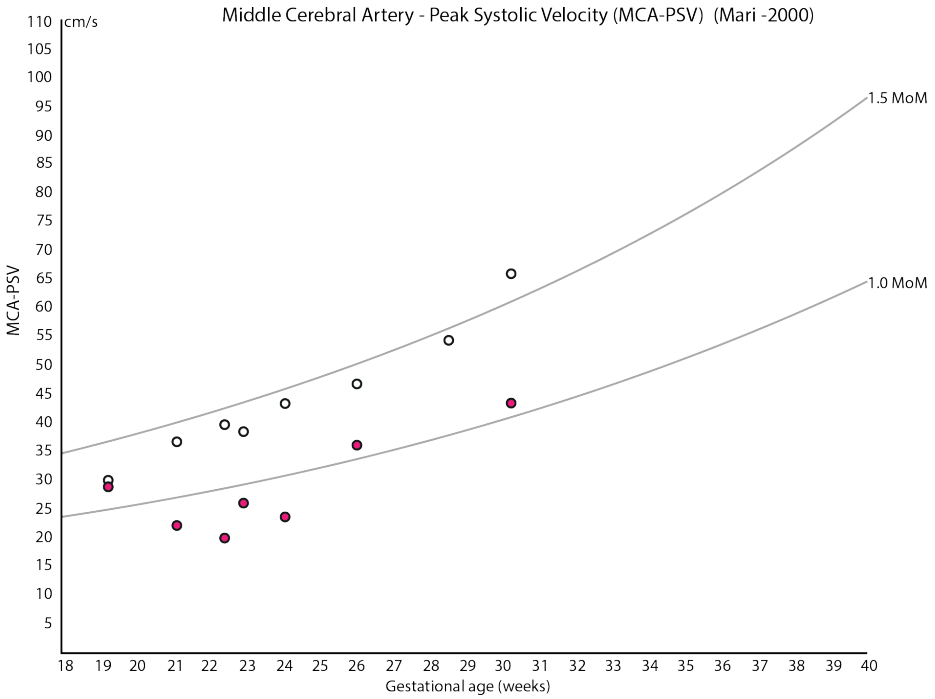


Figure 2. MCA-PSV values for both babies (red dots: bigger baby, white dots: smaller baby) .

At 25 weeks, after two very emotional and stressful weeks, there is finally some good news: the smaller baby is catching up in growth again. Ultrasound check-ups are continued weekly, and the smaller baby keeps showing satisfactory interval growth at 26, 27 and 28 weeks. The inter-twin MCA-PSV difference persists and fluctuates in wideness during the weeks. At 29 weeks, the parents receive counseling from one of the neonatalogists who explains what to expect when the boys are delivered prematurely. For now, it does not seem like the babies will be born anytime soon since the situation is stable. At 30 weeks, Alexandra and Jan Marten are again at the hospital for their routine check-up for their twins. The ultrasound reveals some unexpected bad news: the smaller baby is not doing well. It shows no fetal movements on ultrasound and the heart is enlarged. In addition, the MCA-PSV flow is increased (1.7 MoM) and now crosses the 1.5 MoM line. Furthermore, the sonographer sees that the placental share of the smaller baby is brighter on the ultrasound, and is significantly enlarged. Alexandra confirms that she indeed does not feel movements from

the smaller baby anymore and to further check the condition of the babies, a cardiotocography (CTG) registration is made. This shows that the smaller baby is in serious distress. The doctors explain to the parents that it is no longer safe for the babies to be inside the womb and that they need to be delivered as soon as possible. An emergency cesarean section is performed.

The birth of Daan and Max

A few moments later, Alexandra and Jan Marten become the parents of twin boys. Daan (bigger baby) is born first, and has a relatively good start (Apgar scores (AS): 7/10/10), and leaves for the neonatal intensive care unit (NICU) without any respiratory support. When his brother Max (small baby) is born, he is extremely pale, limp, and has a slow heartbeat (AS: 1/7/7). Resuscitation with continuous positive airway pressure (CPAP) is not sufficient to reach adequate oxygen saturation levels and intubation and mechanical ventilation is needed. During resuscitation, Alexandra is cared for in the operating room and Jan Marten is with the boys.

Figure 3. Max (left) and Daan (right) directly after birth.

New diagnosis

In the NICU, the boys are weighed and their blood is checked. As anticipated, they differ greatly in weight: Daan is 1423 grams and Max only 843 grams (weight discordancy = 44%). But there is also a striking difference in skin color: Max is very pale and Daan is red. A full blood count reveals that Max is severely anemic with a hemoglobin value of 3.0 g/dL and reticulocyte counts of 363‰ (indicating that Max was anemic for a longer time). Daan has a significantly higher hemoglobin value of 19.3 g/dL and substantially lower reticulocytes, 73‰. The large difference in hemoglobin and reticulocytes, plus their pale and red appearance indicates that something else has been going on during pregnancy, aside from the selective growth restriction.

According to routine care in the LUMC, the placenta is examined using color dye injection of the vessels. At first, a striking color difference on the maternal side of the placenta is observed with a dark red placental share for Daan and a pale placental share for Max, in analogy to their skin color at birth. Color dye injection of the placental vessels reveals the presence of only a few minuscule arterio-venous anastomoses. Inspection of the placenta yields an interesting observation: while Max is the smaller infant, his placental share is substantially larger than Daan's placental share. This contradicts the pathogenesis of sFGR, in which growth restriction is a result of a significantly smaller placental share.

Based on the large inter-twin hemoglobin difference (19.3-3.0 = 16.3 g/dL), a high reticulocyte count ratio (363/73 = 5.0) and the presence of only minuscule placental anastomoses, the medical team reaches the diagnosis of **twin anemia polycythemia sequence (TAPS),** with Max being the anemic TAPS donor and Daan the polycythemic TAPS recipient.

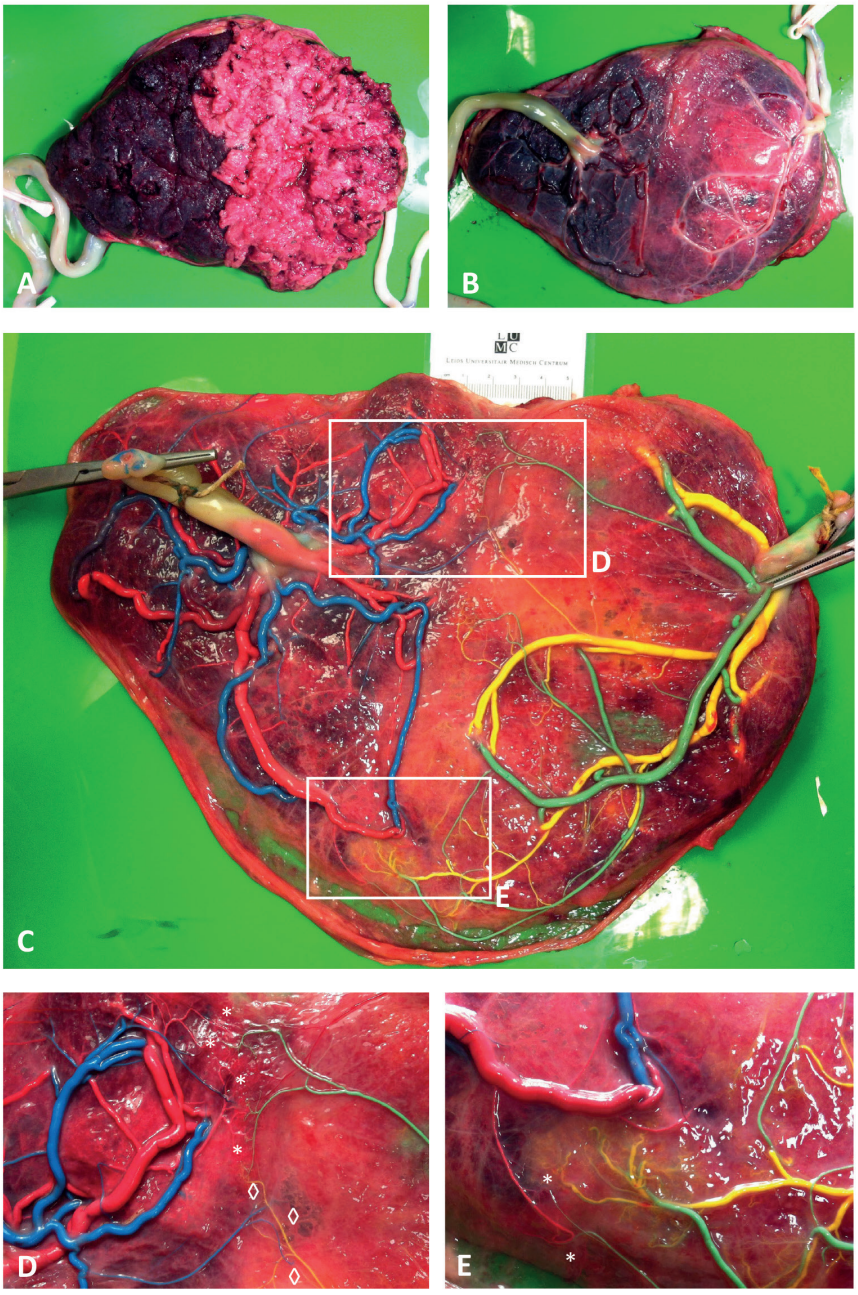


Figure 4. Pictures of the examination of the placenta of Daan and Max. **A.** Maternal side of the placenta showing a plethoric share for Daan and a pale share for Max. **B.** Fetal side of the placenta, demonstrating congested vessels for Daan and empty slender vessels for Max. **C.** Placenta injected with color dye. **D.** Close-ups of the minuscule anastomoses between Daan and Max. Stars (*) indicate VA anastomoses (Daan → Max), diamonds indicate AV anastomoses (Daan ← Max).

The first days

In the first two days, both Daan and Max are in critical condition, leaving the medical team and the parents worried about their prognosis. Although Daan had a good start, both his lungs collapse (bilateral pneumothorax) shortly after birth, for which he needs an intensified form of ventilation (high-frequency oscillation; HFO) and treatment with thoracic drains. Max, on the other hand, is diagnosed with persistent pulmonary hypertension for which nitric oxide and also HFO ventilation is required. In addition, two blood transfusions on day 1 are performed to treat his severe anemia. After two days, the condition of the boys is still worrisome, but more stable, giving the parents time to catch their breath after a very stressful and emotional 48 hours. On day 5, the parents again receive bad news: a cranial ultrasound shows that Daan has a severe brain bleed (intraventricular hemorrhage grade 3 (left) and grade 2 (right)). This is very difficult news for the family to take, especially after they just had a little hope after the boys survived their first days. The neonatologists explain that it is not clear how Daan's brain bleed will progress, but treatment with a ventricular drain in the brain might be needed if his situation worsens. In the upcoming days, his ventricles enlarge (post-hemorrhagic ventricular dilatation), but fortunately they stabilize and no further intervention is required.

Time at the hospital

In the first month after birth, both boys take small steps forward. Alexandra and Jan Marten grow quickly used to the doctors and nurses, other parents, and to all of the different and continuous NICU alarm bells.

After a week, Daan and Max switch from mechanical ventilation to less invasive respiratory support, CPAP. But both boys still have many hurdles to take. Daan battles a staphylococcus aureus sepsis, and Max develops neonatal chronic lung disease (bronchopulmonary dysplasia) due to long-term exposure to mechanical ventilation and oxygen. After 4 intensive weeks on the NICU, Daan (with no respiratory support) and Max (on CPAP) are in sufficient enough shape to be transferred to a secondary care center. The new hospital with different people with different mentality, and with no familiarity with the sensitive history of their boys, feels like a cold shower compared to the warm, nurturing care they had received before. Luckily, after 47 days of hospital admission, and even before his term date, Daan is ready to go home.

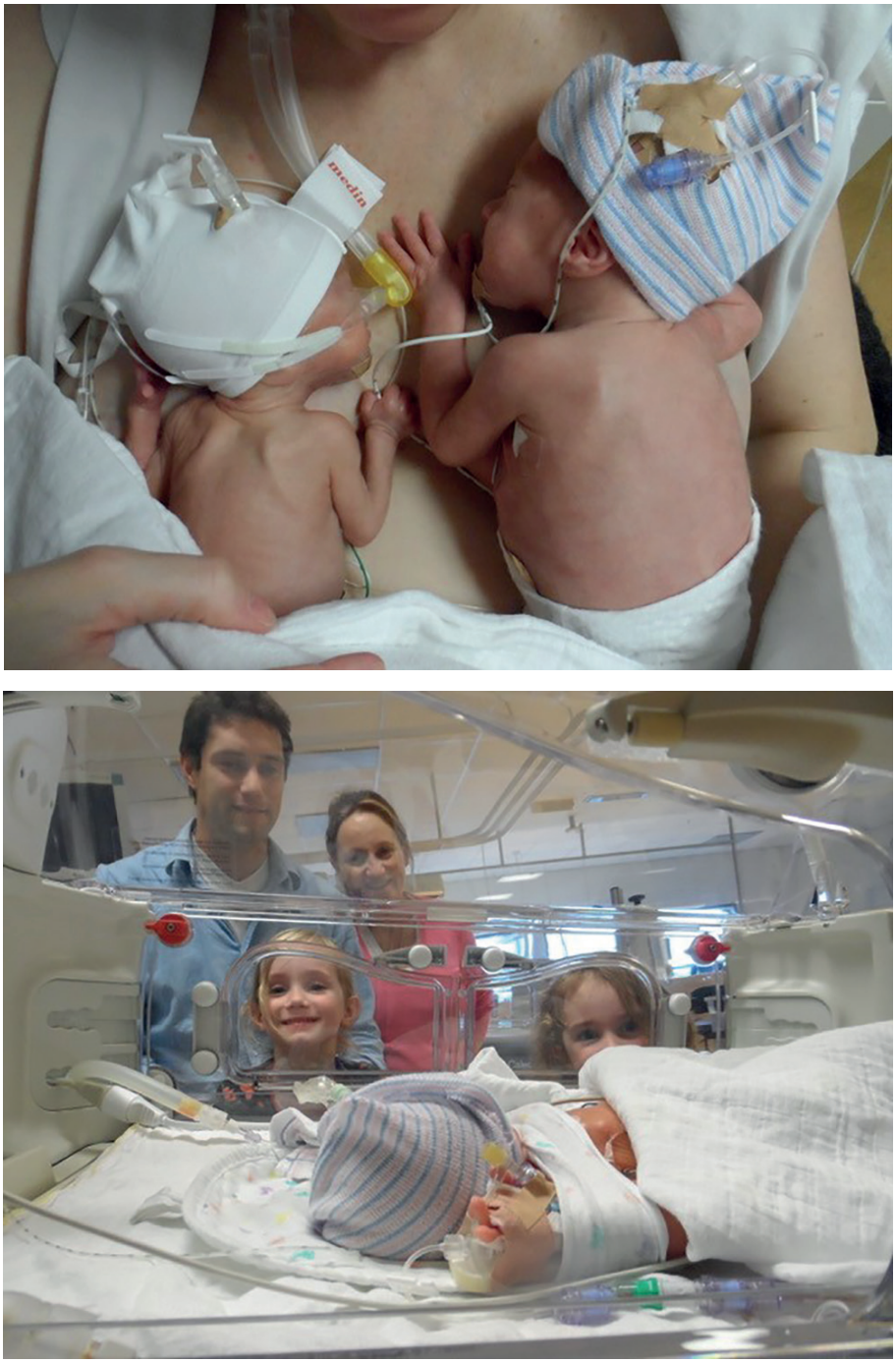


Figure 5. Pictures of Max and Daan at the NICU. Upper picture: Max (left) and Daan (right) kangarooing with Alexandra. Lower picture: The family looking at Daan through the incubator walls.

Due to his chronic lung disease, Max is unfortunately not there yet. Having one baby home is wonderful for the parents, but it is also a logistical and practical challenge to divide their care between an ex-premature baby and two girls at home and their brother in a hospital half an hour away. Luckily, just before Christmas, Max is transferred to yet another hospital, closer by. Finally, after 95 days in the hospital, Max is ready to follow his brother home, and the family is reunited at last.

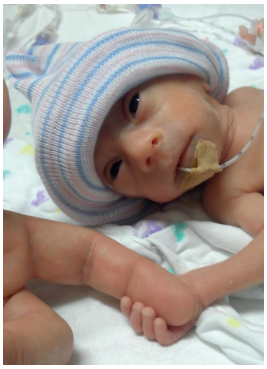


Figure 6. Max at the NICU

First years of life

During the first years of their lives, the twins are doing relatively well. However, both boys appear to be very prone to pulmonary infections resulting in multiple prolonged hospital admissions during their first year. At 1.5 years Max's motor development seems to be lagging behind his brother. Max is still not able to sit up straight, and Daan has already takes his first steps. Max gets physical therapy, helping him sit and eventually walk. Although he needs some help to achieve his developmental milestones, Max appears to be quite a strong boy and has no fear in exploring the world around him. When the boys are 2 years of age, the family moves from the Netherlands to Switzerland due to work-related motives. This is a big change: they need to adapt to a different environment, a new system and a new language.

Around this period, Alexandra and Jan Marten also start noticing that Max is significantly behind his brother Daan in regard to speech and language development. While Daan is already verbally strong and speaks in short sentences, Max is unable to speak at all. A Dutch pediatrician tells them the speech delay is not be anything to be worried about now, and could also be related to the new language Max has to adapt to. Therefore, no additional diagnostics or intervention are required at this moment. The parents try to understand the cause of the remarkable difference between the boys' language development and suggest that a hearing problem might be the cause of his speech delay, but this explanation is disregarded. They should reevaluate the situation when Max is 3 years old.

A year later, Max is still not able to speak and an extensive list of doctors appointments follow. The first ear-nose-throat (ENT) doctor that evaluates his hearing says that his left ear is totally fine, and that there is some slight hearing loss in his right ear, but that it's more a problem of "not wanting to talk" than "not being able to". As this observation does not coincide with Jan Marten and Alexandra's experience with Max, they visit another ENT doctor in the academic hospital in Zurich. Remarkably, this doctor concludes the exact opposite: his right ear is totally fine, but there is a slight hearing loss in his left ear. A very confusing and frustrating message for the family.

Max is now almost four years old, still not able to speak, the cause is unknown and therefore Max is not receiving the care that he should receive. To clear up the uncertainty about the hearing issues Max has, another examination is planned. An 'Auditory Brainstem Response' test is performed under general anesthesia, finally revealing the cause of his problem: Max has profound hearing loss in both ears, caused by auditory neuropathy spectrum disorder. At 4 years of age, Max finally receives two hearing aids and learns to speak within 3 months.

Figure 7. Max, just after he received his hearing aids. As a surprise, the ENT doctors also gave him a stuffed dog with hearing aids.

Before school

Between 4 and 5 years of age Max's vocabulary expands greatly. While forming words was first a big issue, he's now talking his parents' ears off. Some sounds still remain a challenge, but Alexandra and Jan Marten are helping him out. Although Max may be lagging behind his brother in development, he completes his milestones at his own pace, including riding a bicycle, swimming and even skiing in the Swiss mountains. The chronic lung disease that he developed when he was a premature baby still hampers him sometimes, and he is easily exhausted when doing activities. But when the family includes more breaks in their schedule, Max is able to enjoy it just as much as everyone else. In Daan's first years, he quickly shows to be an eager learner and already demonstrated a great vocabulary at age four. He craves for more educational challenges, but

has to wait until he is 5 years old to be allowed to go to school in Switzerland. Therefore, his parents start teaching him to read and write at home.

Going to school

At age 5, Daan and Max are finally allowed to go to school. Already very early on, one of the teachers tells the parents that this school is not the right place for Max and that he would be better off with special education. This is sad news for the parents. Not only do they have to separate their twin boys, this would also mean that they have to drop their 5-year old boy off to a taxi for impaired children that will take him to a school half an hour away, in a foreign country.

Figure 8. Daan (right) and Max (left) on ski holiday

Figure 9. Max (right) and Daan (left) going to school

Fortunately, already after a few weeks at his new school, the transfer appears to be the right choice. Max is thriving at the school for children with impaired hearing, and the attention and care that he receives feels like a warm embrace to the family. At school, the teachers highly stimulate Max's self-confidence. This, and the fact that he now is the best in his class, greatly boosts his self-image. It fills Alexandra and Jan Marten with joy and pride to hear from the school that Max, although he might not always able to express it, has great potential.

Back in the Netherlands

When the boys are 6 years old, the family moves back to the Netherlands. As Daan is already able to write and read, he is allowed to skip first grade. Due to the pleasant experience with special education in Switzerland, Alexandra and Jan Marten again choose for special education in The Netherlands and find a nice school in their neighborhood. There, Max follows the regular curriculum, but with more attention to his impairment and in smaller classes.

Now

Alexandra and Jan Marten are looking back on an emotional time with Daan and Max, but feel happy and grateful to get to see both boys coming from vulnerable premature babies to strong and happy individuals. Together with the ENT doctors, parents are still looking for ways to further improve Max's hearing. However, seeing the progress Max has made in the last few years, Alexandra and Jan Marten feel more confident every day that both boys will be fine.

Figure 10. The family, back in the Netherlands. Left to right: Rosalie, Alexandra, Max (holding his hamster Fransje), Sarah, Jan Marten and Daan.

Researcher's perspective

Daan and Max represent a case of severe TAPS, which was not identified antenatally and was first diagnosed at birth. In order improve our care for TAPS twins, some critical remarks and questions need to be made with regard to the following aspects:

Antenatal diagnosis

Despite the severity of TAPS postnatally, Daan and Max did not meet our proposed antenatal criteria for TAPS. They did however show a large difference in MCA-PSV measurements and presented with additional ultrasound markers including cardiomegaly and placental echogenicity. This raises the following questions: are the currently cut-off levels of < 1.0 MoM (for the recipient) and > 1.5 MoM (for the donor) accurate enough to diagnose TAPS during pregnancy, or should we use an inter-twin MCA-PSV difference? What is known about the prevalence of cardiomegaly and a difference in placental echogenicity in TAPS? Are these findings unique for this case, or are they more ubiquitous in the TAPS population?

Antenatal therapy

Being born at 30^{+1} weeks of gestation has placed Daan and Max at risk for severe prematurity-related complications, including pulmonary problems and brain damage. Early identification of TAPS could have allowed considering antenatal fetal intervention to treat the condition and to prolong pregnancy. If TAPS would have been detected early on, what management strategy would have been best for Daan and Max?

Growth restriction

Growth restriction in Max was not based on unequal placental sharing as seen in selective fetal growth restriction, on the contrary; Max' placental share was paradoxically larger than the placental share of his brother. Therefore, the restricted growth in Max was likely a result of chronic severe anemia. What is the prevalence of severe growth restriction in TAPS? Is Max an exceptional case, or is severe growth restriction more frequent in TAPS?

Postnatal diagnosis

In line with the sonographic observation antenatally, the maternal side of the TAPS placenta showed a striking color difference. Is this feature related to the hemoglobin difference in TAPS twins? Could looking at the maternal side of the placenta be helpful in reaching the diagnosis of TAPS shortly after birth (even before reticulocytes are available and placental injection is performed)? Could it help to differentiate between other causes of large inter-twin hemoglobin discordances such as acute peripartum twin-twin transfusion syndrome?

Long-term outcome

Whereas both boys suffered from severe neonatal problems, they demonstrate a remarkable difference in long-term outcome. Especially Max' development is extensively hampered by bilateral hearing loss. Currently, no information is available on long-term outcome in TAPS survivors. In order to optimize our care and counseling for parents expecting TAPS twins, we need to know: what is the long-term neurodevelopmental and behavioral outcome in TAPS survivors? Are there differences in long-term outcome between TAPS donors and recipients? Does Max represent a unique case of deafness in TAPS, or are hearing problems more prevalent in this population?

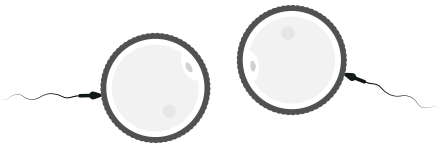
At the end of this thesis, we will try to answer these questions based on the studies we performed in the last three years, and will discuss if and how our new insights could have improved the care for future TAPS twins like Daan and Max.

PART 2

general introduction

dizygotic twins

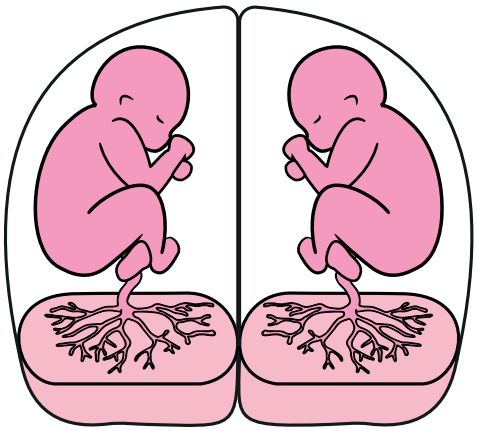
or fraternal twins



all dizogotic twins

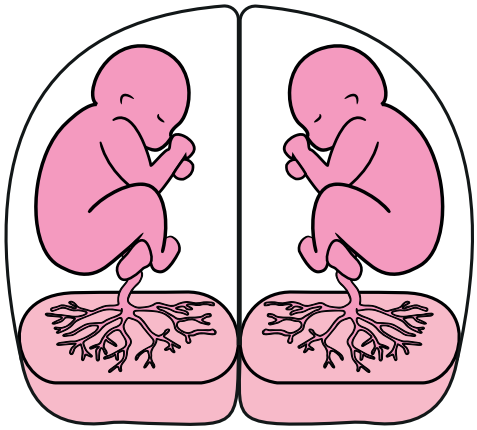
1-3 days

1/3 of monozygotic twins



DICHORIONIC DIAMNIOTIC

2 placentas
2 amniotic sacs



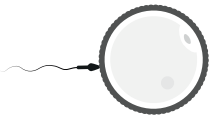
DICHORIONIC DIAMNIOTIC

2 placentas
2 amniotic sacs

Figure 1. Schematic overview of types of twin gestations

monozygotic twins

or identical twins



timing of egg division

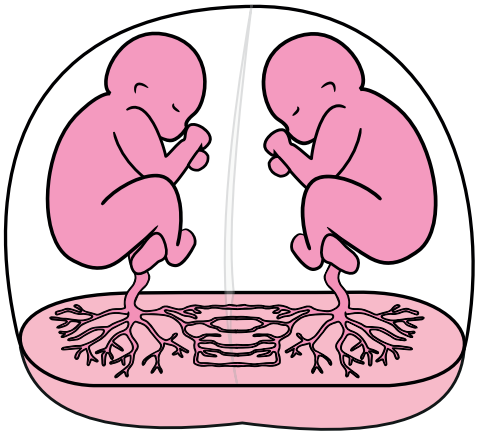
days after fertilization

4-8 days

2/3 of monozygotic twins

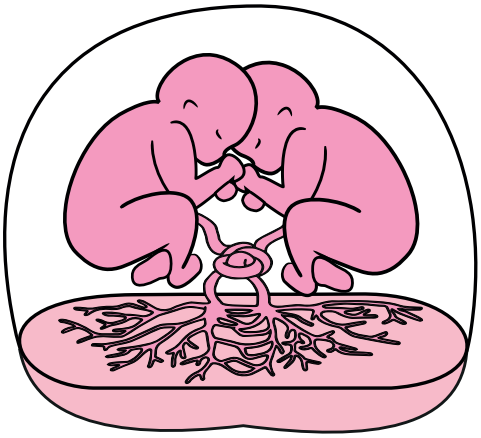
9-12 days

< 1% of monozygotic twins



MONOCHORIONIC DIAMNIOTIC

1 placenta
2 amniotic sacs



MONOCHORIONIC MONOAMNIOTIC

1 placenta
1 amniotic sac

General Introduction

Twins

In the Netherlands, twin pregnancies occur in approximately 2% of all pregnancies annually.[1] In total, 2/3 of these twins is dizygotic (fraternal) and 1/3 is monozygotic (identical). Dizygotic twin fetuses always have their own placenta and amniotic sac, and are therefore dichorionic diamniotic (Figure 1). In monozygotic twins, the number of placentas and amniotic sacs depends on the moment of egg division. If this occurs within 3 days after fertilization, the monozygotic twins will be dichorionic diamniotic as well. When splitting into two embryos takes place after 3 days (which occurs in 2/3 of monozygotic twins), both fetuses will be sharing one placenta while still having separate amniotic sacs, thus being a monochorionic diamniotic twin pregnancy. From 8 days after fertilization, twin pregnancies will be monochorionic monoamniotic, with two fetuses sharing one placenta and one amniotic sac.

Monochorionicity in twins is associated with increased risk for adverse outcome, due to the problems that arise from the feto-fetal blood transfusion through vascular connections (anastomoses) on the shared placenta.[2] Placental anastomoses can either be unidirectional (arterio-venous (AV) or veno-arterial (VA)), or bidirectional (arterio-arterial (AA) or veno-venous (VV)). Under normal conditions, the feto-fetal blood transfusion through these placental anastomoses is balanced and equal. However, in approximately 15% of the monochorionic twins the configuration of placental anastomoses result in an unbalanced blood flow between the twins. Depending on the size of the anastomoses, this can result in two different types of feto-fetal transfusion disorders: twin-twin transfusion syndrome (TTTS) or twin anemia polycythemia sequence (TAPS) (Figure 2).

Twin-twin transfusion syndrome

Large anastomoses allow for the transfer of large proportions of blood volume from one fetus to the other. When unbalanced, this can lead to oliguria in the TTTS donor twin and polyuria in the TTTS recipient twin. Chronic oliguria will eventually result in oligohydramnios in the TTTS donor, whereas chronic polyuria will cause the TTTS recipient to develop a polyhydramnios. This typical finding, also called twin oligohydramnios-polyhydramnios sequence (TOPS), is essential for the diagnosis of TTTS. The first treatment of choice for

this condition is fetoscopic laser surgery of the vascular anastomoses at the placental surface.[3] This is an endoscopic procedure that is aimed at blocking all the anastomoses between the two fetuses, thereby establishing an artificial 'dichorionizition' of the placenta.

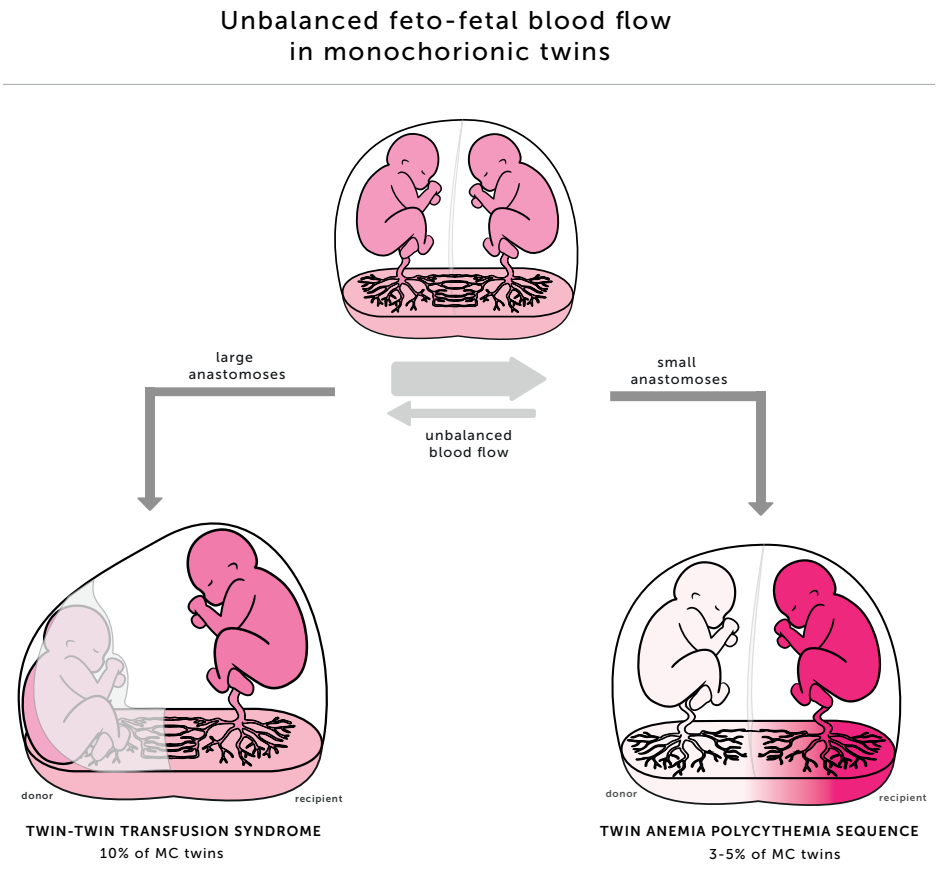


Figure 2. Schematic overview of unbalanced blood flow in monochorionic (MC) twins leading to twin-twin transfusion syndrome or twin anemia polycythemia sequence, depending on the size of the anastomoses.

Twin anemia polycythemia sequence

Whereas the diagnosis of TTTS has been widely established, TAPS is a relatively recently described form of chronically unbalanced feto-fetal transfusion. TAPS arises from a slow blood transfusion through only a few minuscule placental anastomoses leading to chronic anemia in the TAPS donor and chronic polycythemia in the TAPS recipient. At birth, the anemic TAPS donor is pale and the polycythemic TAPS recipient is red (Figure 3). In contrast to TTTS,

TAPS develops in the absence of amniotic fluid discordances.[4] TAPS can occur spontaneously in 3-5% of the monochorionic twin pregnancies (spontaneous TAPS)[5], or can develop iatrogenically In 2-16% of TTTS cases treated with laser surgery (post-laser TAPS).[6, 7] The first cases of post-laser TAPS were described by Robyr et al., although at that time the cause of the disease was still unknown and no clear terminology was used.[7] Shortly thereafter, in 2006, Lopriore et al. reported three cases of spontaneous TAPS, introduced the currently worldwide accepted term 'twin anemia polycythemia sequence' and its acronym TAPS, and proposed the pathophysiologic explanation for the onset of the disease.[4] Since then, knowledge on various aspects of TAPS has expanded greatly. The pathophysiology of TAPS is now largely unveiled and both antenatal and postnatal diagnostic criteria for TAPS have been proposed.[8] Moreover, small studies into outcome of TAPS have shown that both TAPS donors and recipient are at risk for severe neonatal morbidity and neonatal mortality.[9]

Although several studies during the last decade have contributed to the insights of TAPS, some major questions remain unanswered. This thesis focuses on multiple aspects of TAPS, trying to address unsolved issues regarding pathophysiology, diagnostic criteria and treatment. Our aim is to place new pieces of knowledge into the large puzzle of this rare, but intriguing monochorionic twin condition and ultimately improve the management and outcome in this high-risk and vulnerable group of patients.

Pathophysiology

Although TTTS and TAPS have been described as two mutually exclusive entities, recent reports show that these two feto-fetal transfusion disorders can co-exist.[10, 11] We formulated the following questions: what is the prevalence of anemia-polycythemia in TTTS twins before laser surgery? What can we say about the angioarchitecture of the placenta in this subgroup of TTTS twins? Does having TTTS and TAPS at the same time lead to a more detrimental outcome?

Figure 3. Pale anemic TAPS donor on the left-hand side of the picture, plethoric polycythemic TAPS recipient on the right-hand side of the picture.

Antenatal diagnosis

TAPS can be detected antenatally through evaluation of the middle cerebral artery peak systolic velocity (MCA-PSV). The diagnosis of TAPS is reached by an MCA-PSV > 1.5 Multiples of the Median (MoM) in the TAPS donor, suggestive of fetal anemia, and MCA-PSV < 1.0 MoM in the recipient, suggestive of fetal polycythemia.[12] To determine the severity of TAPS, an antenatal classification system was proposed by Slaghekke et al in 2014 (Table 1). However, in the years following the publication, we doubted the diagnostic accuracy of the proposed MCA-PSV cut-off values, since we documented several monochorionic twins with evident postnatal TAPS that did not meet the antenatal criteria for TAPS. We therefore set up a study to evaluate the diagnostic accuracy of an alternative criterion, namely delta MCA-PSV > 0.5 MoM and compared its predictive value to the currently used fixed cut-off values of < 1.0 MoM and > 1.5 MoM for the antenatal diagnosis of TAPS.

Table 1. Antenatal classification system for TAPS

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

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

Although the antenatal diagnosis of TAPS is primarily based on inter-twin MCA-PSV discordance, other additional ultrasound markers suggestive of TAPS have been described in a few anecdotal reports.[13, 14] These markers include cardiomegaly in the TAPS donor, a starry-sky liver in the TAPS recipient and difference in placental echogenicity with a hyperechogenic (enlarged) placental share for the TAPS donor and a hypoechogenic (flattened) placental share for the TAPS recipient. We conducted a retrospective study to investigate the prevalence of these ultrasound markers in a large cohort of TAPS twins. An overview of signs of TAPS that can be detected on ultrasound can be found in Figure 4.

Antenatal management

Treatment options for TAPS include expectant management, preterm delivery, intrauterine transfusion (IUT) with or without a partial exchange transfusion (PET), fetoscopic laser surgery and selective feticide.[15] With expectant management, no intrauterine intervention is performed, but the twins are monitored intensively using MCA-PSV Doppler measurements. A preterm delivery is only feasible after viability is achieved and is aimed at treating TAPS on the NICU, instead of in the womb. With an IUT, the TAPS donor will be provided with erythrocytes to temporarily treat its anemia. In case of signs of severe polycythemia, an IUT in the donor can be combined with a PET in the recipient (Figure 5). During a PET, 5-10 ml of the recipient's blood will be removed slowly and will be replaced with saline, repeatedly. IUT (with PET) is not a definitive treatment and only a temporary solution and therefore reintervention might be required.

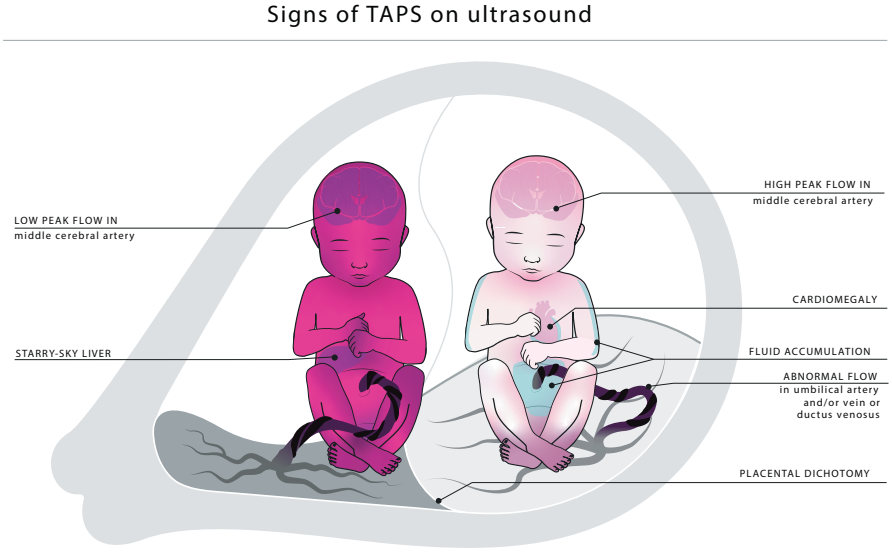
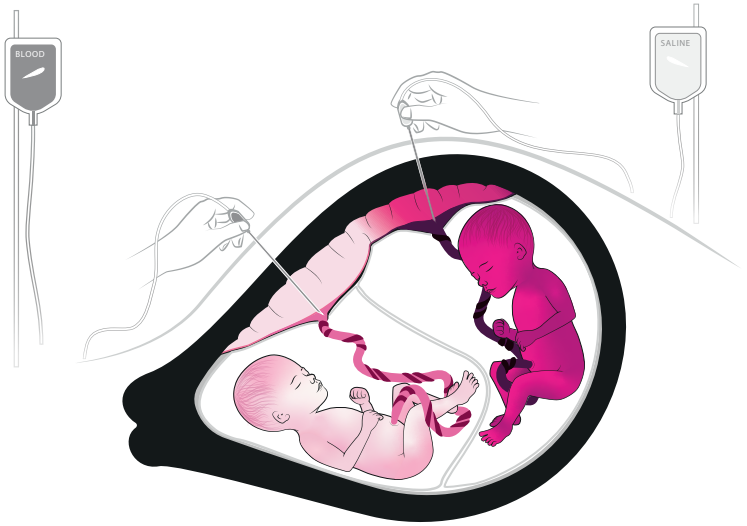


Figure 4. Schematic overview of signs of TAPS that can be detected on ultrasound. TAPS recipients show a decreased MCA-PSV and might demonstrate a starry-sky liver. TAPS donors present with an increased MCA-PSV and might have an enlarged heart (cardiomegaly), abnormal flows in the umbilical artery/vein or ductus venosus (stage 3 TAPS) and accumulation of fluid in one or more body compartments (hydrops, stage 4 TAPS).

Fetoscopic laser surgery is the only causal treatment option for TAPS, but is technically more challenging in TAPS than in TTTS, due to the absence of TOPS and size of the anastomoses, which are small and might be hard to identify on fetoscopy (Figure 6). Selective feticide can be considered in severe cases of TAPS or when other treatment options are infeasible, and is aimed at sacrificing one twin in order to increase the chances for healthy survival in the co-twin. The best antenatal treatment strategy for TAPS is not clear. Since TAPS can lead to perinatal mortality and severe neonatal morbidity[9], investigation into the optimal treatment for this condition is of great importance. Unfortunately, due to the low incidence of the condition, studies are limited to small numbers, hampering generalizability of results and demanding extreme caution when comparing the outcomes. To generate more substantiated knowledge on the effects of management strategies for TAPS twins, we set up the TAPS Registry, an international collaboration aimed at collecting data on diagnosis, management and outcome in TAPS.

Intrauterine blood transfusion with partial exchange transfusion

Figure 5



Fetoscopic laser surgery

Figure 6

Figure 5. Intrauterine blood transfusion in the TAPS donor combined with a partial exchange transfusion in the TAPS recipient.
Figure 6. Fetoscopic laser surgery for TAPS using the Solomon technique. To optimize technical conditions, the amniotic sac of the recipient has been infused with saline and the amniotic fluid in the donor's sac has been drained to artificially create twin oligo-polyhydramnios sequence.

With regard to management, we formulated the following questions: how is TAPS treated amongst different fetal therapy centers across the world? What is the outcome after different treatment strategies for TAPS?

The only way to adequately investigate the best treatment for TAPS is by conducting an international randomized controlled trial. We therefore set up The TAPS Trial, a randomized controlled trial comparing fetoscopy laser surgery with standard treatment (expectant management, preterm delivery, IUT/PET). In April 2019 the first patient was recruited. Aside from the Leiden University Medical Center, 5 other centers agreed to participate.

Postnatal diagnosis

The postnatal diagnosis for TAPS is based on a large inter-twin hemoglobin difference (> 8.0 g/dL).[16] However, acute peripartum TTTS, that results from an acute large feto-fetal blood transfusion during delivery, is characterized by a large inter-twin hemoglobin difference and the presentation of a pale and plethoric twin pair, as well.[17] To distinguish between the two conditions, two additional criteria have been proposed. The first is a reticulocyte count ratio > 1.7. In TAPS, the donor demonstrates a high reticulocyte count as a result of increased erythropoiesis due to chronic anemia (Figure 7). In acute peripartum TTTS, the donor twin is not able to adapt to anemia in such a short amount of time and therefore reticulocyte values are normal, resulting in a reticulocyte ratio < 1.7. The second additional criterion is the size of the placental anastomoses detected through color dye injection. In TAPS the anastomoses are small (diameter < 1mm), whereas in acute peripartum TTTS anastomoses are large (allowing for an acute feto-fetal transfusion).[16, 18]

However, reticulocyte count measures are often not performed and color dye injection is a complex and time-consuming procedure, that is often only carried out by experienced fetal therapy centers. This prompted us to search for an alternative diagnostic criterion that would be more practical and readily available for all caregivers in the perinatal field. Anecdotal reports show that, in line with the typical skin color difference of TAPS twins, the maternal surface of the placenta is also characterized by a striking color difference, with a pale placental share for the donor and a plethoric placental share for the recipient.[19] To explore the usefulness of this unique feature of the TAPS placenta, we conducted two studies. In the first study we tried to quantify the

color difference on the maternal side of the TAPS placenta and in the second study we investigated whether the color difference on the maternal side of the placenta could distinguish between TAPS and acute peripartum TTTS.

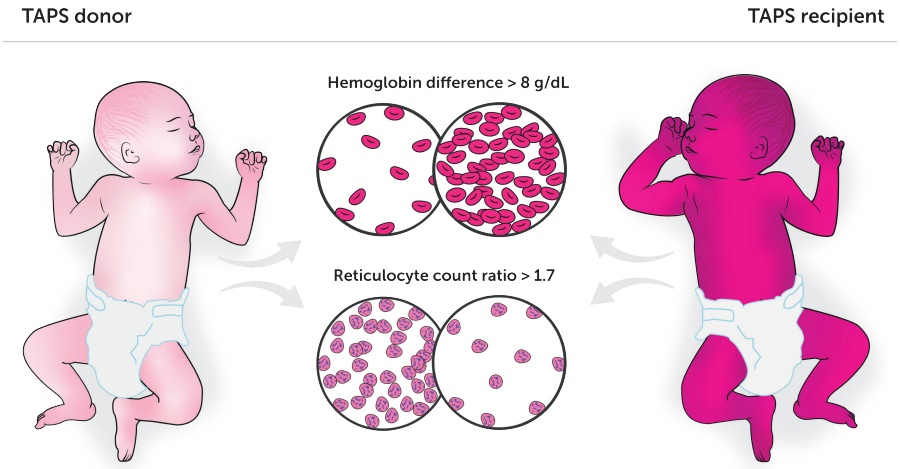


Figure 7. Hematological criteria for TAPS. The TAPS donor has a low hemoglobin value and a high reticulocyte count (‰) due to chronic anemia. The TAPS recipient shows a high hemoglobin value and a low reticulocyte count (‰) as a result of chronic polycythemia. The inter-twin hemoglobin difference should be > 8.0 g/dl and the reticulocyte count ratio (reticulocytes donor/reticulocytes recipient) is > 1.7.

Short- and long-term outcome

Short-term outcome in TAPS can vary from isolated hemoglobin difference to severe cerebral injury and neonatal death.[9] To date, there are no studies available that focus on perinatal outcome in a large cohort of spontaneous TAPS and post-laser TAPS twins. Importantly, looking at outcome in both groups separately might be of added value as the two conditions differ in gestational history (post-laser TAPS twins also suffered from TTTS). To expand our knowledge on this subject, we used the TAPS Registry data to evaluate time of onset, management and outcome in two large international cohorts of spontaneous- and post-laser TAPS twins.

With an increasing number of monochorionic twins being born alive after a complicated pregnancy, attention is shifting from short-term perinatal outcome to long-term neurodevelopmental outcome, focusing more on survival without impairment and quality of life. In TAPS, only one study evaluated the long-term outcome in a cohort of post-laser TAPS twins and showed that severe neurodevelopmental impairment (NDI) occurs in 9% of the survivors.[20] However, in spontaneous TAPS the long-term neurodevelopmental outcome is

unknown, which hampers adequate parent counselling. We therefore carried out a follow-up study in spontaneous TAPS survivors and evaluated long-term neurodevelopmental and behavioral outcome.

Outline of this thesis

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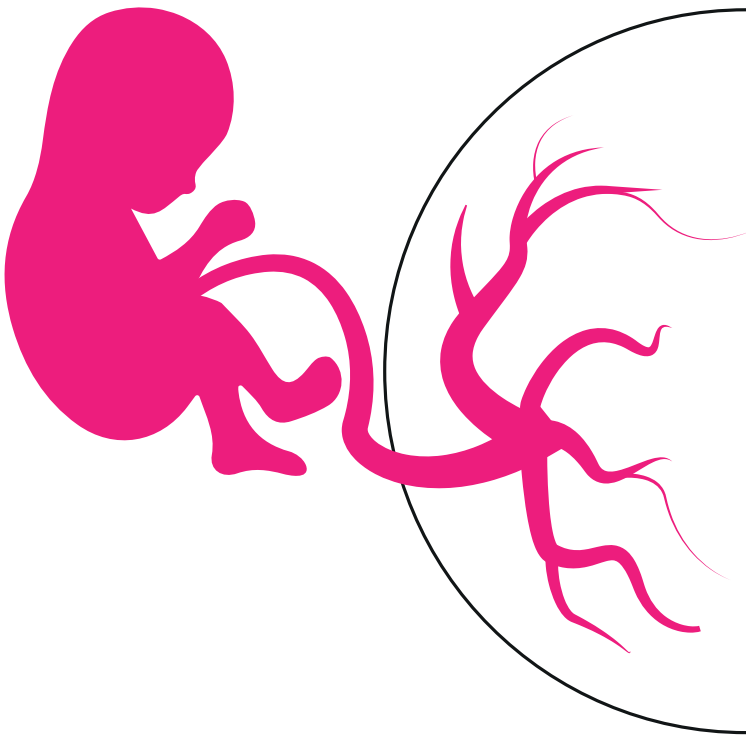
PART 3

review



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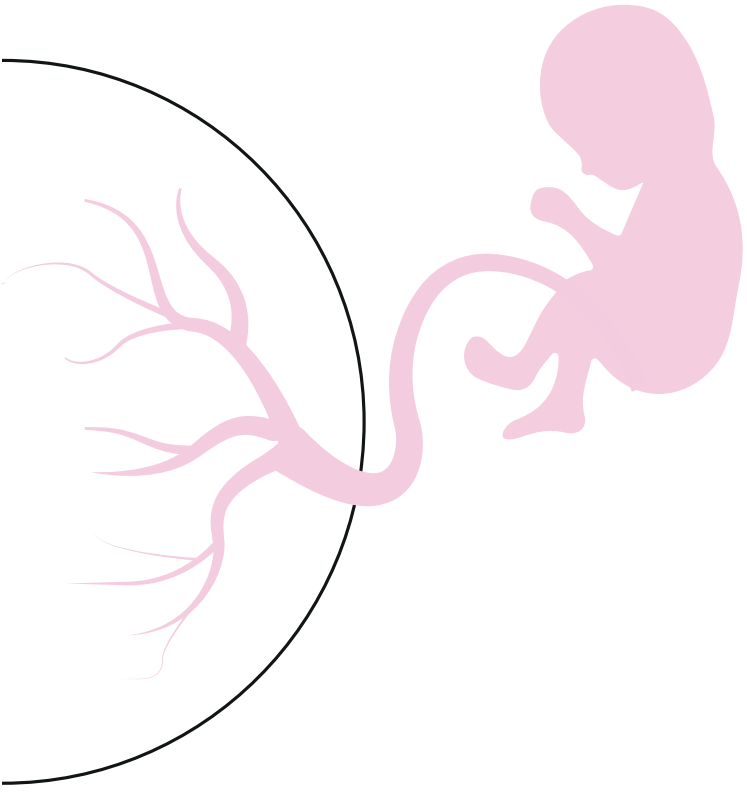
Twin Research and Human Genetics, 2016; 19(3): 222-33

Chapter 1

Twin anemia polycythemia sequence: current views on pathogenesis, diagnostic criteria, perinatal management, and outcome



Abstract

Monochorionic twins share a single placenta and are connected with each other through vascular anastomoses. Unbalanced inter-twin blood transfusion may lead to various complications, including Twin-to-Twin Transfusion Syndrome (TTTS) and Twin Anemia Polycythemia Sequence (TAPS). TAPS was first described less than a decade ago and the pathogenesis of TAPS results from slow blood transfusion from donor to recipient through a few minuscule vascular anastomoses. This gradually leads to anemia in the donor and polycythemia in the recipient, in the absence of twin oligo-polyhydramnios sequence. TAPS may occur spontaneously in 3-5% of monochorionic twins or after laser surgery for TTTS. The prevalence of post-laser TAPS varies from 2-16% of TTTS cases, depending on the rate of residual anastomoses. Prenatal diagnosis of TAPS is currently based on discordant measurements of the middle cerebral artery peak systolic velocity (MCA-PSV) (> 1.5 multiples of the median (MoM) in donors and < 1.0 in recipients). Postnatal diagnosis is based on large inter-twin hemoglobin (Hb) difference (> 8g/dL), and at least one of the following: reticulocyte count ratio > 1.7 or minuscule placental anastomoses. Management includes expectant management, intra-uterine blood transfusion with or without partial exchange transfusion or fetoscopic laser surgery. Post-laser TAPS can be prevented by using the Solomon laser surgery technique. Short-term neonatal outcome ranges from isolated inter-twin Hb differences to severe neonatal morbidity and neonatal death. Long-term neonatal outcome in post-laser TAPS is comparable with long-term outcome after treated TTTS. This review summarizes the current knowledge after 10 years of research on the pathogenesis, diagnosis, management and outcome in TAPS.

Introduction

Monochorionic twin pregnancies are at increased risk for adverse outcome compared to dichorionic twin pregnancies and singletons. This is primarily due to the fact that almost all monochorionic twins share a single placenta with inter-twin anastomoses allowing blood to flow bidirectionally between the two fetuses. Unbalanced net inter-twin blood transfusion may lead to various complications, including Twin-to-Twin Transfusion Syndrome (TTTS) and Twin Anemia Polycythemia Sequence (TAPS). TTTS was first described in the 19th century and results from imbalanced inter-twin blood flow causing hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient twin, the so-called twin oligo-polyhydramnios sequence (TOPS). TAPS is a newly described form of chronic and slow inter-twin blood transfusion characterized by large inter-twin hemoglobin (Hb) differences without signs of TOPS. The pathogenesis of TAPS is based on the presence of a few minuscule vascular anastomoses. TAPS may occur spontaneously in monochorionic twin pregnancies or may develop in TTTS cases after incomplete laser surgery due to a few small residual anastomoses. The post-laser form of TAPS was first reported in 2006 [1], whereas the spontaneous form of TAPS as well as the acronym TAPS was first described in 2007.[2] In the last decade, over a 100 studies have been published on TAPS and our knowledge and awareness has gradually increased. This review focuses on the epidemiology, pathogenesis, diagnostic criteria, management options, and short- and long-term outcome in TAPS and summarizes the data gathered since the first description of TAPS a decade ago.

Epidemiology

TAPS may occur spontaneously (spontaneous TAPS) or after laser treatment for TTTS (post-laser TAPS). Spontaneous TAPS occurs in 3-5% of the monochorionic twin pregnancies,[3-7] whereas post-laser TAPS occurs in 2-16% of TTTS cases after incomplete laser treatment.[1, 8, 9] The wide range in incidence rate in post-laser TAPS can be explained by the use of different laser surgical techniques and/or the existence of different definitions and criteria for TAPS (see sections here below).

Pathogenesis

Placental characteristics

The pathogenesis of TAPS is based on the unique angioarchitecture of the placenta, characterized by the presence of only few minuscule anastomoses. These few small anastomoses between the two placental shares allow a chronic and slow transfusion of blood from the donor to the recipient twin (Figure 1). The actual blood flow through these small anastomoses ranges from 5 to 15 ml per 24 hours.[2, 10] This process gradually leads to highly discordant Hb levels, causing the donor twin to become anemic and the recipient twin to become polycythemic. In monochorionic placentas, three types of anastomoses have been described: arterio-venous (AV), arterio-arterial (AA) and veno-venous (VV) anastomoses. AV anastomoses are unidirectional whereas AA and VV anastomoses are bidirectional. TAPS placentas are characterized by the presence of a few very small AV anastomoses, with a diameter < 1mm. TAPS placentas have on average 3 to 4 anastomoses compared to an average of 8 anastomoses in uncomplicated monochorionic placentas.[11] AA anastomoses are rare in TAPS cases and occur in 10-20% of TAPS placentas. AA anastomoses are considered to protect against the development of TTTS or TAPS because of the bidirectional blood flow allowing inter-twin equilibration of blood volumes.[10, 12-14] The size of AA anastomoses in TAPS appear to be significantly smaller (diameter < 1mm) compared to AA anastomoses in TTTS cases or in uncomplicated monochorionic twins.[11] Spontaneous TAPS placentas also differ from post-laser TAPS placentas. Spontaneous TAPS placentas have a higher total number of anastomoses compared to post-laser TAPS placentas, 4 versus 2, respectively. Miniscule AA anastomoses were detected sporadically in both groups, but the rate of AA anastomoses was slightly higher in the spontaneous group.[15]

TAPS versus TTTS

The absence of oligohydramnios and polyhydramnios is an essential element in the diagnosis of TAPS. Presence of TOPS is only pathognomonic for TTTS. Importantly, 2-8% of TTTS cases may have preoperative signs of severe fetal anemia in the donor and polycythemia in the recipient.[16, 17] The mechanism preventing the donor twin to develop oligohydramnios and the recipient twin to develop polyhydramnios in TAPS cases is not entirely clear.

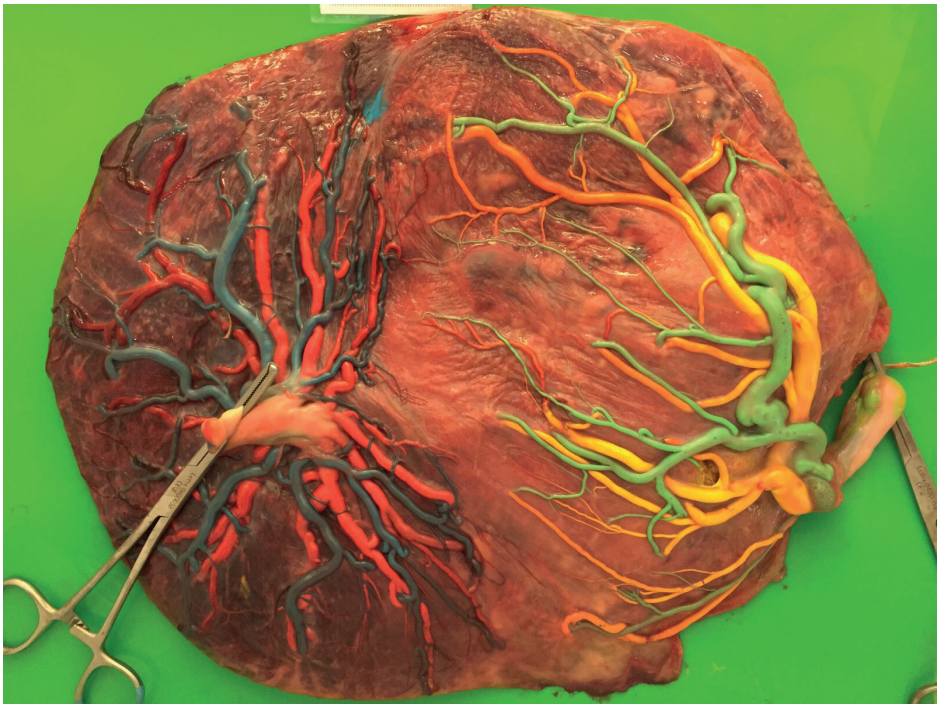


Figure 1. A spontaneous TAPS placenta injected with color dye. Blue and green dye was used for the arteries and yellow and pink for the veins, showing the presence of only a few very small anastomoses

The most probable explanation is that due to the chronic character of TAPS, slow inter-twin blood transfusion allows more time for the hemodynamic compensatory mechanisms to take place.[18] While TTTS results from large unbalanced inter-twin blood transfusion in combination with unbalanced hormonal regulation with rapid deterioration, TAPS probably arises from slow inter-twin blood transfusion without hormonal imbalance and a slow deterioration.[19] In TTTS, donor twins show high renin levels, presumably as a result of renal hypoperfusion,[20] and recipients have absent renin protein, presumably because of down-regulation due to hypervolemia. This hormonal discordance has not been described in TAPS pregnancies. Interestingly, in post-laser TAPS, it is usually the former recipient who becomes anemic, whereas the former donor becomes polycythemic. [1, 21-23] A possible explanation could be that 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 may delay the development of oligohydramnios in the ex-recipient who becomes the TAPS donor.[19]

TAPS versus uncomplicated monochorionic twins

In addition to the small anastomoses which characterize TAPS placentas, TAPS placentas also show a remarkable difference in placental shares. In uncomplicated monochorionic twins, the placental share is the principal element affecting fetal growth and final birth weight, i.e. the smaller twin usually has a relatively smaller placental share.[24] In TAPS twins, the donor twin is usually smaller than the recipient twin but often has a paradoxically larger placental share compared to its co-twin. In contrast to uncomplicated monochorionic twins, fetal growth in TAPS appears to be primarily determined by inter-twin blood transfusion rather than the size of the placental share. Possibly, a relatively larger placental share may enable the fetal survival of the anemic twin in TAPS.[25] In addition, TAPS donor twins also have hypoalbuminemia and hypoproteinemia due to loss of not only erythrocytes but also proteins and nutrients, which may partly also affect their fetal growth.[26]

Diagnosis

TAPS can be diagnosed either antenatally or postnatally. TAPS is characterized by Hb differences in absence of antenatal ultrasound signs of polyhydramnios in the recipient and oligohydramnios in the donor. Antenatal diagnosis is based on ultrasound abnormalities whereas the postnatal diagnosis is derived from large inter-twin hematologic discordances and a characteristic placental angioarchitecture.

Antenatal criteria

The antenatal diagnosis of TAPS is based on Doppler ultrasound abnormalities. Middle cerebral artery peak systolic velocity (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. In TAPS, this test will show an increased MCA-PSV in the donor twin, suggestive of fetal anemia, and decreased velocities in the MCA-PSV in the recipient, suggestive of polycythemia. During the past decade, different MCA-PSV values for the diagnosis of TAPS have been proposed. Robyr et al. initially suggested the use of MCA-PSV > 1.5 multiples

of the median (MoM) for the donor twin and < 0.8 MoM for the recipient twin.[1] However, Slaghekke et al. showed that a MCA-PSV between 0.8 and 1.0 MoM in the recipient was also frequently found in postnatally diagnosed TAPS cases and therefore suggested the currently used cut-off of MCA-PSV < 1.0 MoM in the recipient twin and > 1.5 MoM in the donor twin.[27] This cut-off level is characterized by high sensitivity and specificity of MCA-PSV for both anemia and polycythemia, confirming the clinical usefulness of this non-invasive test. MCA-PSV measurement has a high diagnostic accuracy for predicting abnormal Hb levels in fetuses with TAPS.[28, 29] However, the high predictive value of MCA-PSV measurements reported in the study from Slaghekke et al.[27] was determined in a highly selected group of only TAPS cases followed in a specialized fetal therapy center. In a recent study, Fishel-Bartal et al. did not find a similar predictive value of MCA-PSV for the detection of polycythemia in monochorionic twins. MCA-PSV measurement in polycythemic twins did not differ compared to normal twins.[30] They did however find a significant correlation between a large inter-twin difference in MCA-PSV (delta MCA-PSV) and large inter-twin hematocrit difference and proposed the alternative use of a delta MCA-PSV > 0.5 MoM instead of the currently used fixed cut-off levels of < 1.0 MoM and > 1.5 MoM for the prenatal diagnosis of TAPS.

In some TAPS cases, additional ultrasound findings have been reported. In several cases of spontaneous TAPS, a striking difference in placental thickness and echodensity on ultrasound examination was detected (Figure 2).[27, 31, 32] This difference can be explained by the hydropic and echogenic character of the anemic placental share in contrast to the normal appearance of the polycythemic placental share. Another ultrasound finding described in TAPS is the so-called 'starry-sky liver' (Figure 3).[33] Starry-sky appearance refers to a sonographic pattern of the liver, characterized by clearly identified portal venules (stars) and diminished parenchymal echogenicity (sky) that accentuates the portal venule walls. Starry-sky liver is usually reported in acute hepatitis, but other conditions like heart failure can lead to this typical finding as well. More studies are needed to further investigate the validity and significance of these antenatal ultrasound findings for the diagnosis of TAPS. Since diagnosis of TAPS at an earlier gestational age is associated with more favorable outcomes,[34] we recommend the routine use (at least biweekly) of MCA-PSV Doppler ultrasound measurements in all monochorionic pregnancies to timely detect TAPS.

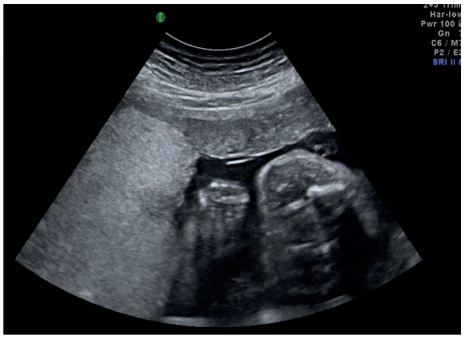


Figure 2. Ultrasound image of a TAPS placenta showing a difference in placental thickness and echodensity. On the left side of the image the hydropic and echogenic placental share of the anemic donor and on the right side the normal aspect of the placenta of the recipient is depicted.

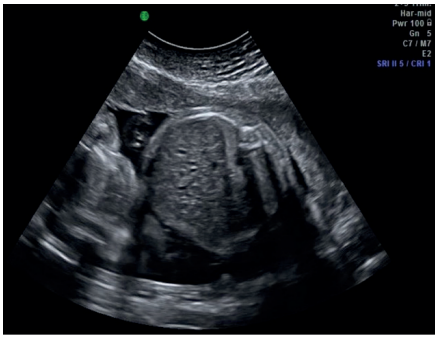


Figure 3. Ultrasound image showing a starry sky liver in a TAPS recipient with clearly identified portal venules (stars) and diminished parenchymal echogenicity (sky) that accentuates the portal venule walls

Postnatal criteria

In 40-63% of cases, TAPS is not detected antenatally, but is only diagnosed after birth.[15, 27] Therefore, postnatal diagnostic criteria have been proposed. These criteria are based on the presence of (chronic) anemia in the donor and polycythemia in the recipient twin (Figure 4) and the characteristic angioarchitecture of the placenta. Different cut-off levels for anemia and polycythemia have been used since the first description of TAPS in the last decade. Lewi et al. first 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.[5] However, absolute Hb levels do not take the positive association between Hb levels and higher gestational age into account. Fetal Hb concentrations are known to increase linearly with gestation. [35-37] Lopriore et al. used gestational-age-dependent cut-off levels to define anemia in the donor (Hb < 5th centile) and polycythemia in the recipient (hematocrit > 65%).[2] However, this contained practical disadvantages since it required the use of specific normograms related to gestational age. Several normograms have been published and differ slightly from one another.[35, 37] Since a fixed inter-twin Hb difference is a more logical and pragmatic criterion, nowadays an inter-twin Hb difference > 8 g/dL is commonly used, based on a case-control study were all TAPS cases had an inter-twin Hb difference > 8g/dL.[38]

Figure 4. Spontaneous TAPS twins at birth. On the left the plethoric polycythemic recipient and on the right the pale anemic donor.

A large Hb difference (> 8 g/dL) at birth is also detected in case of acute peripartum TTTS. Distinction between these two clinical pictures (TAPS and acute peripartum TTTS) is important, as they require a different therapeutic neonatal management. Two additional criteria are required to distinguish TAPS from acute peripartum TTTS.[39] The first criterion is an increased reticulocyte count in the TAPS donor (as a result of increased erythropoiesis due to chronic anemia). An inter-twin reticulocyte count ratio > 1.7 is pathognomonic for TAPS.[38] This ratio is measured by dividing the reticulocyte count of the donor by the reticulocyte count of the recipient. The second criterion for the postnatal diagnosis of TAPS is the presence of small residual anastomoses (diameter < 1 mm) at the placental surface,[27] detected through color dye injection of the placenta.[40] In acute peripartum TTTS, blood transfusion from the donor twin to the recipients occurs rapidly and reticulocyte count in the donor is typically still low. Acute anemia will eventually lead to increased erythropoiesis but the increased reticulocyte production is not detected in the acute phase. In addition, in contrast to TAPS, the pathogenesis of acute peripartum TTTS is

based on large placental AA or VV anastomoses with low resistance allowing large amount of blood to flow directly from the donor to the recipient.[39]

Since reticulocyte count is not always measured and placental injection is difficult to perform, we recently studied a new additional criterion for the postnatal diagnosis of TAPS. In analogy with the difference in skin color of the TAPS twins at birth (the anemic donor is pale and the polycythemic recipient is plethoric), the maternal side of the TAPS placenta also shows a striking color difference (Figure 5 & Figure 6). We developed a new quick and easy tool to determine the color difference ratio (CDR) between the two placental shares using digital pictures.[41] We found that TAPS placentas had a significantly higher CDR (> 1.5) compared to uncomplicated monochorionic twin placentas. Whether this method can eventually be added to the list of postnatal criteria requires further investigations in larger series of placentas with and without TAPS to determine the sensitivity and specificity of the test. Importantly, CDR measurements should also be investigated in placentas with acute TTTS to determine whether this method can help distinguish TAPS cases from cases with acute peripartum TTTS, since in both situations large inter-twin hemoglobin differences are present at birth.

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 recently proposed both an antenatal and postnatal classification system (Table 1, Table 2). Whether this classification has an additional value to adequately stage and treat TAPS, requires further investigation.

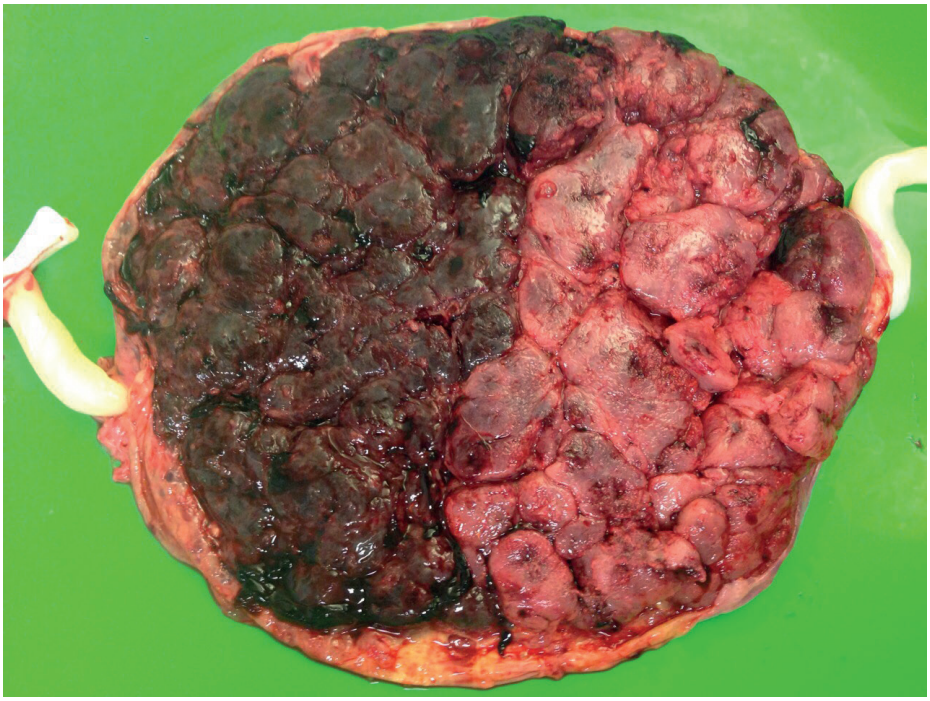


Figure 5. Maternal side of the TAPS placenta showing the difference in color between the plethoric share of the recipient (left side of the placenta) and the anemic share of the donor (right side of the placenta).

Table 1. Antenatal TAPS classification

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 and MCA-PSV recipient < 0.8 MoM, without other signs of fetal compromise
Stage 3	As stage 1 or 2, with cardiac compromise of the 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 the umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in the ductus venosus

Table 2. Postnatal TAPS classification

Postnatal stage	Inter-twin Hb difference, g/dL
Stage 1	> 8.0
Stage 2	> 11.0
Stage 3	> 14.0
Stage 4	> 17.0
Stage 5	> 20.0

Perinatal management and outcome

The optimal perinatal management for TAPS is not clear. Options include expectant management, induction of labor, intrauterine blood transfusion (IUT) in the donor, with or without partial exchange transfusion (PET) in the recipient, selective feticide and (repeat) fetoscopic laser surgery.

Expectant management consists of close monitoring with ultrasound including Doppler measurements of the MCA-PSV. Close monitoring can be considered in less severe cases of TAPS, such as stage 1 and 2. Whether close monitoring is safe enough in these TAPS stages needs to be evaluated in combination with validating the staging system. When TAPS stage 1 quickly progresses to stage 2 or stage ≥ 3, intrauterine intervention or termination of pregnancy should be considered. However, both management options may lead to premature delivery and its associated risks of perinatal morbidity and mortality.

Intrauterine blood transfusion

Treatment with intrauterine blood transfusion in the donor can be performed either intravascularly or intraperitoneal. Intraperitoneal IUT is preferred, since intraperitoneal transfusion may allow slower absorption of red blood cells into the fetal circulation preventing rapid loss of transfused blood in the circulation of the recipient twin.[42] Although treatment with IUT has often been reported, it is not a causal treatment and only a temporary solution. Furthermore, a potential side effect of IUT treatment is worsening of the polycythemia hyperviscosity syndrome in the recipient. Robyr et al. reported skin necrosis of the leg in the recipient twin of a TAPS case treated with several IUTs.[1] To reduce the risk of increasing polycythemia hyperviscosity a combination procedure of IUT in the donor and partial exchange transfusion (PET) in the recipient can be of additional value. The rationale behind this therapy is that PET may help

to decrease the viscosity of the blood of the polycythemic recipient. Genova et al. reported on three different TAPS cases treated with IUT and PET.[43] We recently developed a computational model, to evaluate the effect of IUT with and without PET in post-laser TAPS cases and showed the beneficial effect of PET.[44]

Since TAPS twins share their blood circulation and therefore have the exact same blood cell characteristics, it may be of additional value to transfuse the anemic twin with the recipient's whole blood as a donor source instead of foreign donor blood. Recently, Yarci et al. reported a case of TAPS in which the anemic donor was successfully transfused after delivery with blood obtained from the polycythemic co-twin during PET.[45] The main advantage of this new therapeutic method is avoidance of donor exposure and of the risk of blood product infections. Whether this new approach may lead to decreased morbidity in TAPS pregnancies requires further investigation.

Fetoscopic laser coagulation

The only causal treatment of TAPS is (repeated) fetoscopic laser coagulation of the (residual) anastomoses at the vascular equator of the placental. Fetoscopic laser coagulation in TAPS is more challenging than in TTTS, since the absence of oligo-polyhydramnios sequences and therefore a wavering inter-twin membrane, makes the visualization of the vascular equator more difficult.[27] Moreover, placental anastomoses in TAPS are known to be only few and minuscule and may therefore be missed during fetoscopy.[27] Different case reports show the feasibility of fetoscopic laser coagulation in TAPS placentas.[46-50] In a retrospective study where laser treatment for antenatally detected TAPS is compared to IUT or expectant management, laser therapy appeared to improve perinatal outcome by prolonging pregnancy and reducing respiratory distress syndrome.[51] The 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. In the laser group no residual anastomoses were found after color dye injection. Larger, adequately randomized controlled studies are required to determine the optimal management and to evaluate the possible additional value of fetoscopic laser coagulation for the treatment of TAPS. When performing laser coagulation in TAPS placentas, we recommend using the Solomon technique to reduce the risk of residual anastomoses and

recurrent TAPS.[9] With the Solomon technique, a line is drawn from one placenta margin to the other, connecting the individual laser spots.[52]

In unique circumstances, spontaneous resolution of antenatal TAPS may also occur. Spontaneous resolution has been reported once and was presumably caused by thrombosis of the residual AV anastomosis.[53] Whether expectant management would lead to spontaneous resolution in other TAPS cases is unknown and should be considered unlikely.

Proposal for antenatal management

In the absence of evidence on optimal management, we suggest that management decisions should made after careful evaluation of different factors including TAPS stage, gestational age and the feasibility of the different types of intrauterine intervention. TAPS stage 1 and possibly stage 2 can be observed with close monitoring. In case TAPS progresses quickly to stage 2 or in case of stage ≥ 3, intervention should be considered. If gestational age is below 28 weeks and laser treatment is feasible, laser treatment should be considered since this is the only causal treatment for TAPS and is known to prolong the pregnancy.[51] When laser treatment is not feasible and gestational age is below 30 to 32 weeks of pregnancy intrauterine transfusion should be considered. When repeated intrauterine transfusions are expected or in case of severe polycythemia in the recipient, partial exchange transfusion of the recipient should be envisaged. A management proposal for antenatal TAPS is presented in the flowchart in Figure 6. Whether this flowchart is useful in current practice and will improve outcome still needs to be validated.

Prevention of post-laser TAPS

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 the Solomon technique was introduced. The Solomon randomized trial showed a significant reduction of post-laser TAPS of 16% in the standard treatment group to 3% in the Solomon group.[9] The Solomon technique did not appear to be associated with an increase in any identifiable short-term adverse outcome or complications. A study investigating the neurodevelopmental outcome at 2 years in TAPS survivors randomized for the Solomon trial, showed no difference in the risk of neurodevelopmental

impairment between the groups treated with the Solomon technique and the standard laser technique.[54]

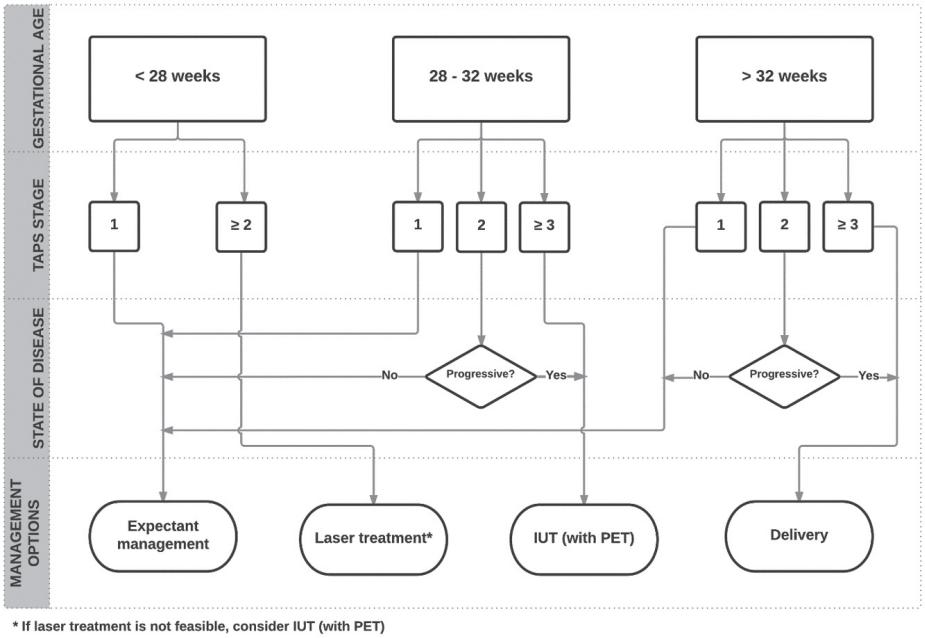


Figure 6. Flowchart with antenatal management options for TAPS.

The Solomon technique should therefore be used in all TTTS cases to reduce the risks of residual anastomoses and prevent the occurrence of post-laser TAPS.

Neonatal and pediatric outcome

Data of perinatal mortality and morbidity rates in TAPS are scarce and mostly based on case reports and small series. The neonatal outcome in TAPS may vary from isolated large inter-twin Hb differences to severe neonatal morbidity including cerebral injury, and neonatal death.[55]

Short-term neonatal outcome

Hematological complications are commonly seen in TAPS donors and recipients, requiring blood transfusion or partial exchange transfusion, respectively. TAPS recipients may develop polycythemia-hyperviscosity syndrome, which may possibly lead to necrosis of the skin and multiple limb ischemia.[1, 56] In

addition, recipients are at increased risk of thrombocytopenia, probably due to impaired production secondary to tissue hypoxia and slow spleen blood flow.[38, 57] Platelet count at birth was inversely related to the severity of polycythemia in recipients.[38] In addition to lower Hb levels, donor twins with TAPS also have significantly lower albumin and total protein levels compared to recipient twins, suggesting that the unbalanced inter-twin transfusion does not only concern red blood cells but also proteins and albumin.[26] Chronic inter-twin transfusion in TAPS may also cause short-term renal dysfunction: Verbeek et al. found that donor twins with TAPS have higher creatinine levels than recipients, probably due to chronic renal hypoperfusion.[58] Whether donor twins may also have permanent renal damage and long-term renal complications is not known. Chronic severe anemia in donor twins and polycythemia in recipient twins may theoretically also lead to cerebral injury. Several small case studies report on severe cerebral injury leading to fatal outcome in TAPS.[43, 59] Genova et al.[43] described a TAPS case in which, despite treatment with IUT with PET, the anemic twin died of extensive cerebral injury including numerous large cysts in the basal ganglia, bilateral white matter injury and multiple microbleeds. Lopriore et al.[59] reported on a spontaneous TAPS case delivered after an emergency Cesarean section at 33 weeks' gestation. The recipient twin suffered from severe cerebral injury due to massive hemorrhage and infarctions and died on day 3 after withdrawal of intensive care.

Long-term neurodevelopmental outcome

The long-term neurodevelopmental outcome in surviving TAPS infants is not well known and data is based on small uncontrolled case series. Severe long-term morbidity such as bilateral deafness and spastic paralysis have recently reported.[60] In another recent study on long-term neurodevelopmental outcome in post-laser TAPS, Slaghekke et al. detected neurodevelopmental impairment or mild-to-moderate cognitive delay in respectively 9% and 17% of TAPS survivors.[61] No difference in impairment was found between donors and recipients. The rate of impairment in TAPS seems to be comparable to the rate of impairment in children treated with laser surgery for TTTS. Risk factors for low cognitive scores in the study from Slaghekke et al. were low gestational age at birth and low birth weight, as well as treatment with intrauterine transfusion.[61] In a recent small study, neonatal outcome in monochorionic twins affected by TAPS appeared to be comparable to gestational age-matched uncomplicated monochorionic twins. However, only 10

TAPS cases were included with mild TAPS (stage 1 and 2) limiting the conclusions.[62] To date, there are no studies reporting on neurological, motor and cognitive outcomes of TAPS twins in childhood and adolescence. Data from these studies could provide us a more complete view on the long-term consequences of TAPS.

Conclusion

TAPS is a recently described form of unbalanced feto-fetal transfusion through small (diameter < 1 mm) anastomoses that either may occur spontaneously monochorionic twins, or that can develop in TTTS cases after incomplete laser surgery. In the last decade, our knowledge on pathogenesis, diagnostic criteria, management options and short- and long-term outcome has greatly increased. However, further studies are required to determine the optimal diagnostic criteria. Whether a delta MCA-PSV > 0.5 MoM (comparable to a delta Hb postnatally) is a better alternative criterion than the currently used fixed cut-off levels of < 1.0 and > 1.5 MoM of MCA-PSV requires further investigation. In addition, we recently introduced a new diagnostic criterion based on the color difference of the maternal side of the placenta. Whether this criterion can eventually be added to the list of postnatal criteria requires further investigations in larger series of placentas with and without TAPS. Although different management options have been proposed, optimal treatment for TAPS is still unclear and remains a challenging problem due to lack of randomized trials. In this review we propose a stage and gestational-age based flow chart for the treatment of TAPS. Large randomized controlled trials are needed to test the clinical usefulness of this proposed flowchart. Studies on long-term outcome in TAPS survivors show that neurodevelopmental outcome is similar to TTTS twins but data on neurological, cognitive and motor function child- and adulthood are still not available. Long-term follow up studies comparing TAPS to TTTS and uncomplicated monochorionic twins are required to determine whether TAPS twins have an increased risk for developing adverse long-term neurodevelopmental outcome at an older age. Since TAPS is a rare disease, collaboration between international fetal therapy centers is of utmost importance to increase sample size and quality of the studies. To facilitate this purpose, we have recently created a web-based registry (www.TAPSregistry.org) to gather information on the short- and long-term outcome in TAPS. This information will provide us with crucial information to set up well-designed studies and investigate the optimal management in the future.

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PART 4

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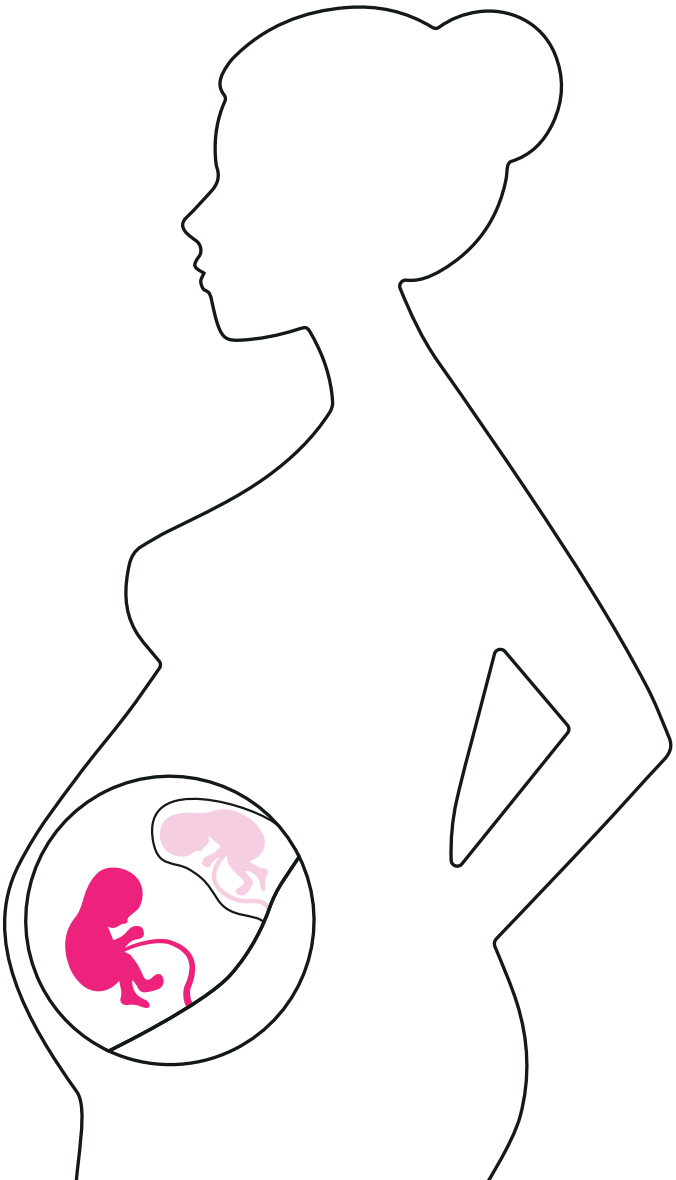
Chapter 2

Twin-twin transfusion syndrome with anemia-polycythemia: prevalence, characteristics and outcome



Abstract

The aim of this study was to estimate the prevalence of co-existing anemia-polycythemia (AP) in twin pregnancies with twin-twin transfusion syndrome (TTTS) prior to laser surgery, and to evaluate the characteristics and outcomes in TTTS twins with and without AP. All TTTS cases treated with laser between 2001 and 2019 were retrospectively reviewed for the presence of AP before surgery. AP was defined as delta middle cerebral artery–peak systolic velocity > 0.5 multiples of the median. The primary outcome was a composite of perinatal survival and severe neurodevelopmental impairment (NDI). Secondary outcomes included procedure-related characteristics, severe neonatal morbidity, and disease-free survival. In total, 66% (461/696) of TTTS twin pregnancies were eligible for analysis. AP was detected in 15% (70/461) of the TTTS twins prior to laser surgery. Gestational age at laser was higher in the TTTS+AP group compared to the TTTS-only group—21.0 weeks (interquartile rage (IQR): 18.8–24.0) versus 19.3 weeks (IQR: 17.3–21.9), respectively ($p < 0.0001$). Fewer placental anastomoses were detected in the TTTS+AP group than in the TTTS-only group—five (IQR: 4–6) versus six (IQR: 5–8), respectively ($p < 0.0001$). Perinatal survival was 77% (599/782) in the TTTS-only group and 83% (118/142) in the TTTS+AP group ($p = 0.130$). Severe NDI was 8% (28/370) in TTTS-only and 3% (2/74) in TTTS+AP. TTTS-only twins showed more severe neonatal morbidity than twins with TTTS+AP—23% (132/575) versus 11% (13/115), respectively ($p = 0.005$). Disease-free survival was lower in the TTTS-only group compared to the TTTS+AP group—62% (341/548) versus 73% (72/98), respectively ($p = 0.046$). Thus, AP complicates 15% of TTTS twins prior to laser. TTTS+AP twins show a different placental angioarchitecture, a later time of onset of the disease, and a more favorable outcome.

Introduction

Monochorionic twins share one placenta and have their blood circulation connected to each other via placental anastomoses, which allow blood to transfer bidirectionally between the two fetuses. Unbalanced feto-fetal blood flow may lead to twin-twin transfusion syndrome (TTTS) or twin anemia polycythemia sequence (TAPS). TTTS occurs in 10% of monochorionic twin pregnancies and arises from an unbalanced net blood flow from donor to recipient through various large placental anastomoses, resulting in large amniotic fluid discordances between donor and recipient.[1] The antenatal diagnosis of TTTS is based on the presence of twin oligohydramnios-polyhydramnios sequence (TOPS) detected through ultrasound. TAPS arises from an unbalanced and chronic net transfusion through only a few minuscule (diameter < 1 mm) vascular anastomoses, resulting in large inter-twin hemoglobin differences, without the development of TOPS.[2] TAPS may occur spontaneously in 2–5% of monochorionic twins or can develop in 1–16% of the TTTS twins treated with laser surgery (post-laser TAPS).[3-6] The antenatal diagnosis of TAPS is reached via ultrasound Doppler, showing a large discrepancy in middle cerebral artery–peak systolic velocity (MCA-PSV).[7]

Although TTTS and TAPS have been described as two separate entities, two recent reports show that a small percentage of TTTS pregnancies is diagnosed with co-existing anemia-polycythemia (AP).[8,9] However, in both studies, the size of the group of twins with TTTS and AP was limited, hampering adequate statistical analysis with respect to clinical outcome. In this study, we sought to evaluate the prevalence of preoperative AP in a large population of TTTS pregnancies, in order to provide more reliable information regarding placental characteristics and the short- and long-term outcomes of this selective subgroup of TTTS.

Methods

In this retrospective study, all consecutive monochorionic twin pregnancies receiving laser surgery for TTTS between 2001 and 2019 were reviewed for the preoperative presence of AP. TTTS twins with complete MCA-PSV measurements at least a week prior to fetoscopic laser surgery were considered eligible for analysis. Reasons for exclusion were incomplete or lacking MCA-PSV records,

triplet pregnancies with TTTS, and TTTS pregnancies with co-existing anemia due to other causes, such as red blood cell alloimmunization.

TTTS was diagnosed using the Eurofoetus criteria.[10] MCA-PSV was assessed in agreement with the previously described technique by Mari et al.[11] The presence of AP was defined as a delta MCA-PSV > 0.5 multiples of the median (MoM), in accordance with the new antenatal classification system for TAPS. To compare the results of this study with previously published studies, a distinction was made between twins with a delta MCA-PSV > 0.5 MoM (with normal values in the donor or recipient) and the old criteria for TAPS: MCA-PSV value > 1.5 MoM in the donor and < 1.0 MoM in the recipient. MCA-PSV values were converted from Vmax into MoMs, according to the reference ranges for monochorionic diamniotic twin pregnancies published by Klaritsch et al.[12]

The following maternal, placental, neonatal, and long-term outcome data were retrospectively obtained from digital medical records: maternal age, gravidity, parity, Quintero stage, gestational age at laser, gestational age at birth, sex, birth-weight discordance, number and type of anastomoses at fetoscopy, residual anastomoses, recurrent TTTS, recurrent TTTS with AP, post-laser TAPS, perinatal survival, severe neonatal morbidity, and severe neurodevelopmental impairment (NDI). The number of anastomoses on the placenta was counted by the operating surgeon during fetoscopic laser surgery. Severe neonatal morbidity was defined as the presence of at least one of the following conditions within 28 days after birth: respiratory distress syndrome requiring mechanical ventilation or surfactant, necrotizing enterocolitis stage 2 or higher,[13] patent ductus arteriosus requiring medical therapy or surgical closure, and severe cerebral injury (at least one of the following: intraventricular hemorrhage grade 3 or higher,[14] cystic periventricular leukomalacia grade 2 or higher,[15] ventricular dilatation > 97th percentile,[16] arterial or venous infarct, or porencephalic or parenchymal cysts). Neurodevelopment was assessed at two years of age using the Bayley Scales of Infant and Toddler Development second and third edition (Bayley-II and III), according to the standard care after fetal therapy in our center. Severe NDI was defined as at least one of the following: severe cognitive or motor delay (IQ score < 70 (–2 standard deviations (SD)), bilateral blindness, bilateral deafness (requiring amplification), or cerebral palsy (Gross Motor Function Classification System (GMFCS) ≥ stage two). The severity of cerebral palsy was classified according to the GMFCS for Cerebral Palsy.[17]

The primary outcomes were perinatal survival and severe NDI. Secondary outcomes included procedure-related characteristics (gestational age at laser, coagulated placental anastomoses, occurrence of post-laser TAPS, and recurrent TTTS), severe neonatal morbidity, and disease-free survival. Disease-free survival was defined as survival without severe NDI. Outcomes were compared between twins with TTTS (TTTS-only) and twins with TTTS and AP (TTTS+AP).

Statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, NY, USA). Data are reported as medians and interquartile ranges (IQR). The Mann–Whitney U test and the chi-squared test were used to calculate differences for continuous and categorical variables, respectively. To account for the fact that observations between co-twins are not independent, the generalized estimating equation module was performed for analyses per fetus or neonate. A p-value < 0.05 was considered to indicate statistical significance.

Results

Between 2001 and 2019, a total of 696 monochorionic twins with TTTS were treated with laser. Details on the derivation of the study population are shown in Figure 1. In total, 235 cases were excluded due to being a triplet (N = 13), incomplete MCA-PSV records prior to laser (N = 220), erythrocyte alloimmunization (N = 1), and TOP based on a genetic disorder (N = 1). Out of the 461 TTTS twins that were eligible for analysis, 391 twins (85%) were diagnosed with TTTS-only and 70 TTTS twins (15%) presented with AP before laser surgery. Of the 70 TTTS+AP twins, 30 cases (6.5%) had an MCA-PSV value > 1.5 MoM in the donor and < 1.0 MoM in the recipient, and 40 cases (8.7%) showed a delta MCA-PSV > 0.5 MoM, with a normal MCA-PSV value in either the donor or recipient.

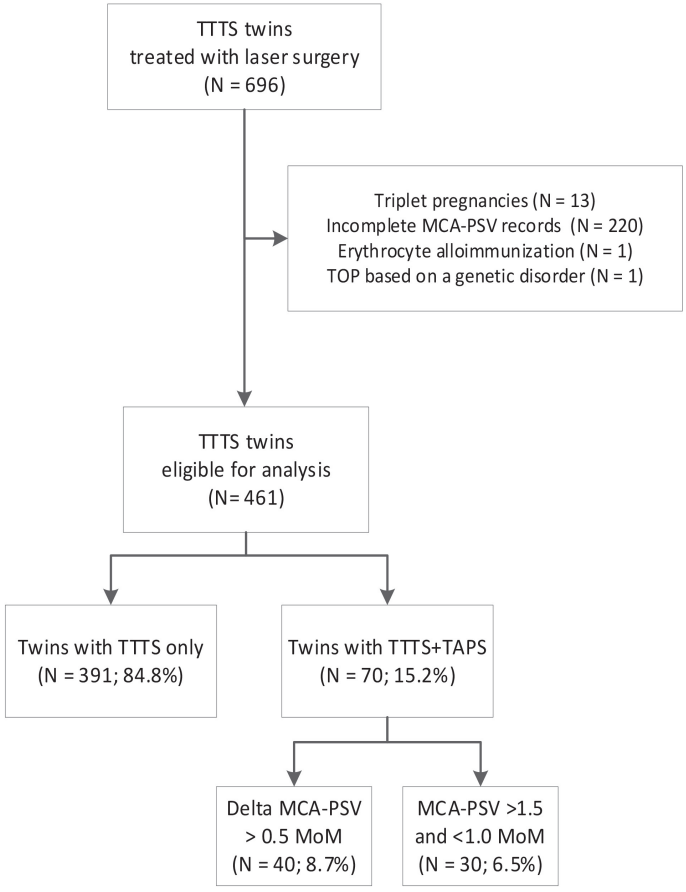


Figure 1. Flowchart of the derivation of the study population. TTTS: twin-twin transfusion syndrome; MCA-PSV: middle cerebral artery–peak systolic velocity; TAPS: twin anemia polycythemia sequence; MoM: multiples of the median.

Baseline characteristics of the population are presented in Table 1. Median maternal age, gravidity, parity, and Quintero stage were similar for both groups. In Table 2, procedure-related characteristics are shown. Compared to twins with TTTS only, TTTS+AP twins were more likely to receive laser treatment at a higher gestational age ($p < 0.0001$), and showed a lower total number of placental anastomoses prior to intervention ($p < 0.0001$). All types of anastomoses (arterio-venous (AV), veno-arterial (VA), arterio-arterial (AA), and veno-venous (VV)) were less frequently present in twins with TTTS+AP, although the difference in the total number of AA anastomoses failed to reach statistical significance. There was no difference in the development of recurrent TTTS or post-laser TAPS.

Table 1. Baseline characteristics for twins with TTTS only and TTTS+AP (anemia-polycythemia) twins.

	TTTS-only (N = 391 pregnancies, n = 782 fetuses)	**TTTS+TAPS (N = 70 pregnancies, n = 140 fetuses)**	***p*-value**
Maternal age (years)	32 (28-35)	30 (27-34)	0.438
Gravidity	2 (1-3)	2 (1-3)	0.380
Parity	1 (0-1)	1 (0-1)	0.778
Male	189/388 (49)	37/69 (54)	0.552
Cesarean	286/776 (37)	58/140 (42)	0.423
Delta MCA-PSV (MoM)	0.2 (0.1-0.3)	0.7 (0.6-0.9)	**<0.0001**
Quintero stage			0.198
I	58/391(15)	11/70 (16)	
II	140/391(36)	16/70 (23)	
III	180/391 (46)	40/70 (57)	
IV	13/387 (3)	3/70 (4)	

Data are median (IQR) or n/N (%)
TTTS, twin-twin transfusion syndrome; TAPS, twin anemia polycythemia sequence; MCA-PSV, middle cerebral artery – peak systolic velocity; MoM, multiples of the median. Bold indicates statistical significance

Table 2. Procedure related characteristics for twins with TTTS-only and twins with TTTS+TAPS

	TTTS-only (N=391)	**TTTS+TAPS (N=70)**	**p-value**
Gestational age at laser	19.3 (17.3-21.9)	21.0 (18.8-24.0)	**<0.0001**
Total number of anastomoses on fetoscopy	6 (5-8)	5 (4-6)	**<0.0001**
Number of AV-anastomoses	3 (3-5)	3 (2-4)	**0.018**
Number of VA-anastomoses	2 (1-3)	2 (1-3)	**0.012**
Presence of AA-anastomoses	54/371 (15)	5/67 (8)	0.118
Presence of VV-anastomoses	35/371 (9)	0/67 (0)	**0.009**
Presence of residual anastomoses	53/277 (19)	9/50 (18)	0.742
Recurrent TTTS	3/291 (1)	1/70 (1)	0.458
Recurrent TTTS with AP	2/391 (1)	0/70 (0)	0.549
Post-laser TAPS	37/387 (10)	6/70 (9)	0.855

Data are median (IQR) or n/N (%)
AV, arterio-venous; VA, veno-arterial; AA, arterio-arterial; VV, veno-venous; TTTS, twin-twin transfusion syndrome; AP, anemia-polycythemia; TAPS, twin anemia polycythemia sequence.

Table 3. Perinatal outcome for TTTS-only twins and TTTS+AP twins

	TTTS-only (N = 391 pregnancies, n = 782 fetuses)	**TTTS+TAPS (N = 70 pregnancies n = 140 fetuses)**	**p-value**
Gestational age at birth (weeks)	33.0 (29.2-35.6)	33.1 (29.9-35.6)	0.556
Birth-weight discordance (%)	11.5 (4.9-21.3)	10.8 (4.6-20.8)	0.953
Perinatal survival	599/782 (77)	118/142 (83)	0.130
Fetal demise	157/782 (20)	22/140 (16)	0.283
Neonatal mortality	26/625 (4)	2/118 (2)	0.090
Severe neonatal morbidity	132/575 (23)	13/115 (11)	**0.005**
Respiratory distress syndrome	114/575 (20)	8/115 (7)	**< 0.0001**
Patent ductus arteriosus	19/575 (3)	1/115 (1)	0.054
Necrotizing enterocolitis	17/575 (3)	3/115 (3)	0.873
Severe cerebral injury	33/575 (6)	3/115 (3)	0.082
Severe NDI	28/370 (8)	2/74 (3)	0.053
Severe cognitive delay	11/370 (3)	0/74 (0)	**0.007**
Severe motor delay	14/370 (4)	2/74 (3)	0.605
Bilateral blindness	0/370 (0)	0/74 (0)	1.000
Bilateral deafness	4/370 (1)	0/74(0)	0.177
Cerebral Palsy	12/370 (3)	2/74 (3)	0.682
Disease free survival	341/548 (62)	72/98 (74)	**0.046**

Data are median (IQR) or n/N (%)
TTTS, twin-twin transfusion syndrome; TAPS, twin anemia polycythemia sequence; NDI, neurodevelopmental impairment

Table 3 depicts characteristics on short- and long-term outcome for twins with TTTS only and twins with TTTS+AP. Twins with TTTS only were delivered at a similar gestational age when compared to twins with TTTS+AP—33.0 (IQR 29.2–35.6) versus 33.1 (IQR 29.9–35.6), respectively (p = 0.556). Perinatal survival was 77% (599/782) and 83% (118/142) for TTTS-only twins and for TTTS+AP twins, respectively (p = 0.130). Fetal demise was observed in 20% (157/182) of twins with TTTS only and 16% (22/140) of TTTS+AP twins (p = 0.289). Neonatal mortality occurred in 4% (26/625) of the live-born twins with TTTS only and in 2% (2/118) of TTTS+AP twins (p = 0.090). In total, 23% (132/575) of the twins with TTTS only had severe neonatal morbidity, as opposed to 11% (13/115) of twins with TTTS+AP (p = 0.005). The rate of respiratory distress syndrome differed significantly between the TTTS-only group and the TTTS+AP group—20% (114/575) versus 7% (8/115), respectively.

At the time of the study, 74% (579/787) of the study population was older than two years of age and eligible for follow-up evaluation. In total, 21% (126/579) was lost to follow-up, primarily explained by the lack of follow-up between 2006 and 2007, due to organizational issues. Follow-up was incomplete in 2% (9/579) of the group. A total of 77% (444/579) of the survivors had complete follow-up and were included in the analyses. Severe NDI was observed in 8% (28/370) of the TTTS-only survivors and in 3% (2/74) of the TTTS+AP survivors, p = 0.053. The rate of severe cognitive delay was significantly higher in the TTTS-only group than in the TTTS+AP group—3% (11/370) versus 0% (0/74), respectively (p = 0.007). Disease-free survival differed significantly between TTTS-only and TTTS+AP survivors—62% (341/548) and 73% (72/98), respectively (p = 0.046).

Discussion

This study showed that 15% of the TTTS twins presented with co-existing anemia in the donor and polycythemia in the recipient prior to fetoscopic laser surgery. Twins with TTTS+AP had a later gestational age at laser, fewer placental anastomoses, and were characterized by a more favorable short- and long-term outcome compared to twins with TTTS-only. Since TTTS+AP twins differed on multiple levels from TTTS-only, an alternative name to distinguish between the two types might be indicated. We therefore proposed the suffix 'AP' for TTTS cases that present with delta MCA-PSV > 0.5 MoM.

We reported the prevalence of co-existing AP in TTTS pregnancies to be much higher compared to the studies previously conducted by Donepudi et al. and Van Winden et al., which showed a prevalence of AP of 2.4% and 8.3% in 369 and 133 TTTS twins, respectively.[8,9] The higher detection rate in our study is likely to be attributed to the use of the new antenatal criterion for TAPS: delta MCA-PSV > 0.5 MoM. This choice was based on a recently published study from our group, which showed that delta MCA-PSV > 0.5 MoM was a superior predictor for TAPS compared to the MCA-PSV cut-off levels of >1.5 MoM and <1.0 MoM.[7] Based on the old criterion, the prevalence of AP (6.5%) was roughly comparable to the findings of Donepudi et al.[8]

We found no difference in maternal baseline characteristics between twins with TTTS- only and TTTS+AP twins. In contrast, Van Winden et al. demonstrated that twins with TTTS+AP were less likely to be multiparous,[9] whereas Donepudi

et al. showed that mothers of TTTS+AP twins had an increased chance of being multiparous and older.[8] Given the limited number of TTTS+AP cases in both studies (N = 11 and N = 9, respectively), these findings are probably due to coincidence, since it is unlikely for these factors to have a causal relationship with the pathogenesis of TTTS or TAPS.

Remarkably, twins with TTTS only showed a higher prevalence of severe neonatal morbidity than twins with TTTS+AP. This finding can be primarily explained by the large difference in respiratory distress syndrome between the two groups. Although strict statistical significance is lacking, there also seems to be a modest trend towards an increased risk for patent ductus arteriosus, severe cerebral injury, and neonatal mortality in twins with TTTS only. Notably, adverse short-term outcomes seem to be translated to an impaired long-term outcome as well; twins with TTTS only had a higher incidence of long-term NDI, almost reaching statistical significance. In addition, the rate of severe cognitive delay was significantly lower in twins with TTTS+AP. Moreover, disease-free survival was significantly higher in twins with TTTS+AP than in twins with TTTS only. The reason for the discrepancy in outcome between the two groups is not clear and has not been reported in the studies from Donepudi et al. and Van Winden et al.[8,9] Since Quintero stage and gestational age at birth were comparable, those factors are not likely to be of influence and other explanations need to be envisaged. Since twins with TTTS only received laser therapy at a significantly lower gestational age, the time of onset of the disease might play a role in perinatal outcome. Possibly, fetal development is more prone to the detrimental effects of TTTS earlier in pregnancy. Further investigation into the role of time of onset of TTTS on perinatal outcome is needed to explore this hypothesis.

Although TTTS and TAPS have been described as two mutually different entities, the subset of TTTS+AP twins in our study seems to suggest that these two feto-fetal transfusion disorders might be part of a spectrum of findings, with a certain degree of overlap between the two. Illustratively, TTTS+AP twins resembled spontaneous TAPS twins in various ways. Confirming findings from previous studies, twins with TTTS+AP displayed a significantly lower total number of anastomoses at the placental surface.[8,9] This lower number of anastomoses was mainly explained by less AV and VA anastomoses, although AA and VV anastomoses were also less frequently present in placentas from twins

with TTTS+AP. TAPS placentas are known to have only a few minuscule (<1 mm) vascular anastomoses, and usually do not show AA and/or VV anastomoses.[18-21] Furthermore, TTTS+AP twins were more likely to receive laser therapy at a higher gestational age, which might reflect a later time of onset of the disease. This finding is in agreement with the time of onset of spontaneous TAPS, which tends to develop later in pregnancy, whereas TTTS is mostly detected early in the second trimester.[22]

A principle limitation of this study is the inability to measure the diameter of the anastomoses during laser treatment. In the last decade, knowledge with respect to the pathophysiology of TTTS and TAPS has increased significantly as result of postpartum macroscopic examination of the vascular anastomoses on monochorionic placentas injected with color dye. However, this retrospective study was only carried out in TTTS+AP twins treated with laser coagulation, thereby hampering any quantification of the original placental anastomoses postnatally. Notably, Donepudi et al. did report on the size of the anastomoses in TTTS+AP pregnancies, classifying them into typical or non-typical TAPS anastomoses, but these findings were solely based on a visual inspection of the vascular equator during fetoscopy and not on quantified measurements.[8] Studies with non-lasered placentas from TTTS+AP twins might help to further unveil the pathophysiological mechanism behind this subgroup of TTTS twins, and more importantly, might offer more insight into the role of anastomoses in the entire spectrum of feto-fetal transfusion disorders.

In conclusion, co-existing AP was detected in 15% of the TTTS twins prior to laser surgery. This study shows that, compared to twins with TTTS only, twins with TTTS+AP show a different placental angioarchitecture, a later time of onset of the diseases and a more favorable short- and long-term outcome. Since TTTS+AP twins differ on multiple levels from twins with TTTS only, we proposed the use of the suffix 'AP' for TTTS cases that present with delta MCA-PSV > 0.5 MoM antenatally. Adequate distinction between the two types of TTTS can only be made when MCA-PSV measures are routinely taken throughout pregnancy.

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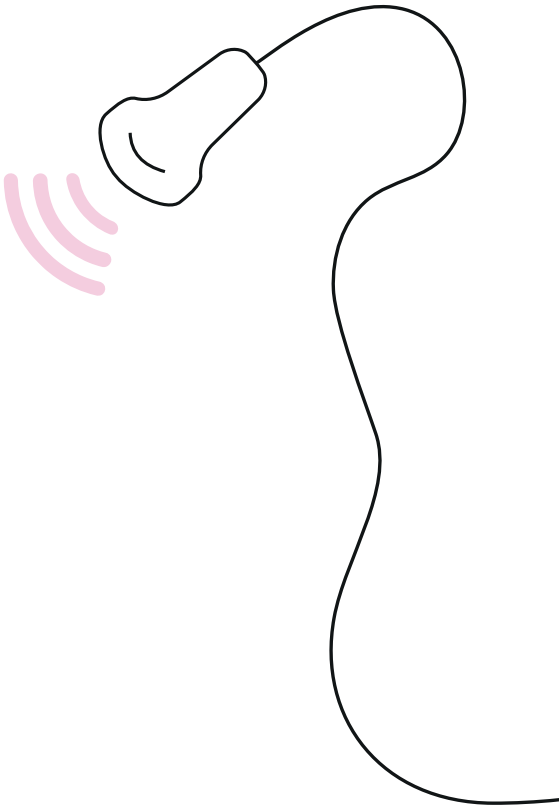
PART 5

antenatal diagnosis



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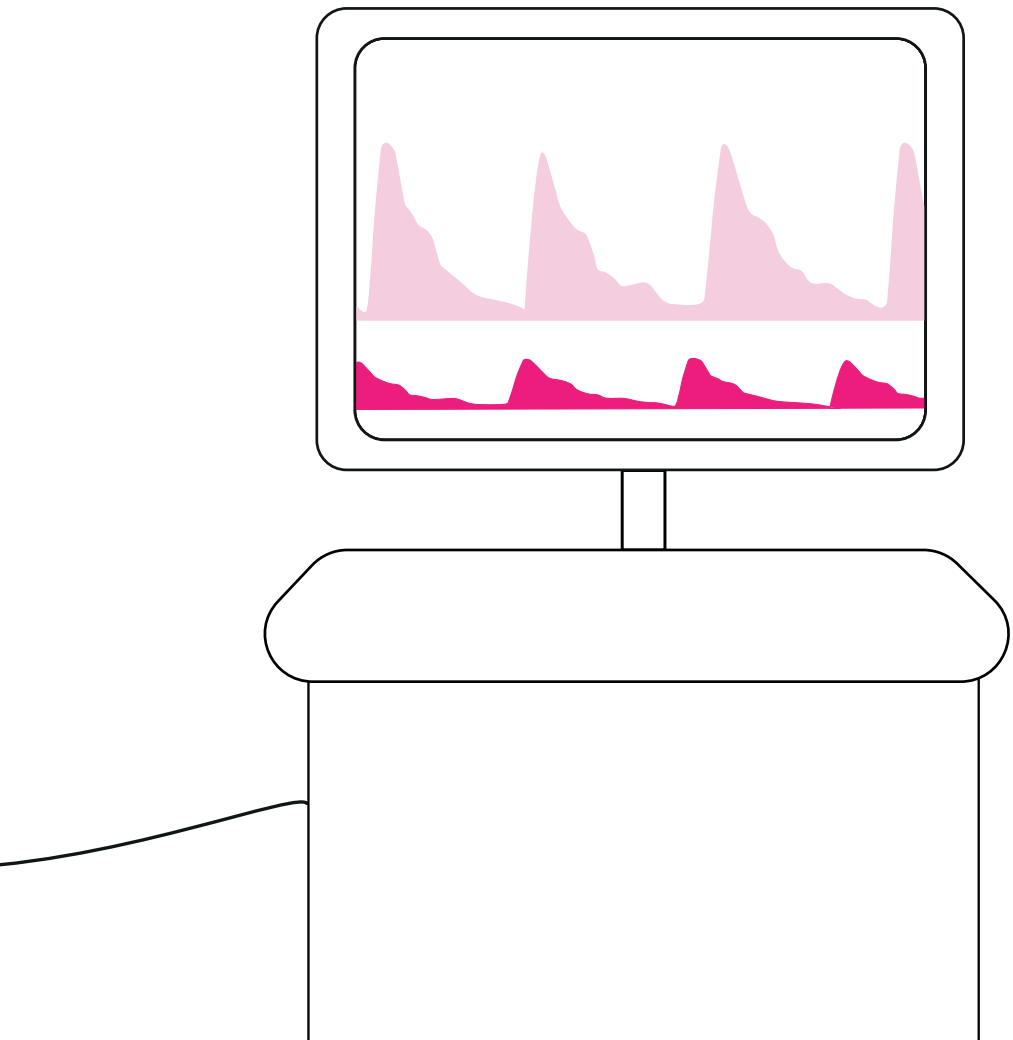
Ultrasound in Obstetrics and Gynecology, 2019; 53(6): 788-793

Chapter 3

Improved prediction of twin anemia polycythemia sequence by delta middle cerebral artery peak systolic velocity: a new antenatal classification system



Abstract

Objective

To investigate the diagnostic accuracy of delta middle cerebral artery peak systolic velocity (MCA-PSV) > 0.5 multiples of the median (MoM) and compare its predictive value with that of the current cut-off MCA-PSV values of >1.5 MoM in the donor and < 1.0 MoM in the recipient, for the diagnosis of twin anemia polycythemia sequence (TAPS).

Methods

This was a retrospective consecutive cohort study comprising all uncomplicated monochorionic twin pregnancies and twin pregnancies with a postnatal diagnosis of TAPS between 2003 and 2017 in the Dutch national referral center for fetal therapy. Cases with incomplete MCA-PSV Doppler measurements 1 week prior to delivery or with incomplete hemoglobin measurements within 1 day after birth were excluded. The postnatal diagnosis of TAPS was based on an inter-twin hemoglobin difference > 8 g/dL and at least one of the following: reticulocyte count ratio > 1.7 or presence of minuscule anastomoses on the placental surface. We compared the predictive accuracy of the current diagnostic method using MCA-PSV cut-off values of > 1.5 MoM in the donor and < 1.0 MoM in the recipient with that of a new method based on inter-twin difference in MCA-PSV > 0.5 MoM for prediction of TAPS.

Results

In total, 45 uncomplicated monochorionic and 35 TAPS twins were analyzed. The sensitivity and specificity of the cut-off MCA-PSV values (donor >1.5 MoM, recipient <1.0 MoM) to predict postnatal TAPS was 46% (95%CI 30-62%) and 100% (95%CI 92-100%), respectively; positive predictive value was 100% (95%CI 81-100%) and negative predictive value 70% (95%CI 58-80%). Delta MCA-PSV showed a sensitivity of 83% (95%CI 67-92%) and a specificity of 100% (95%CI 92-100%); the positive predictive value and negative predictive value were 100% (95%CI 88-100%) and 88% (95%CI 77-94%), respectively. Of the 35 cases with TAPS diagnosed postnatally, 13 twin pairs showed a delta MCA-PSV > 0.5 MoM, but did not fulfill the cut-off MCA-PSV criteria. Of these 13 TAPS twins, nine donors and four recipients had normal MCA-PSV values. There was a high

correlation between delta MCA-PSV and inter-twin difference in hemoglobin level (R= 0.725, $p < 0.01$).

Conclusion

Delta MCA-PSV > 0.5 MoM has a greater diagnostic accuracy for predicting TAPS compared to the current MCA-PSV cut-off criteria. We therefore propose a new antenatal classification system for TAPS. In monochorionic twin pregnancies with delta MCA-PSV > 0.5 MoM on Doppler ultrasound, but normal MCA-PSV values in the donor or recipient, obstetricians should be aware of the therapeutic implications and neonatal morbidities associated with TAPS.

Introduction

Twin anemia polycythemia sequence (TAPS) is a feto-fetal transfusion syndrome in monochorionic twins, in which chronic net inter-twin blood transfusion through minuscule placental anastomoses leads to large hemoglobin differences between donor and recipient, without signs of twin oligohydramnios polyhydramnios sequence (TOPS).[1] TAPS occurs spontaneously in 2-5% of monochorionic twin pregnancies, and develops in 3-16% of twins with twin-twin transfusion syndrome (TTTS) after fetoscopic laser surgery, as a result of the presence of small residual anastomoses.[2-5]

Antenatal diagnosis of TAPS is currently based on discordant middle cerebral artery peak systolic velocity (MCA-PSV) Doppler measurements. To identify TAPS before birth, the following MCA-PSV cut-off values have been proposed: > 1.5 multiples of the median (MoM) in the donor twin, suggestive of fetal anemia, and MCA-PSV < 1.0 MoM in the recipient, indicating fetal polycythemia.[6, 7] Recently, the predictive value and clinical usefulness of the lower cut-off level for polycythemia has been questioned in a recent study by Fishel-Bartel et al., which revealed that monochorionic twins diagnosed with polycythemia at birth often showed MCA-PSV values > 1.0 MoM prior to delivery.[8] In the same study, the delta MCA-PSV correlated strongly with the inter-twin hematocrit difference and was thus proposed a better indicator for the antenatal detection of TAPS. However, the study consisted of only nine TAPS cases, highlighting the need for additional studies with a larger population to investigate the potential value of this alternative antenatal diagnostic criterion in TAPS.

This study sets out to evaluate the diagnostic accuracy of delta MCA-PSV > 0.5 MoM and to compare its predictive value to that of the fixed cut-off values of MCA-PSV (< 1.0 MoM in the recipient and > 1.5 MoM in the donor) used currently for the detection of TAPS in monochorionic twin pregnancy.

Methods

This was a retrospective study of all consecutive uncomplicated monochorionic diamniotic twin pairs and monochorionic twins with TAPS diagnosed postnatally, managed between 2003 and 2017 in the Dutch national referral center for fetal therapy. Cases in which MCA-PSV ultrasound Doppler measurements were performed in both fetuses within 1 week before delivery were considered

eligible for analysis. The postnatal diagnosis of TAPS was based on an inter-twin hemoglobin difference > 8 g/dL and at least one of the following: reticulocyte count ratio > 1.7 or the presence of minuscule anastomoses (diameter < 1.0 mm) on the placental surface, detected through placental color dye injection.[9] Since a large difference in hemoglobin levels is essential for the postnatal diagnosis of TAPS, all cases with incomplete postnatal hemoglobin values were excluded from this study.

MCA-PSV values were obtained retrospectively from obstetrical records. MCA-PSV was measured according to the technique described by Mari et al.[10] The reference ranges for monochorionic diamniotic twin pregnancies published by Klaritsch et al. were used to convert MCA-PSV (cm/s) values to MoM.[11] When twins exceeded both cut-off values, i.e. > 1.5 MoM in one twin *and* < 1.0 MoM in the co-twin, this was named a cut-off MCA-PSV diagnosis. When there was an inter-twin difference in MCA-PSV > 0.5 MoM, the term delta MCA-PSV > 0.5 MoM diagnosis was used.

The following obstetric, fetal and neonatal data were collected from our database: gestational age at birth, antenatal fetal intervention, indication of TAPS on ultrasound, type of TAPS (spontaneous or post-laser), Quintero stage of TTTS preceding post-laser TAPS, mode of delivery, birth weight, postnatal hemoglobin values, postnatal intervention, severe neonatal morbidities and neonatal mortality. Adverse outcome was defined as either neonatal mortality or severe neonatal morbidity. Severe neonatal morbidity included at least one of the following: respiratory distress syndrome requiring mechanical ventilation or surfactant, necrotizing enterocolitis stage 2 or higher, patent ductus arteriosus requiring medical therapy or surgical closure, severe cerebral injury (at least one of the following: intraventricular hemorrhage grade 3 or higher, cystic periventricular leukomalacia grade 2 or higher, ventricular dilation > 97th percentile or porencephalic or parenchymal cysts) or severe anemia/polycythemia requiring blood transfusion or partial exchange transfusion respectively, within 24h after birth.

Statistical analysis was performed using SPSS version 23.0 (IBM, Armonk, NY, USA). Data are reported as medians and interquartile ranges (IQR). Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio were calculated using 2 x 2 tables and standard formulas for binominal proportions. Wilson's interval method

was used to calculate the 95% confidence interval (CI).[12] Group differences of continuous variables were compared using the Mann-Whitney U-test. The Chi square test was applied when calculating differences in proportions. Spearman's correlation coefficient was used to measure the correlation between delta MCA-PSV and inter-twin hemoglobin difference. 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. A p-value < 0.05 was considered statistically significant.

Results

A total of 45 uncomplicated monochorionic twins and 35 twin pairs diagnosed postnatally with TAPS were included in this study. Figure 1 shows the derivation of the study population.

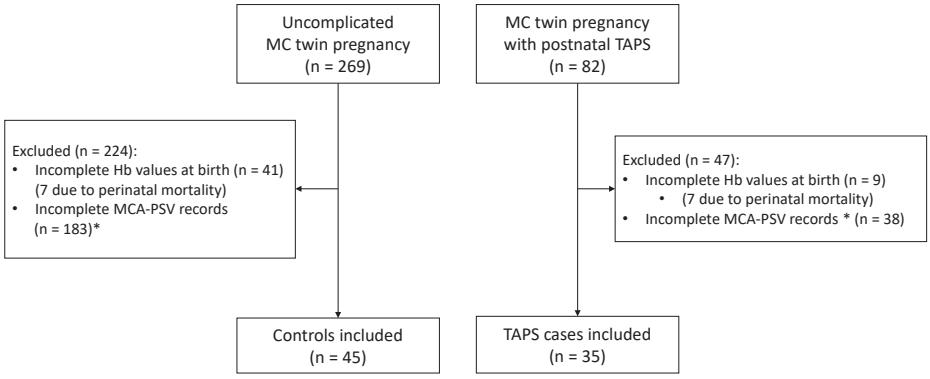


Figure 1 Flowchart showing derivation of study population consisting of uncomplicated monochorionic (MC) twin pregnancies (controls) and twins diagnosed postnatally with twin anemia polycythemia sequence (TAPS). *Within 1 week prior to delivery. Hb, hemoglobin; MCA-PSV, middle cerebral artery peak systolic velocity.

In total, 183 uncomplicated monochorionic twins and 38 TAPS twins were excluded due to lacking or incomplete MCA-PSV records 1 week prior to delivery. Baseline characteristics for both groups are presented in Table 1. Compared with uncomplicated monochorionic twins, TAPS twins were delivered more frequently via Cesarean section, showed a lower gestational age at birth, and were characterized by a larger inter-twin difference in hemoglobin and birth weight.

Table 1. Baseline characteristics of uncomplicated monochorionic twin pregnancies (controls) and pregnancies diagnosed postnatally with twin anemia polycythemia sequence (TAPS)

Characteristic	Controls (N=45)	TAPS (N=35)	P
Female sex	23/45 (51)	14/35 (40)	0.163
Cesarean section	39/90 (43)	52/70 (74)	<0.0001
Gestational age at birth (weeks)	35 (33-36)	32 (29-34)	<0.0001
Birth-weight discordance (%)	11.6 (5.9-17.3)	14.5 (7.9-20.8)	0.114
Birth-weight discordance ≥ 20%	4/45 (9)	12/35 (34)	0.005
Inter-twin Hb difference (g/dL)	1.2 (0.3-3.6)	12.7 (10.8-15.1)	<0.0001

Data are given as n/N (%) or median (interquartile range). Hb, hemoglobin

In Table 2 a 2x2 cross table on the diagnostic accuracy of the cut-off MCA-PSV diagnosis for postnatal TAPS is presented. Out of the 35 cases with a postnatal TAPS diagnosis, 16 pregnancies fulfilled the cut-off MCA-PSV diagnosis antenatally, reflected by a sensitivity rate of 46% (95%CI 30-62%). The specificity for this antenatal diagnostic criterion was 100% (95%CI 92-100%): out of the 45 control cases none showed a cut-off MCA-PSV diagnosis. Positive predictive value was 100% (95%CI 81-100%) and negative predictive value 70% (95%CI 58-80%). The negative likelihood ratio was 0.54. The cross table on de diagnostic accuracy of delta MCA-PSV > 0.5 MoM for postnatal TAPS is shown in Table 3. The sensitivity for this second antenatal diagnostic criterion was 83% (95%CI 67-92%): 29 out of the 35 postnatally diagnosed TAPS cases were characterized by a delta MCA-PSV > 0.5 MoM ultrasound measurement prior to delivery. In the control group there was no case which fulfilled the delta MCA-PSV > 0.5 MoM criterion, reflected by a specificity rate of 100% (95%CI 92-100%). The positive predictive value and negative predictive value were 100% (95%CI 88-100%) and 88% (95%CI 77-94%), respectively. The negative likelihood ratio was 0.17. Due to a specificity rate of 100% for both antenatal MCA-PSV criteria, the positive likelihood ratio could not be calculated.

Table 2. Prediction of twin anemia polycythemia sequence (TAPS) using fixed cut-off values of middle cerebral artery peak systolic velocity (MCA-PSV) < 1.0 multiples of the median (MoM) and > 1.5 MoM in recipient and in donor twin, respectively, in monochorionic twin pregnancy diagnosed postnatally with TAPS

Met MCA-PSV cut-off criteria	**Postnatal diagnosis of TAPS**		
	Yes	No	Total
Yes	16	0	16
No	19	45	64
Total	35	45	80

Sensitivity = 46% (95%CI 30-62%), specificity = 100% (95%CI 92-100%), positive predictive value = 100% (95%CI 81-100%) negative predictive value = 70% (95%CI 58%-80%), positive likelihood ratio not calculable, negative likelihood ratio = 0.54

Table 3. Prediction of twin anemia polycythemia sequence (TAPS) based on inter-twin difference in middle cerebral artery peak systolic velocity (MCA-PSV) > 0.5 multiples of the median (MoM), in monochorionic twin pregnancy diagnosed postnatally with TAPS

Met Delta MCA-PSV > 0.5 MoM	**Postnatal diagnosis of TAPS**		
	Yes	No	Total
Yes	29	0	29
No	6	45	51
Total	35	45	80

Sensitivity = 83% (95% CI 67-92%), specificity = 100% (CI95%, 92-100%), positive predictive value =100% (95%CI 88-100%), negative predictive value = 88% (95% CI 77%-94%), positive likelihood ratio not calculable, negative likelihood ratio = 0.17

In total, 13 TAPS cases did not fulfill the cut-off MCA-PSV criteria, having normal MCA-PSV values in either the donor (n = 9) or recipient (n = 4), but showed a delta MCA-PSV > 0.5 MoM. Table 4 shows fetal and neonatal characteristics of these 13 cases in comparison with the TAPS cases that met the cut-off MCA-PSV criteria (n=16). TAPS pregnancies that did not reach both MCA-PSV cut-off levels but had delta MCA-PSV > 0.5 MoM were non-significantly less likely to be treated antenatally with intrauterine transfusion and/or PET, were delivered at a later gestational age and were characterized by a larger birth-weight discordance compared with twin pregnancies that fulfilled the cut-off MCA-PSV criteria. There were no noteworthy differences with respect to postnatal treatment and neonatal outcome between the two groups.

Table 4. Fetal and neonatal characteristics of 16 pregnancies with twin anemia polycythemia sequence (TAPS) that fulfilled cut-off middle cerebral artery peak systolic velocity (MCA-PSV) criteria and 13 TAPS pregnancies that did not reach MCA-PSV cut-off levels in both twins but had delta MCA-PSV > 0.5 multiples of the median (MoM)

	Met Cut-off MCA-PSV criteria (N=16)	**Normal MCA-PSV but delta MCA-PSV > 0.5 MoM (N=13)**	**P**
Male sex	6/10 (37)	11/13 (85)	0.007
Type of TAPS			0.452
Spontaneous	3/16 (19)	4/13 (31)	
Post-laser	13/16 (81)	9/13 (69)	
Quintero stage			0.646
I	1/13 (8)	2/9 (22)	
II	4/13 (31)	2/9 (22)	
III	7/13 (54)	5/9 (56)	
IV	1/13 (8)	-	
Antenatal Therapy			0.087
None	8/16 (50)	11/13 (85)	
IUT	4/16 (25)	2/13 (15)	
IUT and PET	4/16 (25)	-	
Difference in placental echogenicity on ultrasound	6/16 (38)	6/13 (46)	0.716
Starry-sky liver in recipient	8/16 (50)	3/13 (23)	0.135
Gestational age at birth (weeks)	31 (28-32)	34 (31-35)	0.430
Inter-twin Hb difference (g/dL)	13.9 (12.3-16.0)	12.7 (10.2-15.8)	0.350
Reticulocyte count ratio	3.9 (2.7-4.6)	4.5 (2.5-5.8)	0.384
Postnatal therapy on day 1			
BT	11/16 (68)	9/13 (69)	0.978
PET	11/16 (68)	6/13 (46)	0.219
BT and PET	7/15 (47)	4/13 (31)	0.296
Birth-weight discordance (%)	11.5 (5.8-20.3)	19.7 (13.4-38.2)	0.070
Birth-weight discordance > 20%	4/16 (25)	7/13 (54)	0.111
Severe neonatal morbidity*	14/32 (44)	7/26 (27)	0.227
Neonatal mortality	2/32 (6)	1/26 (4)	0.665

Data are given as n/N (%) or median (interquartile range). *Severe neonatal morbidity defined as at least one of: respiratory distress syndrome, patent ductus arteriosus requiring medical or surgical intervention, necrotizing enterocolitis and severe cerebral injury. BT, blood transfusion; Hb, hemoglobin; IUT, intrauterine transfusion; PET, partial exchange transfusion.

The correlation between delta MCA-PSV and inter-twin hemoglobin level difference is displayed in Figure 2. There was a strong correlation between delta MCA-PSV and inter-twin difference in hemoglobin level (R = 0.725, P < 0.01)

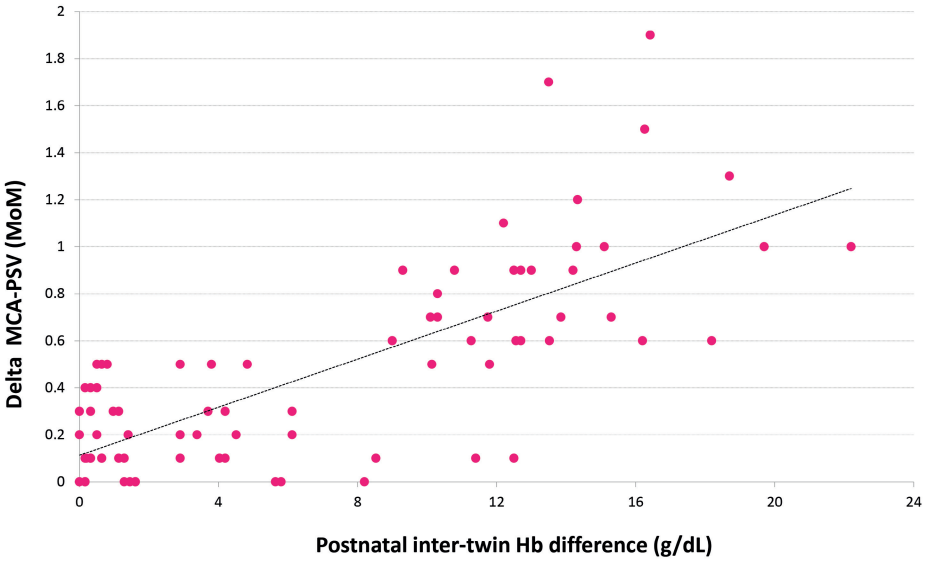


Figure 2 Correlation between delta middle cerebral artery peak systolic velocity (MCA-PSV) and inter-twin difference in hemoglobin (Hb) levels in 45 uncomplicated monochorionic twin pregnancies and 35 with twin anemia polycythemia sequence. Strong correlation was observed (R = 0.725; P < 0.01). MoM, multiples of the median.

Discussion

This is the first study evaluating the diagnostic accuracy of delta MCA-PSV for the prediction of TAPS. The criterion of delta MCA-PSV > 0.5 MoM showed high sensitivity and specificity for the prediction of TAPS and proved to be a superior predictor of postnatal TAPS compared with the current criteria of fixed MCA-PSV cut-off values. Moreover, we showed that TAPS twins with delta MCA-PSV > 0.5 MoM but with normal MCA-PSV values (in either the donor or recipient) were comparable with respect to perinatal mortality and neonatal morbidity to the TAPS twins that met the MCA-PSV cut-off criteria. Based on these findings, we propose a new antenatal classification system for TAPS (Table 5).

Table 5. Proposed antenatal classification system for twin anemia polycythemia sequence (TAPS)

Antenatal stage	Previous Criteria	Proposed criteria
Stage 1	MCA-PSV donor > 1.5 MoM and MCA-PSV recipient < 1.0 MoM without signs for fetal compromise	Delta MCA-PSV > 0.5 MoM without signs for fetal compromise
Stage 2	MCA-PSV donor > 1.7 MoM and MCA-PSV recipient < 0.8 MoM without signs for fetal compromise	Delta MCA-PSV > 0.7 MoM without signs for fetal compromise
Stage 3	As stage 1 or 2, with cardiac compromise of the donor, defined as critically abnormal flow*	As stage 1 or 2, with cardiac compromise of the donor, defined as critically abnormal flow*
Stage 4	Hydrops of donor	Hydrops of donor
Stage 5	Intra-uterine demise of one or both fetuses preceded by TAPS	Intra-uterine demise of one or both fetuses preceded by TAPS

*Defined as critically abnormal flow: Doppler shows absent or reversed end-diastolic flow in umbilical artery, pulsatile flow in umbilical vein and/or increased pulsatility index or reversed flow in ductus venosus. MCA-PSV, middle cerebral artery peak systolic velocity; MoM, multiples of the median.

In the new classification system, Stage 1 TAPS is changed from MCA-PSV > 1.5 MoM in the donor and <1.0 MoM in the recipient to delta MCA-PSV value > 0.5 MoM, while Stage 2 TAPS is changed from MCA-PSV > 1.7 MoM in the donor and < 0.8 MoM in the recipient to delta MCA-PSV > 0.7 MoM. We chose a lower delta MCA-PSV value (> 0.7 MoM) for TAPS Stage 2 than would be expected based on the previous criteria (MCA-PSV cut-off values > 1.7 MoM and < 0.8 MoM in the donor and recipient, respectively, would indicate delta MCA-PSV > 0.9 MoM) because our data show that using delta MCA-PSV > 0.9 MoM as the criterion, five of seven TAPS twins with delta MCA-PSV of 0.8 and 0.9 MoM would have adverse outcome. These twins could benefit from antenatal treatment with intra-uterine transfusions or laser surgery. Based on the proposed treatment flowchart for TAPS which indicates that antenatal intervention is recommended from Stage 2, we therefore accept delta > 0.7 MoM for Stage 2 TAPS.[13]

Our findings show that TAPS twins with a delta MCA-PSV > 0.5 but with normal MCA-PSV values in either the donor or recipient have a similar perinatal outcome as twins that fulfilled the MCA-PSV cut-off criteria. Notably, these TAPS twins were delivered at a later gestational age and were treated less often with an intrauterine intervention than the twins that fulfilled MCA-PSV cut-off diagnosis, although the differences were not statistically significant.

Perhaps, this group was regarded as having mild or as atypical TAPS, resulting in a more reluctant attitude towards antenatal intervention. The group with delta MCA-PSV > 0.5 MoM but with normal MCA-PSV values in either the donor or the recipient comprised significantly more male twin pairs than the group that fulfilled the MCA-PSV cut-off criteria but this difference may be a result of small sample size and is not likely to be related to the pathophysiology of the disease. Interestingly, a higher rate of birth-weight discordance was found in TAPS twins with delta MCA-PSV > 0.5 MoM compared to the cut-off MCA-PSV group. It is possible that coexisting selective intrauterine growth restriction is of influence on the hemodynamic balance in this population. Between the two groups, no statistically significant differences were found with respect to the type of TAPS, placental anastomoses, and perinatal outcome, corroborating the fact that these two groups probably share the same elemental pathological mechanism responsible for the inter-twin difference in hemoglobin level.

Although TAPS derives its acronym from the presence of anemia and coexisting polycythemia, our results show that the inter-fetal net blood transfusion responsible for this condition does not necessarily lead to equally discordant MCA-PSV levels or an equally severe anemia or polycythemia. This idea has already been accepted when the primary postnatal criterion for TAPS was changed from presence of anemia and polycythemia based on fixed hemoglobin cut-off levels into an inter-twin hemoglobin difference, because it was a more logic approach to describe this form of feto-fetal transfusion.[14] To provide continuity between antenatal and postnatal diagnostic criteria, changing the fixed MCA-PSV cut-off values into delta MCA-PSV would be a suitable next step, and the greater diagnostic accuracy of this new criterion, combined with its correlation with postnatal inter-twin hemoglobin difference postnatally, shows its potential clinical benefits.

Our findings seem to contravene the previously published studies regarding the diagnostic accuracy of MCA-PSV cut-off values for the antenatal detection of TAPS, including one performed by our own research group in which we found high sensitivity rates for both MCA-PSV > 1.5 MoM for anemia and < 1.0 MoM for polycythemia.[6] It should, however, be stressed that this particular study was performed in TAPS pregnancies only, which might have resulted in an overestimation of these criteria. Moreover, in the current study, the MCA-PSV criteria were not tested for the presence of anemia and polycythemia at birth,

but for a postnatal TAPS diagnosis, which is primarily based on an inter-twin hemoglobin difference.

These results should be interpreted with caution due to the retrospective nature of this study and the limited sample size. Since TAPS was discovered only a decade ago, MCA-PSV measurements are not performed routinely in every monochorionic twin pregnancy. Therefore, many TAPS cases in our database were only diagnosed postnatally and were excluded due to missing MCA-PSV values. Furthermore, in this study, the diagnostic accuracy of delta MCA-PSV > 0.5 MoM is only assessed in a TAPS group and in a control group consisting of uncomplicated monochorionic twin pregnancies. MCA-PSV > 0.5 MoM as a diagnostic tool for TAPS may perform less well in a larger, more heterogeneous monochorionic twin population. To further evaluate the true potential of delta MCA-PSV > 0.5 MoM as an accurate and reliable screening tool to detect TAPS, a large prospective study of monochorionic twin pregnancies is needed. In addition, the effect of the new proposed classification on intervention protocols, perinatal survival and long-term neurodevelopmental outcome needs to be evaluated.

In conclusion, this study shows that delta MCA-PSV > 0.5 MoM has a greater accuracy for diagnosis of TAPS than the cut-off MCA-PSV criteria used currently; to improve antenatal detection of TAPS, we propose a new antenatal classification system. In monochorionic twin pregnancies with an inter-twin difference in MCA-PSV > 0.5 MoM, but with normal MCA-PSV values in either the donor or recipient, obstetricians should be aware of the pathogenesis, therapeutic implications and neonatal morbidities associated with TAPS.

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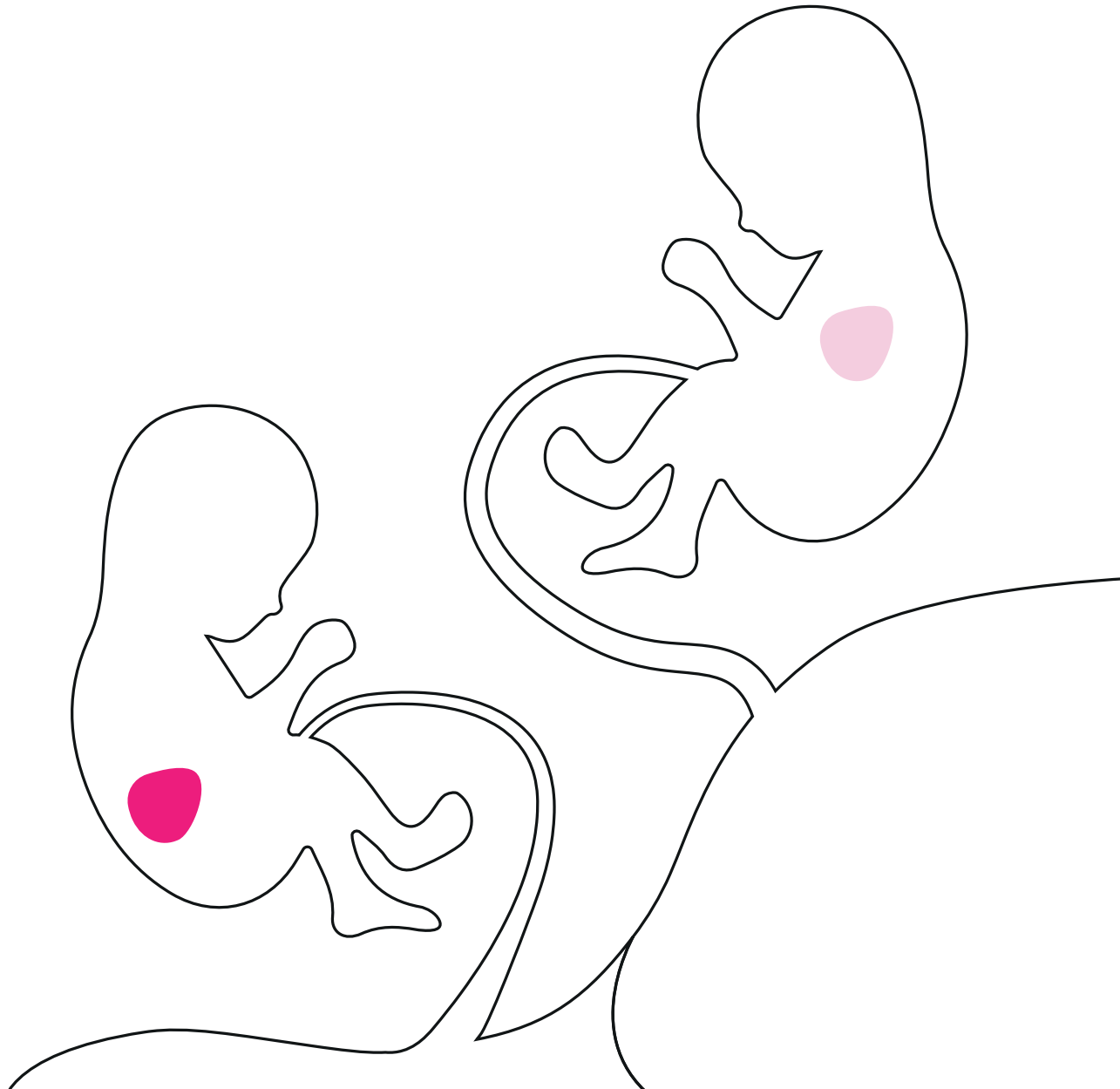
Chapter 4

The prevalence of fetal cardiomegaly, placental dichotomy and starry-sky liver in twin anemia polycythemia sequence



Abstract

Objective

To investigate the prevalence of additional ultrasound markers including placental dichotomy, cardiomegaly, and starry-sky liver in pregnancies with twin anemia polycythemia sequence (TAPS).

Methods

All monochorionic twins antenatally diagnosed with TAPS at our center between 2006-2019 were retrospectively reviewed for the presence of placental dichotomy, cardiomegaly in the donor and a starry-sky liver in the recipient. TAPS was diagnosed based on delta middle cerebral artery – peak systolic velocity > 0.5 multiples of the median. The primary outcome was the prevalence of placental dichotomy, cardiomegaly, starry-sky liver and at least one of these markers in both spontaneous and post-laser TAPS. Secondary outcome included the prevalence of these ultrasound markers per antenatal TAPS stage.

Results

Between 2006 and 2019, 91 monochorionic twins with TAPS were eligible for analysis. Placental dichotomy was observed in 44% (43/91) of TAPS cases. A total of 70% (64/91) of the TAPS donors developed cardiomegaly. A starry-sky liver was identified in 66% (53/80) of the TAPS recipients. The prevalence of cardiomegaly and starry-sky liver was roughly comparable between spontaneous TAPS and post-laser TAPS. Spontaneous TAPS showed a higher prevalence of placental dichotomy than post-laser TAPS twins, 63% (30/48) vs. 23% (10/43) respectively. In 86% (78/91) of the TAPS cases, at least one additional ultrasound finding was detected, meaning that 14% (13/91) of the cases presented solely with discordant MCA-PSV values. For all ultrasound markers, prevalence increased with incrementing antenatal TAPS stage.

Conclusion

Placental dichotomy, fetal cardiomegaly and a starry-sky liver are commonly found in TAPS pregnancies. Looking for these ultrasound markers can be of additional help in improving antenatal detection of TAPS in monochorionic twin pregnancies.

Introduction

Twin anemia polycythemia sequence is a chronic form of unbalanced feto-fetal transfusion through minuscule placental anastomoses leading to anemia in the donor and polycythemia in the recipient.[1] In contrast to twin-twin transfusion syndrome (TTTS), TAPS occurs in the absence of amniotic fluid discordances. TAPS can develop spontaneously in 3-5% of monochorionic twins or can occur iatrogenically in 2-15% of TTTS cases treated with laser, due to residual anastomoses.[2-4] Antenatal diagnosis is based on discordant middle cerebral artery peak systolic velocity (delta MCA-PSV > 0.5 MoM), with an increased MCA-PSV value in the donor, suggestive of anemia, and a decreased MCA-PSV level in the recipient, suggestive of polycythemia.[5]

Although identification of TAPS is primarily based on inter-twin MCA-PSV discrepancy, other ultrasound markers suggestive of TAPS have also been reported. Firstly, the placenta in TAPS can show dichotomy, with a hyperechogenic placental share for the donor and hypoechogenic placental share for the recipient (Figure 1).[6] In some TAPS cases, this observation is accompanied by placental size discordance, with a hydropic enlarged placental share for the donor and a flattened placental share for the recipient.[7] Secondly, polycythemic recipients can present with a starry-sky liver, a term that was coined for the sonographic pattern of the liver, characterized by clearly identified portal venules (stars) and diminished parenchymal echogenicity (sky).[8] Lastly, cardiomegaly can develop in anemic fetuses, because their hypoxic environment demands a higher cardiac output to continue to provide the body with adequate blood flow.[9]

The presence of these ultrasound markers has been described in a few case reports and case series.[6-8, 10] It is unknown whether these cases represent a small subgroup of TAPS, or that these sonographic findings are more ubiquitous in TAPS. Information on the true prevalence could contribute to enhanced understanding of the presentation of TAPS antenatally, and might lead to a timelier detection of the disease, especially in clinics where routine MCA-PSV Doppler measurements are not standard practice.

The aim of the current study was to investigate the prevalence of additional ultrasound markers including placental dichotomy, fetal cardiomegaly, and starry-sky liver in a large cohort of TAPS cases.

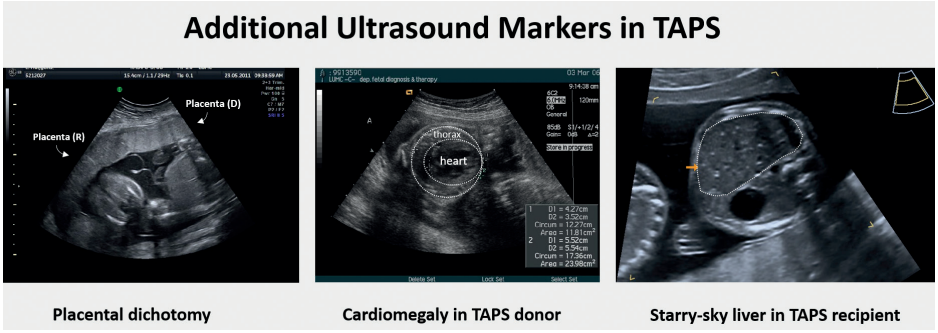


Figure 1. Ultrasound images of placental dichotomy, cardiomegaly in the donor, and starry-sky liver in the recipient in pregnancies with twin anemia polycythemia sequence (TAPS).

Methods

In this retrospective study, all monochorionic twins diagnosed with TAPS at the Leiden University Medical Center (LUMC) between 2006-2019 were reviewed for the presence of placental dichotomy, cardiomegaly in the donor and a starry-sky liver in the recipient. The LUMC is the national referral center for fetal therapy and complicated monochorionic twin pregnancies in The Netherlands. To be eligible for inclusion, TAPS cases needed to have ultrasound records from the moment of diagnosis to birth of the twins. TAPS cases with incomplete or lacking ultrasound reports, or cases that were only diagnosed postnatally were excluded from the study.

MCA-PSV assessment was performed in accordance with the previously described technique by Mari et al.[11] TAPS was diagnosed according to aforementioned antenatal criteria for TAPS (delta MCA-PSV > 0.5 MoM). [5] The following maternal, fetal and neonatal data were retrospectively obtained from digital medical records: maternal age, gravidity, parity, mode of delivery, antenatal TAPS stage, antenatal management (including expectant management, immediate delivery (within 7 days after diagnosis), intrauterine blood transfusion (IUT) with or without a partial exchange transfusion (PET), laser surgery, selective feticide and termination of pregnancy), sex of twins, and gestational age at birth.

The primary outcome was the prevalence of placental dichotomy, cardiomegaly in the donor, a starry-sky liver in the recipient and at least one of these findings, for the total group and per TAPS type (spontaneous and post-laser). The secondary outcome was the prevalence of these ultrasound markers

per antenatal TAPS stage (stage 1-5). For antenatal TAPS stage, the highest TAPS stage that was detected during pregnancy was recorded. All ultrasound examinations were carried out by experienced sonographers specialized in monochorionic twins at our center. For the current study, all available ultrasound reports were checked for the description of the aspect of the placenta (for placental dichotomy), heart of the donor (for cardiomegaly) and liver of the recipient (for starry-sky liver). In case of an inconclusive or absent description regarding any of the ultrasound markers, ultrasound images were reassessed by two of the authors (LSAT and FS). Any disagreements between LSAT and FS were resolved by a third observer (DO). Ultrasound images were eligible for assessment if there was a complete view of the placenta (allowing observation of placental dichotomy), an abdominal transverse section for the TAPS donor (allowing observation of the heart), and a thoracic abdominal section for the TAPS recipient (allowing observation of the liver).

Analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Descriptive statistics were generated for all variables. Data were presented as median with inter-quartile ranges (IQR) or n/N (%), where appropriate.

Results

Between 2006 and 2019, a total of 120 monochorionic twins were diagnosed with TAPS at our center. A total of 29 cases were excluded, either due to a postnatal diagnosis (n = 22) or incomplete or lacking ultrasound records (n = 7), yielding a total population of 91 TAPS twins eligible for analysis. The presence of placental dichotomy and cardiomegaly could be evaluated in all eligible cases. In 11 TAPS recipients, a description of the aspect of the liver was lacking in all ultrasound reports, and corresponding ultrasound images of the liver were unavailable or inconclusive. Consequently, these cases were left out of the prevalence estimation for starry-sky liver.

Table 1. Baseline characteristics for 91 TAPS pregnancies managed in the Leiden University Medical Center between 2006-2019

	TAPS cases (N = 91)
Maternal age (years)	32 (29-35)
Gravidity	2 (1-3)
Parity	1 (0-1)
Female – n/N (%)	50/91 (55)
Cesarean	42/91 (46)
Delta MCA-PSV (MoM)	1.3 (0.9-1.7)
Spontaneous TAPS	48/91 (47)
Post-laser TAPS	43/91 (53)
Gestational age at birth (weeks)	32.4 (29.4-35.0)
Antenatal TAPS stage	
I	16/91 (18)
II	40/91 (44)
III	27/91 (30)
IV	2/91 (2)
V	6/91 (7)
Antenatal treatment	
Expectant management	40/91 (44)
Immediate delivery	1/91 (1)
IUT (with PET)	22/91 (24)
Laser surgery	18/91 (23)
Selective feticide	8/91 (8)
Termination of pregnancy	2/91 (2)

Data are presented as n/N (%) or median (IQR)
TAPS, twin anemia polycythemia sequence; MCA-PSV, middle cerebral artery – peak systolic velocity; MoM, multiples of the median; IUT, intra-uterine transfusion; PET, partial exchange transfusion.

Baseline characteristics of the studied population are presented in Table 1. The population consisted of 48 (53%) spontaneous TAPS twins and 43 (47%) post-laser TAPS twins. Management included expectant management in 44% (40/91), IUT (with PET) in 24% (22/91), laser surgery in 20% (18/91) selective feticide in 9% (8/91), termination of pregnancy in 2% (2/91), and an immediate delivery in 1% (1/91) of cases.

Table 2. The prevalence of placental dichotomy, cardiomegaly in the TAPS donor, starry-sky liver in the TAPS recipient, and at least one of these ultrasound markers in TAPS twins.

	TAPS twins Total (N = 91)	**Spontaneous TAPS (N = 48)**	**Post-laser TAPS (N = 43)**
Placental dichotomy	40/91 (44)	30/48 (63)	10/43 (23)
Cardiomegaly in TAPS donor	64/91 (70)	33/48 (69)	31/43 (72)
Starry-sky liver in TAPS recipient[a]	53/80 (66)	25/39 (64)	28/41 (68)
At least one ultrasound marker	78/91 (86)	41/48 (85)	37/43 (86)

Data are presented as n/N (%).
[a]A total of 11 TAPS cases had inconclusive descriptions regarding a starry-sky liver and were therefore excluded from the prevalence estimation. The 11 cases consisted of nine spontaneous TAPS and two post-laser TAPS.
TAPS, twin anemia polycythemia sequence

Table 2 presents the prevalence of the three additional ultrasound markers for the total TAPS population, and for spontaneous and post-laser TAPS separately. Placental dichotomy was observed in 44% (40/91), cardiomegaly in 70% (64/91) of the TAPS donors, and a starry-sky liver was identified in 66% (53/80) of the TAPS recipients. The prevalence of cardiomegaly and starry-sky liver was roughly comparable between spontaneous TAPS and post-laser TAPS. Spontaneous TAPS twins showed a higher prevalence of placental dichotomy than post-laser TAPS twins, 63% (30/48) vs. 23% (10/43) respectively. In 86% (78/91) of the TAPS cases, at least one additional ultrasound finding was detected, meaning that 14% (13/91) of the cases presented solely with discordant MCA-PSV values. Figure 2 depicts the prevalence of placental dichotomy, cardiomegaly, starry sky liver, and at least one of these ultrasound markers, per TAPS stage. Overall, the prevalence of these ultrasound markers increased with incrementing TAPS stage.

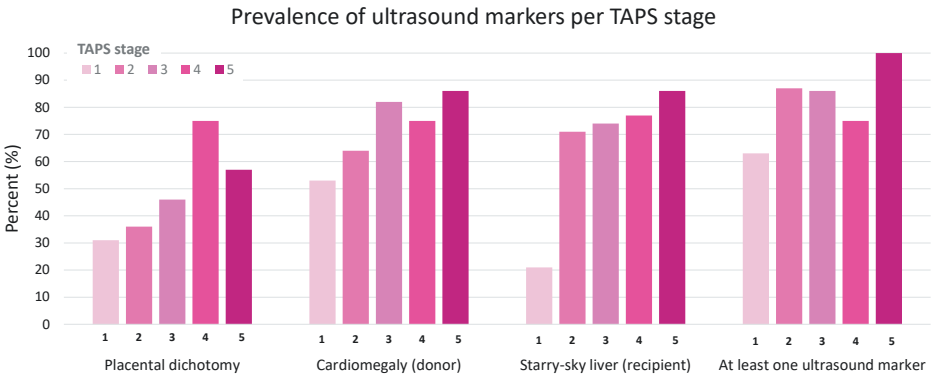


Figure 2. Prevalence (%) of placental dichotomy, cardiomegaly in the donor and starry sky liver in the recipient, per antenatal TAPS stage. In total, 16 TAPS cases were stage 1, 40 cases were stage 2, 27 cases were stage 3, two cases were stage 4 and six cases were stage 5.

Discussion

This study was aimed to assess the prevalence of three additional ultrasound markers in TAPS. We found that the prevalence of placental dichotomy was 44%, that cardiomegaly was detected in 70% of the TAPS donors and that 66% of the TAPS recipients presented with a starry-sky liver.

Since TAPS is characterized by a substantial risk for perinatal mortality and long-term neurodevelopmental impairment, timely antenatal detection is of utmost importance.[14] Unfortunately, there are still many clinics and guidelines across the world where MCA-PSV Doppler screening is not part of standard care for monochorionic twin pregnancies. Consequently, TAPS remains a frequently missed diagnosis. Our study shows that searching for these additional ultrasound markers could be of great value in centers that do not perform MCA-PSV measurements regularly, since the majority of TAPS cases present with at least one of these findings. However, a small subgroup of TAPS twins (14%) does not show any of these markers, and presents solely with MCA-PSV discordancy. Routine MCA-PSV examination therefore remains the cornerstone in the detection of TAPS antenatally.

This is the first study reporting on the prevalence of additional ultrasound findings in a large cohort of TAPS pregnancies. Until now, only a few small studies have been published with regard to this topic, and the majority concerned case reports. Stritzke et al. described a TAPS case that was missed

antenatally, because MCA-PSV Dopplers were not registered.[6] However, a large difference in placental echogenicity was observed four weeks prior to delivery. Movva et al. reported on two TAPS cases in which the observation of placental dichotomy prompted sonographers to perform MCA-PSV Dopplers, that in their turn revealed the diagnosis of TAPS.[15] A starry-sky liver was described in two cases published by Soundararajan et al., where it was seen as an early manifestation of severe TAPS.[8]

Although cardiomegaly in the donor has not been described as a typical marker for TAPS in the literature, our data show that it was the most frequently observed finding on ultrasound, both in spontaneous TAPS and in post-laser TAPS twins. Despite the fact that TAPS stage 1 is usually regarded as mild, this study demonstrated that more than half of the donors in this stage already showed signs of cardiac remodulation to the anemic environment. The exact role of the other two ultrasound markers in the pathophysiology of TAPS is not fully understood. Severe anemia can lead to fetal hydrops, a condition that is accompanied by excessive fluid accumulation in the fetal body. Since the placenta is an indispensable part of the fetal circulation, fluid accumulation in the placental tissue could be in line with the manifestation of the disease. However, our data show that placental dichotomy is detected before the fetus becomes hydropic. Whether placental dichotomy can be considered as a prehydropic sign that indicates a poorer prognosis is unclear. Notably, our results indicate that the prevalence seemed to increase with incrementing TAPS stage, suggesting it might be linked to the severity of the condition. Interestingly, Bamberg et al. and a study of or own research group underline the association between severity of TAPS and the degree of dichotomy in TAPS placentas, both on ultrasound and on post-partum macroscopic placental examination.[10, 16] Of note, placental dichotomy was more prevalent in spontaneous TAPS twins than in post-laser TAPS twins, suggesting there might be a different response of the placenta to anemia, once it has been lasered for TTTS.

A starry-sky liver might be the hardest of the three markers to identify on ultrasound, however, it is the only additional finding that can be detected for polycythemia. Whereas anemia can be accompanied by many signs of fetal decompensation, severe polycythemia in the recipient generally goes without additional fetal sequelae. Identification of a starry-sky liver as a reliable marker for polycythemia might therefore be of great value. Unfortunately, the exact

pathophysiological mechanism behind the development of starry-sky liver in fetal polycythemia remains to be unveiled. In general, the starry-sky pattern in the liver is thought to occur due to the edematous swelling of hepatocytes with a resultant decrease in the hepatic echogenicity.[17] The altered acoustic properties between the portal venules and hepatic lobules cause sonographic accentuation of the venule walls, creating a starry sky appearance. Hepatocyte swelling can arise from many causes including leukemic or neoplastic infiltration, infections, or right heart failure. However, none of them are present in TAPS recipients. Nonetheless, in line with placental dichotomy and cardiomegaly, the prevalence of starry-sky liver also seems to increase with incrementing TAPS stage, indicating an underlying mechanism for severe polycythemia and the development of this specific sonographic feature.

Due to the retrospective nature of the study, our results may be subject to selection bias. Possibly, TAPS cases with additional ultrasound markers were more easily detected and referred to our center than cases that progressed without any additional sonographic signs, resulting in an overestimation of the true prevalence. Another limitation is the fact that these ultrasound markers were based on subjective impressions of individual specialized sonographers and not on predefined standardized criteria, hampering overall reproducibility of the results. Lastly, we were unable to assess the specificity and sensitivity of the markers. Future studies should be aimed at prospective identification of placental dichotomy, fetal cardiomegaly and starry-sky liver in the general population of monochorionic twins, in order assess the exact clinical values of these (combined) ultrasound markers in the detection of TAPS. The most important strength of this study is high number of TAPS cases with ultrasound records and therefore this study is a valuable contribution to the knowledge of the presentation of TAPS prenatally.

In conclusion, this study shows that placental dichotomy, fetal cardiomegaly and a starry-sky liver are commonly found in TAPS pregnancies. Looking for these ultrasound markers can be of additional help in improving antenatal detection of TAPS in monochorionic twin pregnancies.

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PART 6

antenatal therapy

L.S.A. Tollenaar, F. Slaghekke, L. Lewi, Y. Ville, M. Lanna, A. Weingertner, G. Ryan, S. Arévalo, A. Khalil, C.O. Brock, P. Klaritsch, K. Hecher, G. Gardener, E. Bevilacqua, K.V. Kostyukov, M.O. Bahtiyar, M. D. Kilby, E. Tiblad, D. Oepkes, E. Lopriore

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Chapter 5

Treatment and outcome in 370 cases with spontaneous or post-laser twin anemia polycythemia sequence managed in 17 different fetal therapy centers

Abstract

Objective

To investigate antenatal management and outcome in a large international cohort of spontaneous and post-laser twin anemia polycythemia sequence (TAPS).

Methods

Data of monochorionic twins diagnosed antenatally with TAPS collected in the TAPS Registry between 2014-2019 were included in this study. Antenatal diagnosis of TAPS was based on middle cerebral artery peak systolic velocity (MCA-PSV) > 1.5 Multiples of the Median (MoM) in the TAPS donor and < 1.0 MoM in the TAPS recipient. Cases were assigned to the management groups based on the first treatment that was received. The primary outcome included perinatal mortality and severe neonatal morbidity. The secondary outcome was diagnosis-to-birth interval.

Results

In total, 370 TAPS cases were antenatally diagnosed and managed either with expectant management in 31% (113/370), laser surgery in 30% (110/370), intrauterine transfusion (IUT) (with or without partial exchange transfusion (PET)) in 19% (70/370), delivery in 12% (43/370), selective feticide in 8% (30/370) or termination of pregnancy in 1% (4/370). Perinatal mortality occurred in 17% (37/225) of the expectant group, in 18% (38/215) of the laser group, in 18% (25/140) in the IUT (± PET) group, in 10% (9/86) in the delivery group and in 7% (2/30) of the co-twins in the selective-feticide group (p = 0.177). Severe neonatal morbidity was 49% (41/84) in delivery, 46% (56/122) in IUT (± PET), 31% (60/193) in expectant management, 31% (57/182) in laser surgery and 25% (7/28) in selective feticide (p = 0.027). Median diagnosis-to-birth interval was longest after selective feticide (10.5 weeks; IQR: 4.2-14.9), followed by laser surgery (9.7 weeks, IQR: 6.6-12.7), expectant management (7.8 weeks; IQR: 3.8-14.4), IUT (± PET) (4.0 weeks, IQR: 2.0-6.9 weeks) and delivery (0.3 weeks, IQR: 0.0-0.5), p < 0.001. Treatment for TAPS varied greatly within and between the 17 fetal therapy centers.

Conclusions

Antenatal treatment for TAPS differs considerably amongst fetal therapy centers. Perinatal mortality and morbidity were high in all management groups. Prolonging pregnancy was best achieved in expectant management, laser surgery and selective feticide.

Introduction

Twin anemia polycythemia sequence (TAPS) occurs as the result of chronic unbalanced feto-fetal transfusion through minuscule placental anastomoses in monochorionic twins, leading to anemia in the donor and polycythemia in the recipient.[1] Unlike twin-twin transfusion syndrome (TTTS), TAPS develops in the absence of twin oligohydramnios-polyhydramnios sequence (TOPS). TAPS can occur spontaneously in 3-5% of monochorionic twins, or can arise in 2-16% after incomplete laser surgery for TTTS due to the presence of minuscule residual anastomoses.[2, 3]

TAPS is a relatively new disease, with its first description originating from 2006.[4] Since then, knowledge of TAPS has greatly increased and insights into pathophysiology, diagnosis and outcome have gradually been established.[5] However, the best antenatal management option for TAPS is still unknown. Options include expectant management, preterm delivery, intrauterine transfusion (IUT) in the donor with or without partial exchange transfusion (PET) in the recipient, fetoscopic laser surgery of the placental vascular anastomoses and selective feticide. Since TAPS is associated with high rates of adverse short- and long-term outcome, it is crucial to investigate which management strategy provides TAPS twins the best outcome.[6-8] Unfortunately, due to the low incidence of the condition, studies are limited to small numbers, hampering generalizability of results and demanding extreme caution when comparing the outcomes. To generate more substantiated knowledge on the effects of management strategies for TAPS twins, we set up the TAPS Registry, an international collaboration aimed at collecting data on diagnosis, management and outcome in TAPS.

The aim of the current study is to investigate perinatal outcome of different antenatal management strategies and to report the antenatal management choices for TAPS in various fetal therapy centers across the world.

Methods

Registry

The TAPS Registry (www.tapsregistry.org) was established in 2014 as a web-based registry for anonymous data collection. Fetal therapy centers across

the world were invited to participate. Participating centers were supplied with personal credentials to enter data of their TAPS cases into the online registry. Between 2014 and 2019, a total of 17 centers contributed to data collection (see Appendix 1).

Inclusion criteria

Women were eligible for the study if they were pregnant with monochorionic twins diagnosed with spontaneous or post-laser TAPS. The diagnosis for TAPS was based on a MCA-PSV discrepancy, with an increased MCA-PSV value (>1.5 Multiples of the Median (MoM)) in the TAPS donor combined with a decreased MCA-PSV value (<1.0 MoM) in the TAPS recipient, in absence of TOPS.[9] Cases were excluded if they only had a postnatal diagnosis of TAPS (and were missed antenatally) and/or if they were diagnosed with post-laser TAPS within one week after laser for TTTS, unless TAPS was ongoing after one week and/or if they were first diagnosed with TAPS at stage 5. Of note, the outcome from postnatally diagnosed cases are presented in two other studies investigating outcome in spontaneous and post-laser TAPS separately.[10, 11]

Collected information

Data on maternal characteristics, diagnosis, management, delivery, placental injection studies, and perinatal outcome were collected. The following information was retrieved from local medical records: gravidity, parity, location of the placenta, moment of diagnosis (ante- or postnatal), gestational age (GA) at diagnosis and TAPS stage at diagnosis. For antenatal management for TAPS, the type of management was recorded: expectant management, preterm delivery, IUT (± PET), fetoscopic laser surgery, selective feticide or termination of pregnancy (TOP). For each management decision the GA and TAPS stage were noted, as well as the indication. The severity of antenatal TAPS was determined according to the previously published staging system by Slaghekke et al.[12] For delivery, the following parameters were retrieved: type of delivery (spontaneous or planned), mode of delivery (vaginal or cesarean) and type of cesarean (elective or emergency). Based on placental color dye examination, the type, size and number of placental anastomoses were recorded. Perinatal outcome included: donor/recipient status, hemoglobin and reticulocyte values, treatment with blood transfusion for anemia or partial exchange transfusion

for polycythemia on day 1, the presence of severe neonatal morbidities and/or severe cerebral injury and the occurrence of perinatal mortality.

Management-group allocation

We defined the following antenatal management groups for TAPS: expectant management, delivery (defined as a delivery within 7 days after diagnosis), IUT (± PET), laser surgery and selective feticide. Since TAPS cases can be managed according to different strategies in the same pregnancy, management-group allocation was based on the first treatment that was performed. The following rules were applied to management-group allocation: cases were assigned to the laser, IUT (± PET), or selective-feticide group if that was the first treatment they received within 14 days after diagnosis of TAPS (we allowed a one-week re-examination to confirm the diagnosis of TAPS). If this treatment was performed after 14 days, cases were included in the expectant management group. If cases received laser surgery combined with an IUT during the same procedure, they were assigned to the laser group. When cases had an incomplete laser surgery and other interventions were needed to manage persisting or recurring TAPS, they were assigned to the laser group.

Characteristics for the population

The following parameters were studied for all management groups: type of TAPS (post-laser or spontaneous), location of the placenta, GA at diagnosis, TAPS stage at diagnosis, preterm premature rupture of the membranes (PPROM), GA at PPROM, type of delivery (spontaneous or planned), mode of delivery (vaginal or cesarean), GA at birth, the presence of TAPS postnatally, treatment for postnatal TAPS (defined as a blood transfusion for the donor and/or a partial exchange transfusion for the recipient at birth) and number of survivors per case. The postnatal diagnosis for TAPS was established on the presence of an inter-twin hemoglobin difference > 8.0 g/dL combined with least one of the following: a reticulocyte count ratio > 1.7 or the presence of only minuscule vascular anastomoses detected through color dye injection of the placenta.[13,14] Furthermore, we studied specific management-related characteristics for each management group. For expectant management we investigated spontaneous resolution of TAPS (defined as the absence of TAPS postnatally). For IUT (±PET), the number of interventions, time interval between interventions (in days), and site(s) of transfusion were examined. In cases with multiple IUT (± PET)

procedures, the median number of days between interventions was used. For laser surgery we examined recurrent/persistent TAPS, the presence of residual anastomoses, and delivery within 24 hours after the procedure. For selective feticide, donor/recipient status of the treated fetus and the reason for selective feticide were evaluated. For expectant management, IUT (± PET), and laser surgery any additional treatment after the initial intervention was recorded.

Primary and secondary outcomes

The primary outcomes of this study were perinatal mortality and severe neonatal morbidity. Secondary outcome was diagnosis-to-birth interval. Outcomes were compared between expectant management, delivery, IUT (± PET), laser surgery and selective feticide, for the total group, and for spontaneous and post-laser TAPS separately. Perinatal mortality was defined as fetal demise or neonatal death within 28 days after birth. In the selective-feticide group, perinatal mortality was only reported for the co-twin. Severe neonatal morbidity was defined as the presence of at least one of the following, diagnosed within 28 days after birth or before discharge to home: respiratory distress syndrome requiring mechanical ventilation and surfactant, patent ductus arteriosus requiring treatment, necrotizing enterocolitis ≥ stage 2,[15] retinopathy of prematurity ≥ stage 3,[16] amniotic band syndrome, ischemic limb injury or severe cerebral injury. Severe cerebral injury was diagnosed in case of one of the following abnormalities was identified on cerebral imaging: intraventricular hemorrhage ≥ stage 3,[17] ventricular dilatation (including post-hemorrhagic ventricular dilatation),[18] cystic periventricular leukomalacia ≥ grade 2,[19] porencephalic or parenchymal cysts, arterial infarction or other severe cerebral lesions associated with adverse outcome.

Statistical analyses were carried out using SPSS version 25.0 (IBM, Armonk, NY, USA). Data are presented as medians and interquartile ranges (IQR) or range (minimum-maximum), or n/N (%), as appropriate. A p-value < 0.05 was considered statistically significant. To compare management groups, the outcome in the expectant-management group was set as the reference value. Continuous data on pregnancy level was compared using the one-way ANOVA with Tukey correction. A Chi-square test was used for categorical data on pregnancy-level. To account for the fact that observations between co-twins are not independent, outcomes on fetal or neonatal level were compared using the Generalized Estimated Equation (GEE) module. As a GEE cannot be

carried out when an outcome event does not occur in one of the groups, an adjustment to the data was applied. With this adjustment, an unaffected child was changed into an affected child, for all groups. This correction generates more conservative p-values.

Results

Of the 422 TAPS cases that were entered in the TAPS Registry, 10% (43/422) was diagnosed postnatally and excluded from the study. From the remaining 379 cases, nine cases were excluded based on post-laser TAPS diagnosed within one week after laser for TTTS (n = 8) and antenatal TAPS stage 5 at diagnosis (n =1). A total of 370 cases were included in the study. Information on the cases contributed by each fetal therapy are detailed in Appendix 1. Antenatal management consisted of expectant management in 31% (113/370), laser surgery in 30% (110/370), IUT (± PET) in 19% (70/370), delivery in 12% (43/370), selective feticide in 8% (30/370) and termination of pregnancy in 1% (4/370). Table 1 shows diagnosis-, pregnancy- and delivery-related characteristics for expectant management, laser surgery, IUT (± PET), delivery and selective feticide.

Expectant management

The median GA at diagnosis in the expectant management group was 22.6 weeks (IQR: 19.9-27.1, range: 15.1-35.1). The median antenatal TAPS stage at diagnosis was 2 (IQR: 1-2). Spontaneous resolution was seen in 16% (18/111)1 of cases that were managed expectantly, and occurred after stage 1 in 17% (9/52), stage 2 in 13% (6/45), stage 3 in 20% (2/11) and in stage 4 in 20% (1/5). In 11% (13/113) of cases, an alternative management strategy was performed after 14 days of expectant management. An IUT (± PET) was elected in eight TAPS cases (after 15-97 days from diagnosis), based upon progression of TAPS stage (n = 5), ongoing stage 1 TAPS (n = 2) and initial recovery followed by recurrence of TAPS after 13 weeks (n = 1). In five cases managed expectantly, laser surgery was performed for progression of TAPS (after 15-38 days from diagnosis). In two cases managed with laser surgery, a delivery took place within 24 hours after the procedure, resulting in miscarriage (23 weeks) and premature (28 weeks) birth, with double survival in the latter. In the other three cases, perinatal survival was seen in 5/6 neonates.

1 Missing values of the results presented in this paper are reported in the corresponding tables

Table 1. Diagnosis-, pregnancy-, and delivery-related characteristics for expectant management, laser surgery, IUT (±PET), delivery and selective feticide

	Expectant management (N = 113, 226 fetuses)	**Laser surgery (N=110, 220 fetuses)**	IUT (± PET) **(N=70, 140 fetuses)**	**Delivery (N=43, 86 fetuses)**	**Selective feticide (N=30, 60 fetuses)**
GA at diagnosis (weeks)	22.6 (19.9-27.1; 15.1-35.0)	21.7 (19.3-23.9; 16.1-28.9)	25.8 (23.3-28.0; 17.0-32.1)	31.3 (28.6-34.0; 26.0-35.0)	21.4 (19.1-22.9; 15.1-24.0)
GA at intervention (weeks)	-	22.0 (19.5-24.3; 16.7-30.1)	26.3 (23.6-28.8; 18.0-32.1)	31.9 (29.1-34.1; 26.0-36.0)	22.1 (19.9-23.2; 17.1-24.6)
Spontaneous TAPS	51/113 (45)	86/110 (78)	26/70 (37)	34/43 (79)	18/30 (60)
Anterior placenta	55/113 (49)	47/110 (43)	42/70 (60)	22/43 (51)	19/30 (63)
TAPS stage at diagnosis	2 (1-2)	2 (2-3)	2 (1-2)		
1	52/113 (46)	25/110 (23)	18/70 (26)	1 (1-2)	2 (2-3)
2	45/113 (40)	51/110 (46)	37/70 (53)	23/43 (53)	5/30 (17)
3	11/113 (10)	27/110 (25)	10/70 (14)	13/43 (30)	12/30 (40)
4	5/113(4)	7/110 (6)	5/70 (7)	5/43 (12)	11/30 (37)
5	0/113 (0)	-	0/70 (0)	2/43 (5)	2/30 (7)
Alternative treatment				-	-
Expectant	13/113 (12)	16/110 (15)	10/70 (13)		
IUT (± PET)		2/110 (2)	-		
Laser	8/113 (7)	5/110 (5)	-		
(reintervention)		2/110 (2)	3/70 (4)		
Selective feticide	5/113 (4)	7/110 (6)	7/70 (10)		
PPROM	29/113 (26)	40/107 (37)†	17/69 (25)§	4/43 (9)	13/29 (45)¶
GA at PPROM (weeks)	29.0 (25.1-31.3; 21.0-36.4)	29.7 (25.9-32.1; 16.9-35.9)	29.0 (25.8-31.5; 17.7-34.0)	29.3 (26.6-33.4; 26.2-34.2)	27.9 (24.8-31.6; 20.2-33.3)
Spontaneous start of delivery	43/113 (38)	60/106 (57)‡	20/69 (29)§	3/43 (7)	24/29 (83)¶
Cesarean	138/226 (61)	160/212 (75)‡	100/138 (72)	76/86 (88)	26/58 (45)¶

Data are presented as median (IQR; range) or n/N (%). † 3 missing values , ‡ 4 missing values (same as '†' plus one case with missing delivery data) §1 missing value (one case with missing delivery and PPROM information), ¶ 1 case with missing delivery and PPROM information
IUT, intrauterine transfusion; GA, gestational age; BT, blood transfusion; PET, partial exchange transfusion; TAPS, twin anemia polycythemia sequence; PPROM, preterm premature rupture of the membranes

Laser surgery

Laser surgery was performed at a median GA of 22.0 weeks (IQR: 19.5-24.3, range: 16.1-30.1). Spontaneous TAPS cases made up the majority of this treatment group (78%; 86/110). In total, 43% (47/110) of the TAPS cases treated with laser surgery had an anterior placenta. Laser surgery was combined with an IUT in the same procedure in 11% (12/110) of the cases. In 4% (4/108) of cases treated with laser, a delivery took place within 24 hours after the procedure (at 21, 22, 24 and 28 weeks). Recurrent TAPS was seen in 15% (16/106) of the cases treated with laser surgery. Out of the 16 cases with recurrent TAPS, one was diagnosed with TAPS only postnatally. The remaining 15 were managed expectantly in 2% (3/110), with IUT (± PET) in 5% (5/110), laser reintervention in 2% (2/110) and selective feticide in 6% (5/110). In the cases managed expectantly, spontaneous resolution of TAPS was seen in one case. In the other two cases neonatal mortality occurred in three of four liveborn infants. In the recurrent-TAPS cases that were managed with IUT (± PET), fetal demise of the donor occurred in two out of the five twins after the first IUT. In both cases the co-twin survived. In the other three cases, two or three IUT (± PET) interventions were performed and all infants survived. Both laser reinterventions for recurrent TAPS were successful resulting in perinatal survival of the twins. Five recurrent TAPS cases were treated with selective feticide; four were performed in the donor twin, one in the recipient twin. In one case, fetal demise of the co-twin occurred. Aside from the recurrent-TAPS cases, a selective feticide was performed in two other cases treated with laser surgery, based on severe cerebral injury in the donor detected after laser intervention. In 9% (6/65) of liveborn twin pairs treated with laser surgery, postnatal TAPS was diagnosed. Placental injection information was available in 32% (36/110) of cases treated with laser surgery. Residual anastomoses, which were always minuscule, were detected in 19% (7/36). All cases with residual anastomoses (100%; 7/7) had recurrent TAPS.

IUT (± PET)

An IUT (± PET) was performed at a median GA of 26.3 weeks (IQR: 23.6-28.8, range: 18.0-32.1). The median antenatal TAPS stage at diagnosis was 2 (IQR: 1-2). An IUT was combined with PET in the recipient in 21% (15/70). In total, 73% (51/70) of the IUT (± PET) group had one intervention, 13% (9/70) had two, 7% (5/70) had three, 6% (4/70) had four, and 1% (1/70) had six interventions with IUT (± PET). The median time between interventions was 13.0 days (IQR: 8.6-

16.8; range: 6.5-21.0). The transfusion site was only intravenous in 70% (15/67), only intraperitoneal in 10% (7/67), and combined in 19% (13/67). An alternative management strategy was decided in 14% (10/70) of the cases treated with IUT (±PET). Three cases were treated with laser surgery, all within one week after the first IUT and based on progressive or recurrent TAPS. One laser procedure was complete, the other two were incomplete and both had recurrent TAPS. In seven cases treated with IUT (± PET), a selective feticide in the TAPS donor was performed based on recurrent or progressive TAPS (n = 5) or severe cerebral injury (n = 2).

Delivery

Delivery (within 7 days after diagnosis) took place at a median GA of 31.9 weeks (IQR: 29.1-34.1; range: 26.0-36.0). The median antenatal TAPS stage for cases treated with delivery was 1 (IQR: 1-2). In total, 88% (76/86) had a cesarean section.

Selective feticide

Selective feticide was performed at a median GA of 22.1 weeks (IQR: 19.9-23.2, range: 17.1-24.6). Reasons for selective feticide included TAPS (67%; 20/30), or TAPS with co-existing: severe growth restriction (10%; 3/30), severe cerebral injury (10%; 3/30), or congenital anomalies (10%; 3/30) In one case, selective feticide was performed on request of the parents (3%; 1/30). In 87% (26/30) of the group, selective feticide was performed in the TAPS donor.

Comparison of outcome between groups

Table 2a provides further information on the outcome for each management strategy. The rate of perinatal mortality was comparable for expectant management (17%; 39/225), laser surgery (18%; 38/215), IUT (± PET) (18%; 25/140), delivery (11%; 9/86), and selective feticide (7%; 2/30), p = 0.177. Severe neonatal morbidity was significantly higher in twins treated with delivery (49%; 41/84) and IUT (± PET) (46%; 56/122) than in twins managed expectantly (31%; 60/193), treated with laser surgery (31%; 57/182) or selective feticide (25%; 7/28), p = 0.027. Diagnosis-to-birth interval was 7.8 weeks (IQR: 3.8-14.4) in the expectant management group, 9.7 weeks (IQR: 6.6-12.7) after laser surgery and 10.5 weeks (IQR: 4.2-14.9) after selective feticide and was significantly shorter in twins treated with delivery (0.3 weeks, IQR: 0.0-0.5) and IUT (± PET) (4.0 weeks,

IQR: 2.0-6.9), p < 0.001. The prevalence of postnatal TAPS was comparable for expectant management (74%; 66/89), IUT (± PET) (71%; 36/51), and delivery (84%; 36/43), and significantly lower in twins treated with laser surgery (9%; 6/65), p < 0.001. In table 2b and 2c, outcome for management strategies are presented for spontaneous TAPS and post-laser TAPS separately.

Management choices for 17 fetal therapy centers

Figure 1 shows management choices for TAPS amongst 17 fetal therapy centers. Overall, management varied considerably. Some centers, like Leiden, Milan and Brisbane, adopt a more conservative attitude and manage a considerable number of cases expectantly. In contrast, London, Paris, and Houston treat TAPS cases more invasively, with laser treatment or selective feticide. Fetal therapy centers in Hamburg and Barcelona generally refrain from doing in-utero interventions and manage the majority of cases expectantly or with delivery. The remaining centers do not show a remarkable trend or preference in management and apply the different treatment options alternately.

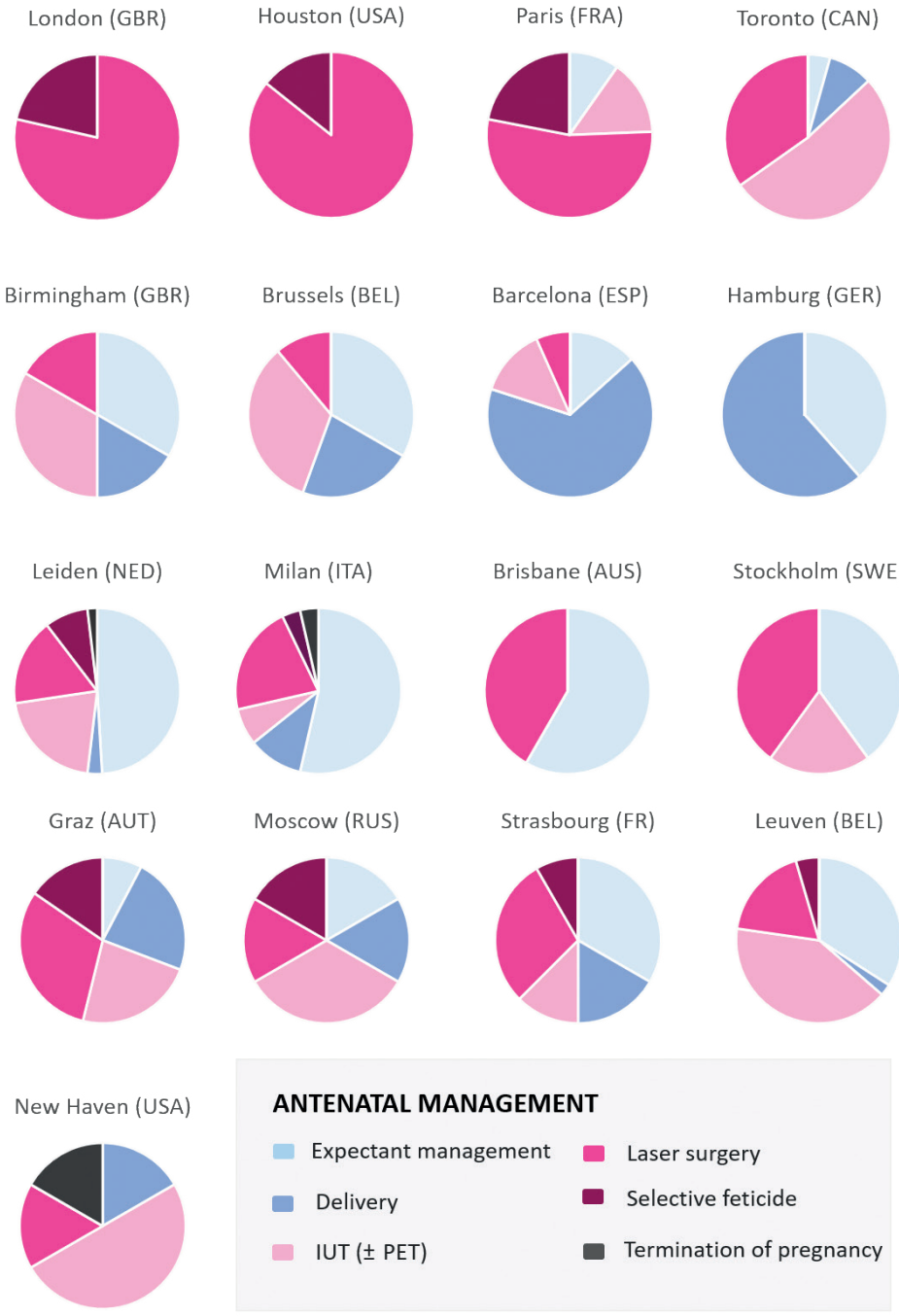

Figure 1. Antenatal management for TAPS in 17 fetal therapy centers

Table 2a. Outcome of expectant management, laser surgery, IUT (± PET), delivery, and selective feticide for all TAPS twin

TOTAL GROUP	Expectant management (N = 113 pregnancies; 226 fetuses)	Laser surgery (N= 110 pregnancies; 220 fetuses)	IUT (± PET) (N=70 pregnancies; 140 fetuses)	Delivery (N=43 pregnancies; 86 fetuses)	Selective feticide (N=30 pregnancies; 30 co-twins)	p-value
GA at birth (weeks)	33.0 (30.1-34.9)	31.8 (29.1-34.1)	**31.1 (28.3-33.0)***	31.9 (29.1-34.1)	32.1 (27.7-34.8)	**<0.001**
Diagnosis-to-birth interval (weeks)	7.8 (3.8-14.4)	9.7 (6.6-12.7)	**4.0 (2.0-6.9)**	**0.3 (0-0.5)***	10.5 (4.2-14.9)	**< 0.001**
Perinatal mortality	39/225 (17)†	38/215 (18)¶	25/140 (18)	9/86 (10)	2/30 (7)*	0.177
Fetal demise	24/226 (11)	28/215 (13)	18/140 (13)	**0/86 (0)***	2/30 (7)	0.024
Neonatal mortality$^{\psi}$	15/201 (7)†	10/187 (5)¶	7/122 (6)	9/86 (10)*	0/28 (0)	0.280
Survivors						
None	5/112 (4)†	8/107 (7)*	3/70 (4)	1/43 (2)	2/30 (7)	0.359
One	27/112 (24)†	20/107 (19)	18/70 (26)	7/43 (16)	**28/30 (93)***	**<0.001**
Two$^{\psi}$	80/112 (71)†	78/107 (73)	49/70 (70)	35/43 (81)	**0/30 (0)***	**<0.001**
At least one	107/112 (96)†	99/107 (93)	67/70 (96)	42/43 (98)	28/30 (93)	0.304
Severe neonatal morbidity	60/193 (31)‡	57/182 (31)¥	**56/122 (46)**	**41/84 (49)***	7/28 (25)	0.027
Severe cerebral injury$^{\psi}$	10/193 (5)‡	6/182 (3)¥	13/122 (11)*	8/84 (10)	0/28 (0)	0.098
Postnatal TAPS	66/89 (74)	**6/65 (9)***	36/51 (71)	36/43 (84)	-	**<0.001**
BT/PET at birth for TAPS$^{\psi}$	81/188 (43)§	**13/171 (8)*£**	60/118 (51)∫	48/84 (57)	0/23 (0)◊	**<0.001**

Data are presented as median (IQR) or n/N (%). To compare treatments, expectant management was set as a reference. Bold numbers represent significant p-values, an * indicates the smallest p-value that is presented in the p-value column.

† 1 missing value (1 infant with incomplete neonatal outcome) ‡ 8 missing values (same as '†' plus 3 cases that died shortly after birth, and 4 cases with unknown neonatal morbidity), § 13 missing values (same as '‡' plus 5 cases with unknown BT/PET information ¶ 5 missing values ¥ 10 missing values (as '¶' plus 5 cases with missing neonatal outcome) £ 21 missing values (same as '¥' plus 11 cases with missing data on BT/PET at birth), ∫ 4 missing values, ◊ 5 co-twin with missing data on BT/PET

$^{\psi}$ Statistical correction for non-occurring events is applied

IUT, intrauterine transfusion; GA, gestational age; BT, blood transfusion; PET, partial exchange transfusion; TAPS, twin anemia polycythemia sequence

Table 2b Outcome of expectant management, laser surgery, IUT (± PET), delivery and selective feticide for spontaneous TAPS twins

SPONTANEOUS TAPS	Expectant management (n = 51 pregnancies; 102 fetuses)	Laser surgery (n = 86 pregnancies; 172 fetuses)	IUT (± PET) (n = 26 pregnancies; 52 fetuses)	Delivery (n = 34 pregnancies; 68 fetuses)	Selective feticide (n= 19 pregnancies; 19 co-twins)	p-value
GA at birth (weeks)	33.6 (31.3-35.4)	**31.9 (29.1-34.4)**	31.3 (30.1-33.1)	32.2 (31.1-34.3)	**30.6 (27.2-35.5)***	**0.024**
Diagnosis-to-birth interval (weeks)	7.7 (2.5-15.4)	10.3 (6.7-14.0)	**2.4 (1.3-5.3)**	**0.3 (0.0-0.8)***	11.1 (3.6-16.3)	**<0.001**
Perinatal mortality	12/101 (12)†	26/168 (15)¶	2/52 (4)*	5/68 (7)	2/19 (11)	0.118
Fetal demiseΨ	5/102 (5)	20/168 (12)¶	2/52 (4)	0/68 (0)	2/19 (11)*	0.104
Neonatal mortalityΨ	7/96 (7)†	6/148 (4)¶	0/50 (0)*	5/68 (7)	0/17 (0)	0.165
Survivors						
None Ψ	1/50 (2) †	5/84 (6) ¶	0/26 (0)	0/34 (0)	2/19 (11)	0.178
One	8/50 (16) †	16/84 (19) ¶	2/26 (8)	5/34 (15)	**17/19 (89)***	**<0.001**
TwoΨ	41/50 (82) †	63/84 (75) ¶	24/26 (92)	29/34 (85)	**0/19 (0)***	**<0.001**
At least one	49/50 (98) †	79/84 (94) ¶	26/26 (100)	34/34 (100)	17/19 (89)	0.174
Severe neonatal morbidity	26/93 (28)‡	45/145 (31)¥	22/50 (44)	**32/67 (48)*∫**	4/17(24)	**0.046**
Severe cerebral injuryΨ	2/93 (2)‡	3/145 (2) ¥	4/50 (8)*	5/67 (7) ∫	0/17 (0)	0.099
Postnatal TAPS	31/46 (67)	4/51 (8)	17/24 (71)	28/34 (82)	-	**<0.001**
BT/PET at birth for TAPSΨ	36/89 (40)§	**9/135(7)***£	27/50 (54)	**40/67 (60)∫**	0/13 (0) ◊	**<0.001**

Data are presented as median (IQR) or n/N (%). To compare treatments, expectant management was set as a reference. Bold numbers represent significant p-values, an * represents the smallest p-value that is presented in the p-value column

† 1 missing value (unknown neonatal outcome), ‡ 4 missing values (same as '†' plus 3 cases with unknown neonatal outcome). § 8 missing values (same as '‡' plus 4 cases with missing information on BT/PET at birth ¶ 4 missing values (2 pregnancies missing outcome), ¥ 7 missing values (same as '¶', plus 3 infants with unknown neonatal morbidity). £ 17 missing values (same as '¥' plus 10 infants without BT/PET information)), ∫ 1 missing value (one infant died directly after birth) ◊ 5 missing values

Ψ Statistical correction for non-occurring events is applied

IUT, intrauterine transfusion; GA, gestational age; BT, blood transfusion; PET, partial exchange transfusion; TAPS, twin anemia polycythemia sequence

Table 2c Outcome of expectant management, laser surgery, IUT (± PET), delivery and selective feticide for post-laser TAPS twins

POST-LASER TAPS	Expectant management (n = 62 pregnancies; 124 fetuses)	Laser surgery (n = 24 pregnancies; 48 fetuses)	IUT (± PET) (n = 44 pregnancies; 88 fetuses)	Delivery (n = 9 pregnancies; 18 fetuses)	Selective feticide (n= 11 pregnancies; 22 fetuses)	p-value
GA at birth (wks)	32.6 (29.4-34.6)	31.7 (29.1-33.7) §	**29.9 (29.0-33.0)***	29.0 (27.7-31.8)	32.6 (31.13-34.0)	**0.027**
Diagnosis-to-birth interval (wks)	8.0 (4.7-14.3)	8.1 (5.9-11.4)	**4.8 (2.5-8.9)**	**0.3 (0.2-0.4)***	10.4 (9.2-14.4)	**<0.001**
Perinatal mortality$^{\Psi}$	27/124 (22)	12/47 (26) §	23/88 (26)	4/18 (22)	0/11 (0)*	0.217
Fetal demise$^{\Psi}$	19/124 (15)	8/47 (17) §	16/88 (18)	0/18 (0)*	0/11 (0)*	0.268
Neonatal mortality$^{\Psi}$	8/105 (8)	4/39 (10)	7/72 (10)	**4/18 (22)***	0/11 (0)	**0.040**
Survivors						
None$^{\Psi}$	4/62 (6)	3/23 (13)	3/44 (7)	1/9 (11)	0/11 (0)	0.111
One	19/62 (31)	4/23 (17)	16/44 (36)	2/9 (22)	**11/11 (100)***	**<0.001**
Two$^{\Psi}$	39/62 (63)	16/23 (70)	25/44 (57)	6/9 (67)	**0/11 (0)***	**<0.001**
At least one	58/62 (94)	20/23 (87)	41/44 (93)	8/9 (89)	11/11 (100)	0.111
Severe neonatal morbidity	34/100 (34)†	12/37 (32)¶	34/72 (47)	9/17 (53)* ∫	3/11 (27)	0.158
Severe cerebral injury$^{\Psi}$	8/100 (8)	3/37 (8)¶	9/72 (13)	3/17 (18)* ∫	0/11 (0)	0.141
Postnatal TAPS	35/43 (81)	**2/14 (14)***	19/27 (70)	8/9 (89)	-	**<0.001**
BT/PET at birth for TAPS$^{\Psi}$	45/99 (45)‡	**4/36 (11)*** ¥	33/68 (49)£	8/17 (47) ∫	0/10 (0) ◊	**0.011**

Data are presented as median (IQR) or n/N (%). To compare treatments, expectant management was set as a reference. Bold numbers represent significant p-values, an * represents the smallest p-value that is presented in the p-value column

† 5 missing values (2 infants died directly after birth, 3 infants with missing outcomes) ‡ 6 missing values (same as '†', plus one case with missing BT/PET information) § 1 missing value (unknown outcome) ¶ 2 missing values, ¥ 3 missing values (same as '¶' plus one case with missing BT/PET information) £ 4 missing values (4 neonates unknown BT/PET information). ∫ 1 missing values (1 infant died directly after birth) , ◊ 1 missing value

IUT, intrauterine transfusion; GA, gestational age; BT, blood transfusion; PET, partial exchange transfusion; TAPS, twin anemia polycythemia sequence

$^{\Psi}$ Statistical correction for non-occurring events is applied

Discussion

This is the first large international study investigating outcome after antenatal management for TAPS. We found that perinatal mortality and severe neonatal morbidity rates were high in all treatment groups. Management for TAPS varied considerably within and between fetal therapy centers, reflecting the lack of international consensus on the most optimal management strategy. With this study we present new information on treatment for TAPS, thereby providing a more detailed context to management decisions, leading to a more enhanced understanding of TAPS and the clinical implications of each treatment strategy.

Perinatal outcome

Confirming findings from previous smaller studies,[20-22] we found comparable perinatal mortality rates for all management strategies, for the total cohort as well as for spontaneous and post-laser TAPS separately. Notably, post-laser TAPS twins showed substantially higher rates of perinatal mortality than spontaneous TAPS twins in all management groups, illustrating the impact of preceding TTTS on the outcome of twins with post-laser TAPS. Severe perinatal morbidity rates were high in all groups, but were significantly increased in cases treated with IUT (± PET) or delivery. Notably, twins managed with IUT (± PET) were delivered at a significantly earlier gestation, which is known to have significant impact on short-term outcome.[10, 11] However, twins that had a delivery were born at a comparable gestation as twins treated with laser, which might suggest that other factors might also play a role. Our results show that expectant management, laser surgery and selective feticide generate a prolongation of pregnancy of 7-10 weeks after the diagnosis of TAPS. A prolonged pregnancy after laser surgery compared to expectant management and IUT (± PET) was previously reported by Slaghekke et al.[20] Our study shows that TAPS cases treated with IUT (± PET) had a significantly shorter diagnosis-to-birth interval. Although gestation can be prolonged by reintervention with IUT (± PET), the majority of TAPS cases had only one intervention. A possible explanation could be that due to the relatively high GA at diagnosis, caregivers preferred delivery with subsequent postnatal treatment over continuous exposure of TAPS, as soon as an acceptable gestation was achieved. The shortest diagnosis-to-birth interval was seen in the delivery group, in accordance with the management-group definition.

Optimal treatment?

Determining the most optimal treatment option is crucial to improve outcome in TAPS. Laser surgery is the only management option that directly treats the cause of TAPS, and has shown to drastically improve outcome in TTTS.[23] However, laser in TAPS is technically more challenging than in TTTS, due to the absence of TOPS, which may lead to reduced accessibility and visibility of the placental surface. This can be especially problematic in case of an anterior placenta. To optimize technical conditions, TOPS can be artificially created with amnioinfusion in one sac and amniodrainage of the other, but this requires more needle insertions and might increase chances of PPROM and premature birth. However, we report PPROM in 37% and delivery within 24 hours after laser in 4%, which is comparable to laser for TTTS.[3] A second technical problem comes with the size of TAPS anastomoses, which are known to be minuscule and might therefore be harder to find during procedure. In line, our data showed that TAPS recurred in 15% of cases treated with laser surgery, which is more than twice as high as the recurrence rate of TTTS after laser.[3] Moreover, we have shown that residual anastomoses after laser for TAPS always lead to the recurrence of the disease. To prevent residual anastomoses and to ensure coagulation of anastomoses that cannot be visualized, the Solomon technique might be of added value[3]. Nevertheless, the rate of residual anastomoses in TAPS was comparable to the rate of residual anastomoses in TTTS (both 19%),[3] and 43% of lasers were performed in cases with an anterior placenta, showing that, despite the practical limitations, laser for TAPS is technically feasible.

Although promising in approach, our data show that laser surgery does not seem to improve (nor deteriorate) perinatal outcome when compared to expectant management. However, laser surgery was associated with a high diagnosis-to birth interval, especially in contrast to treatment with IUT (± PET). As prematurity has a profound impact on short- and long-term health in TAPS twins, prolonging pregnancy is of utmost importance to improve outcome.[6, 7, 10, 11] Notably, a comparable prolongation of pregnancy was achieved with selective feticide and expectant management. However, selective feticide comes with a high price, as parents lose at least one baby and do not have a guarantee of healthy survival for the co-twin. Alternatively, in expectant management, prolonging of pregnancy likely results in continuous exposure to potential detrimental effects of TAPS, as only 16% showed spontaneous resolution. As risk for perinatal mortality and morbidity increases with incrementing

antenatal TAPS stage, definitive treatment with laser might be the most optimal intervention to improve perinatal outcome for this condition.[10, 11]

One should be extremely cautious with drawing conclusions based on the results of this registry. Due to the retrospective nature of this study, management groups are very likely to be subject to selection bias. As our data have indicated, management groups differed in terms of GA at diagnosis, severity of TAPS, and type of TAPS. Since higher TAPS stages and post-laser TAPS are associated with poorer prognosis, these factors could have significantly influenced perinatal outcome rates.[10, 11] Moreover, long-term outcome was not investigated in this study. Previous studies have shown that the detrimental effects of TAPS are not limited to the perinatal period, but also manifest later in life.[6, 7] Therefore, the true effect of management for TAPS can only be properly investigated when TAPS cases are randomized between treatment groups, when stratification for risk factors is applied, and when long-term consequences are taken into account.

In conclusion, this registry shows that there is an extensive heterogeneity in management for TAPS, both within and amongst fetal therapy centers. To improve outcome in TAPS, and to generate an international consensus on optimal management, a randomized controlled trial (RCT) is urgently needed. Recently, the TAPS Trial, an international multicenter open-label RCT comparing laser surgery to standard care (expectant management, IUT (± PET), preterm delivery) has started recruiting patients.[24]

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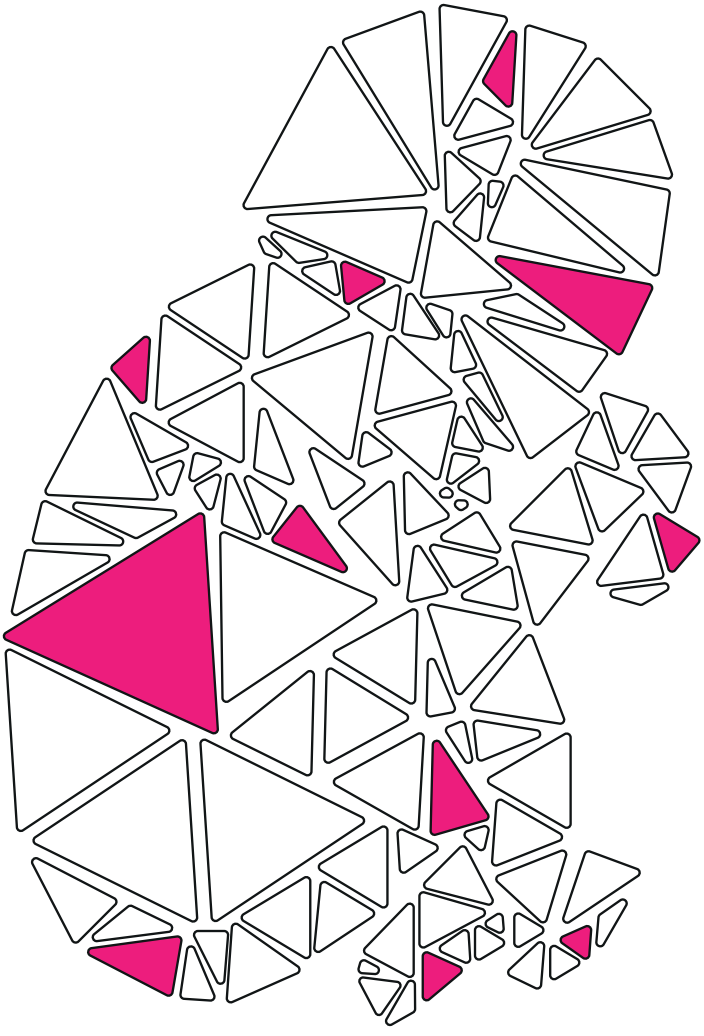
Appendix 1. Participating centers and the number of TAPS cases they contributed to this study

Center	Country	Number of TAPS cases
Leiden University Medical Center	The Netherlands	105
Leuven University Hospital	Belgium	41
Necker-Enfants Malades Hospital Paris	France	39
Children's Hospital V. Buzzi Milan	Italy	28
Center Medico-Chirurgical Obstetrical Strasbourg	France	23
Mount Sinai Hospital Toronto	Canada	22
Hospital Universitari Vall d'Hebron Barcelona	Spain	15
Saint George's Hospital London	United Kingdom	14
University of Texas McGovern Medical School at Houston	United States of America	14
Medical University of Graz	Austria	13
University Medical Center Hamburg-Eppendorf	Germany	13
Mater Hospital Brisbane	Australia	12
Brugmann University Hospital	Belgium	8
Birmingham Women's and Children's NHS Foundation Trust	United Kingdom	6
V.I. Kulakov National Medical Research Center of Obstetrics, Gynecology and Perinatology Moscow	Russia	6
Yale New Haven Hospital	United States of America	6
Karolinska University Hospital Stockholm	Sweden	5



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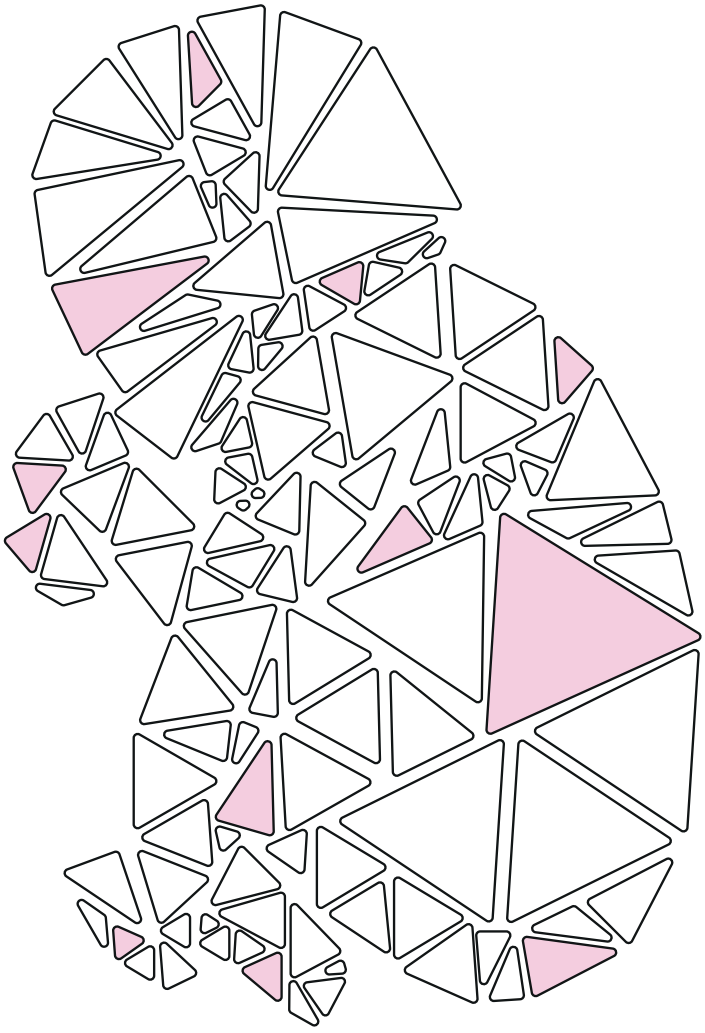
Chapter 6

The TAPS Trial: fetoscopic laser surgery for twin anemia polycythemia sequence - an open-label international multicenter randomized controlled trial (protocol)



Abstract

Background

Twin anemia polycythemia sequence (TAPS) can arise from chronic unbalanced feto-fetal blood flow through miniscule vascular placental anastomoses in monochorionic twins, leading to anemia in the TAPS donor and polycythemia in the TAPS recipient. TAPS is associated with severe short- and long-term outcomes. Management options for TAPS include expectant management, preterm delivery, intra-uterine transfusion in the donor with or without a partial exchange transfusion in the recipient, fetoscopic laser surgery of the placental anastomoses and selective feticide. The best treatment option for TAPS is unknown. We propose to conduct a multi-center open-label randomized controlled trial to assess if fetoscopic laser surgery improves the outcome of TAPS compared to standard care.

Methods

We will randomly assign 44 monochorionic twin pregnancies with TAPS ≥ stage 2, diagnosed between 20+0 and 27+6 weeks of gestation, to the fetoscopic laser surgery group or the standard treatment group (IUT/PET, expectant management, preterm delivery), using a web-based application with a computer-generated list with random permuted blocks, stratified by gestational age at inclusion (20+0-23+6 weeks vs. 24+0-27+6 weeks) and TAPS type (spontaneous vs. post-laser TAPS). Primary outcome will be gestational age at birth; secondary outcomes include a composite of perinatal mortality and severe neonatal morbidity, haematological complications, procedure-related complications and long-term neurodevelopmental outcome at the corrected age of 2 years. Analysis will be intention-to-treat.

Discussion

As TAPS is associated with adverse short- and long-term outcome, investigation of the optimal antenatal management is of utmost importance. This study seeks to gather evidence on the potential beneficial role of laser surgery in the outcome of twins diagnosed with TAPS.

Trial Registration:

NL6879, available at https://www.trialregister.nl/trial/6879

Background

Monochorionic twins share a single placenta and are connected to each other through inter-twin vascular anastomoses, which allow the blood to transfer bidirectionally between the two fetuses. Unbalanced net inter-twin blood transfusion may lead to various complications, including twin-twin transfusion syndrome (TTTS) and twin anemia polycythemia sequence (TAPS). TTTS was first described in the 19th century and results from an imbalanced inter-twin blood flow causing hypovolemia and oligohydramnios in the donor and hypervolemia and polyhydramnios in the recipient twin, the so-called twin oligohydramnios-polyhydramnios sequence (TOPS). TAPS is a newly described form of chronic and slow inter-twin blood transfusion through minuscule placental anastomoses leading to anemia in the TAPS donor and polycythemia in the TAPS recipient, without sings of TOPS.[1] TAPS may occur spontaneously in 3-5% of monochorionic twin pregnancies (spontaneous TAPS) or may develop in 2-16% of TTTS cases after incomplete laser surgery of the placental equator resulting in a few small residual anastomoses (post-laser TAPS).[2-6]

TAPS can be diagnosed either antenatally or postnatally. Antenatal diagnosis is based on discrepant Doppler ultrasound measurements of the middle cerebral artery - the peak systolic velocity (MCA-PSV), showing an increased MCA-PSV in the donor twin suggestive of fetal anemia, and decreased velocities in the MCA-PSV in the recipient, suggestive of polycythemia. An inter-twin MCA-PSV differences > 0.5 MoM is indicative for the diagnosis of TAPS.[7] Postnatal diagnosis is based on a large inter-twin hemoglobin difference (> 8 g/dL), and at least one of the following: reticulocyte count ratio > 1.7 (reticulocyte count of donor/ reticulocyte count of recipient) or minuscule placental anastomoses (diameter < 1 mm), detected through color dye injection of the placenta after birth.[8, 9] Since TAPS is a heterogeneous disease, a classification system for both antenatally and postnatally diagnosed TAPS is proposed to help discriminate between different forms of TAPS (Table 1 & 2).

Table 1. Antenatal TAPS classification

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 and MCA-PSV recipient < 0.8 MoM, without other signs of fetal compromise
Stage 3	As stage 1 or 2, with cardiac compromise of the 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 the umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in the ductus venosus

Table 2. Postnatal TAPS classification

Postnatal stage	Inter-twin Hb difference, g/dL
Stage 1	> 8.0
Stage 2	> 11.0
Stage 3	> 14.0
Stage 4	> 17.0
Stage 5	> 20.0

Neonatal outcome in TAPS may vary from isolated large inter-twin hemoglobin differences to severe cerebral injury and neonatal death.[10-12] Due to polycythemia-hyperviscosity syndrome, TAPS recipients are at risk of developing thrombocytopenia and ischemic limb injury.[8, 13] TAPS donors have increased chances to develop hypoalbuminemia, hypoproteinemia, leukopenia and short-term renal dysfunction.[14-16] Long-term outcome studies show that severe neurodevelopmental impairment occurs in 9% of TAPS survivors.[17, 18] TAPS donors had an increased risk for adverse outcome and showed high rates of cognitive delay and hearing problems.

Antenatal management options for TAPS include expectant management, fetoscopic laser coagulation of the vascular equator of the placenta, intra-uterine blood transfusion (IUT) in the donor with or without a partial exchange transfusion (PET) in the recipient, induced preterm delivery and selective feticide. However, the best management option for TAPS is not clear. Fetoscopic laser coagulation of the anastomoses at the vascular equator of the placenta is

the only causative treatment. Although laser surgery is proven to be effective in decreasing perinatal mortality and morbidity in TTTS,[19] data on the beneficial effect of laser therapy in TAPS are scarce. Importantly, laser surgery in TAPS may be technically more challenging than in TTTS, due to the absence of TOPS, preventing optimal visualization of the vascular equator. An alternative treatment option is performing an IUT in the anemic donor. However, IUT is only a symptomatic treatment and therefore reintervention up to 2-4 times (depending on the gestational age at diagnosis, severity of the disease and occurrence of complications) might be necessary. Moreover, a potential side effect of IUT is worsening of polycythemia in the recipient. To reduce the risk of increasing polycythemia a combination procedure of IUT in the donor and PET in the recipient can be of additional value. However, despite its temporary character, IUT (with PET) may be easier to perform and technically more feasible than laser therapy.

There are only a few studies on outcome of laser therapy for TAPS pregnancies.[20-24] In a small multicenter retrospective study where laser treatment is compared to IUT or expectant management, laser therapy appeared to improve the perinatal outcome by prolonging the pregnancy and reducing the incidence of respiratory distress syndrome.[25] Sananes et al. found the same result in a single center prospective cohort study, comparing in-utero therapy (laser surgery and IUT) to expectant management in TAPS twins.[26] A recent analysis of our own data, performed in October 2018 for the purpose of this trial proposal, shows that laser therapy might have a beneficial effect on gestational age at birth, severe neonatal morbidities and long-term neurodevelopmental outcome when compared to IUT(+ PET) or expectant management (Table 3) However, in all the above-mentioned studies analyses, study groups were small (N= 6 – 15) and due to the retrospective character of these analyses, data might have been subject to selection bias.

We therefore propose to conduct a randomized controlled trial to evaluate the possible beneficial effect of fetoscopic laser surgery and to determine the most optimal management option for TAPS.

Table 3. The outcome of TAPS twins ≥ stage 2, gestational age at diagnosis < 28w, based on the analysis of our own data (2003-2018) in October 2018.

	Expectant (N=16)	**IUT (+PET) (N=16)**	**Laser (N=15)**
GA at birth	31.0 (27.8-24.6)	28.2 (25.8-31.7)	32.3 (30.1-35.7)
Fetal demise	3/32 (9)	6/32 (19)	5/30 (17)
Neonatal mortality	5/32 (16)	2/32 (6)	0/30 (0)
Perinatal survival	24/32 (75)	24/32 (75)	25/30 (83)
Severe neonatal morbidity	12/28 (43)	10/16 (39)	2/25 (8)
NDI	10/19 (53)	6/19 (32)	2/18 (11)
Severe NDI	4/19 (21)	1/21 (5)	1/18 (6)

Data are presented as median (IQR) or n/N (%)
IUT = intrauterine transfusion, PET = partial exchange transfusion, GA = gestational age, NDI = neurodevelopmental impairment

Methods

Aim

The primary aim of this trial is to investigate whether laser surgery will prolong pregnancy in TAPS twins. Prolonging pregnancy is known to be of paramount importance for neonatal and long-term neurodevelopmental outcome. Low gestational age at birth is independently associated with increased risk for severe neonatal morbidity.[27, 28] In addition, we will study perinatal mortality, neonatal morbidity, hematological complications, procedure-related complications and long-term neurodevelopmental outcome in this population.

Design

The design of this study will be a multicenter open-label randomized controlled trial. We will randomly assign monochorionic twin pregnancies diagnosed with TAPS ≥ stage 2 between 20+0-27+6 weeks of gestation to the fetoscopic laser surgery group or the control group, using a web-based application (*Castor*) with a computer-generated list with random permuted blocks, stratified by gestational age at inclusion (20+0 – 23+6 weeks vs. 24+0-27+6 weeks) and TAPS type (spontaneous vs. post-laser TAPS). Analysis will be performed according to the intention-to-treat principle. The outcome adjudication will be performed blinded to group allocation.

Participants

Our study population will consist of patients diagnosed with a monochorionic twin pregnancy complicated with TAPS. In order to be eligible to participate in this study, the patient must meet all of the following criteria (1) having monochorionic twin pregnancy complicated by either spontaneous or post-laser TAPS, stage ≥ 2, diagnosed between 20+0 - 27+6 weeks of gestation (2) being 18 years or more (3) be able to give written informed consent. Women were not eligible when one of the following criteria was applicable (1) TAPS stage 1 (2) TAPS stage ≥ 2, diagnosed within 1 week after laser surgery for TTTS (3) TAPS diagnosed in a triplet or higher order multiple pregnancy (4) TAPS who already underwent an intrauterine treatment (except for laser surgery for TTTS in post-laser TAPS cases) (5) TAPS with co-existent congenital anomalies (including severe cerebral injury) in one or both twins.

Procedure

Eligible women will be randomly allocated to the experimental group or the control group. Women allocated to the experimental group will receive laser therapy within 72 hours after randomization. Women allocated to the control group will be treated with standard care. Standard care includes IUT (with or without PET), preterm delivery or expectant management, depending on the judgement of the fetal surgeon and the preference of the patient. In both groups, antenatal, peripartum and postnatal care of the mother will be similar to that of uncomplicated monochorionic twin pregnancies. After birth, a full blood count (including reticulocyte count) is performed as part of the standard postnatal care for TAPS twins. In addition, all participants will be asked for permission to inject the placenta with color dye. During neonatal period, no additional follow-up examinations other than the regular care for premature neonates will be performed. At the corrected age of 2 years (corrected age is defined as chronological age in months – #months premature), all TAPS survivors of both groups will undergo a neurological examination and an assessment of cognitive and motor development (BSID-III) and parents will fill in a screening list for developmental delay (ASQ) and a checklist for child behavior (CBCL). An overview of the design of the study, including a timeframe for assessment for each outcome, is depicted in Figure 1.

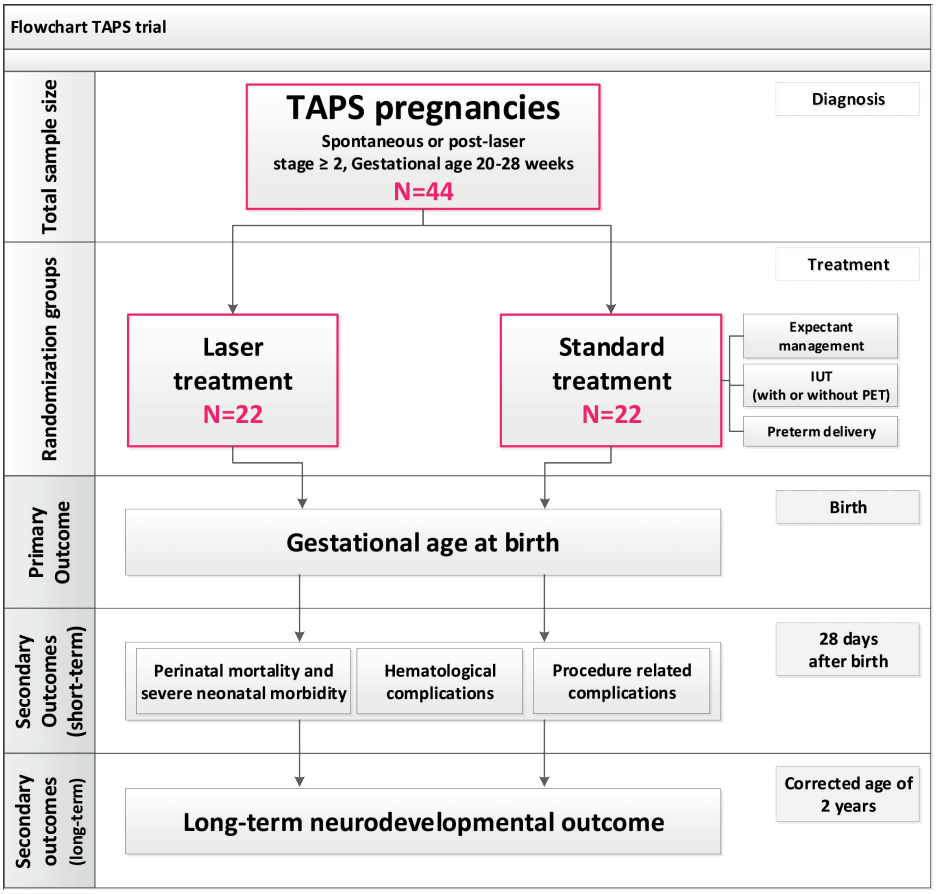


Figure 1. Overview of the design of the TAPS Trial, including a time frame for assessment for each outcome parameter. TAPS; twin anemia polycythemia sequence; IUT, intrauterine transfusion; PET, partial exchange transfusion; GA, gestational

Experimental treatment

In the experimental group, laser surgery must be performed within 72 hours after randomization. Fetoscopic laser surgery will be done under local or regional anesthesia by experienced fetal surgeons. Each surgeon has performed at least 60 previous laser procedures and is competent to undertake the Solomon technique. First, by continuous ultrasound visualization, a cannula will be introduced 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 will be used. After identifying the vascular anastomoses, a 400 µm or 600 µm laser fiber connected to a diode or Nd:YAG laser device

(Dornier MedTech, Westling Germany) will be introduced trough the opening sheath. All visible anastomoses will be coagulated using one to three bursts of a few seconds each with a power setting of 20-50 W, depending on the vessel diameter. After coagulation of all the visible anastomoses, the Solomon technique will be performed: to connect the white areas that resulted from coagulation of the anastomoses, a thin line of tissue at the placental surface will be coagulated from one edge of the placenta to the other at a power setting of 20-50 W. Follow-up will consist of close monitoring with ultrasound including Doppler measurements of the MCA-PSV at least biweekly.

Standard treatment

In the control group the choices of treatment include expectant management, IUT (with or without PET) or preterm delivery, depending on the judgment of the fetal surgeon with regard to the gestational age and stage of TAPS. Treatment with laser surgery will not be performed in the control group; this procedure will solely be performed in the experimental group.

- *Expectant management:* Expectant management will consist of close monitoring with ultrasound including Doppler measurements of MCA-PSV, at least every week. Since no (intrauterine) intervention is performed to mitigate or resolve the symptoms of TAPS, twins managed expectantly might deteriorate during the course of pregnancy. Depending on the judgement of the caretaker regarding the condition of the fetuses, ultrasound evaluation can be performed more frequent and admission to the hospital for fetal monitoring with cardiotocography (CTG) can take place. In case of progression, the caretaker can decide to perform IUT (with or without PET) or preterm delivery.

- *IUT (with PET):* Treatment with IUT can be performed either intravascularly into the umbilical cord, intrahepatic in umbilical vein or indirectly via the peritoneal cavity. The choice of transfusion site is left up to the caretaker, and will be based on the position of the fetus. Since IUT is not a definitive treatment and is only a temporary solution, the donor may become anemic and the recipient even more polycythemic, and the MCA-PSV levels may return to their pre-transfusion levels. Therefore, repeated IUT may be required. In case of polycythemia in the recipient, a combination of IUT in the donor with PET in the recipient may be envisaged. With PET, 5-10 ml of

the recipient's blood will be removed slowly and will be replaced with saline, repeatedly. A follow-up scan will be performed the same day or following day and one week after intervention to check for the condition of the fetuses. Further follow-up during the course of the pregnancy will be the same as in the pregnancies managed expectantly and will consist of close monitoring with ultrasound including Doppler measurements of the MCA-PSV at least every week.

- *Preterm delivery:* Generally, all monochorionic twin pregnancies receive induction of labor or cesarean section at 36 weeks of gestation. However, the caretaker might opt for a preterm delivery in some cases.
    - Criteria for performing a preterm delivery (for both the experimental and control group) are: signs of fetal distress (suboptimal or abnormal CTG in one or both fetuses, worsening of the condition of one or both fetuses without the option of (re)intervention with IUT (+PET)), intrauterine infection or iatrogenic monoamnionicity.

Outcome variables

The primary outcome will be gestational age at birth (in completed weeks and additional days). The secondary outcomes will include a composite outcome of perinatal mortality or severe neonatal morbidity, hematological complications, procedure-related complications, and long-term neurodevelopmental outcome at the corrected age of 2 years. Perinatal mortality will be defined as fetal demise or neonatal death within 28 days after birth. Severe neonatal morbidity is based on the presence of at least one of the following: respiratory distress syndrome requiring mechanical ventilation or surfactant, proven early onset neonatal sepsis (positive blood cultures within 72 hours post-partum), retinopathy of prematurity (ICROP stage 3 or higher[29]), necrotizing enterocolitis (stage 2 or higher[30]), patent ductus arteriosus requiring medical therapy or surgical closure, and/or severe cerebral injury. Severe cerebral injury was diagnosed if one of the following was identified on cerebral imaging: intra-ventricular hemorrhage (grade 3 or higher[31]), cystic periventricular dilatation (> 97th percentile[32]), porencephalic or parenchymal cysts, or other severe cerebral lesions associated with adverse neurological outcome. Hematological complications were defined as: anemia in the donor requiring a blood transfusion within 24 hours after birth, polycythemia in the recipient requiring

a partial exchange transfusion within 24 hours after birth, necrotic skin injury, limb ischemia, thrombocytopenia (platelet count < 150/microL), severe hypoalbuminemia (albumin levels < 20 g/dL), severe hypoproteinemia (protein levels < 40 g/L). The definition of procedure-related complications was based on one of the following: amniotic band syndrome, iatrogenic monoamnionicity, preterm premature rupture of the membranes, placental abruption, clinical chorioamnionitis, histological chorioamnionitis and/or funisitis. Long-term neurodevelopmental outcome at the corrected age of 2 years was assessed with the following instruments: Bayley Scales of Infant and Toddler Development version 3 (BSID-III)[33], and/or Ages and Stages Questionnaire (ASQ-3)[34], Child Behavior Checklist (CBCL 1.5-5y).[35] Neurodevelopmental impairment (NDI) was defined as: cerebral palsy (gross motor function classification system (CMFCS) grade 1[36]), cognitive or motor delay (score < 85 according to BSID-II(33)), impaired functioning in communication/fine and gross motor/problem solving personal and social functioning (score > 1SD below the mean according to the ASQ-3[34]), severe visual loss (blind or partially sighted), severe hearing loss (needing hearing aids). Severe NDI is classified as: cerebral palsy (GMFCS > grade 1[36]), severe cognitive or motor delay (score < 70 according to the BSID-III[33]), severely impaired functioning in communication/fine and gross motor/problem solving/personal and social functioning (as assessed with the ASQ-3[34]), bilateral blindness (visual acuity of less than 3/60 in the better eye[37]), bilateral deafness (severe or profound hearing loss in both ears[38]). Behavioral problems were defined as a T-score ≥ 64 for one of the following broad band scales: total problem score, internalizing problems (anxious/depressed, withdrawn, somatic complaints), externalizing problems (rule-breaking, aggressive behavior) as assessed with the CBCL 1.5-5y.[35]

Data collection of non-randomized patients

In order to demonstrate a representative sample of included patients, coded data of non-randomized patients will be collected. Data will include maternal, fetal and neonatal baseline characteristics as well as outcome parameters: gestational age at birth, perinatal mortality and severe neonatal morbidity, hematological complications and procedure related complications. The collected data for the non-randomized patients will include the same parameters as used for the randomized patients. Data will be stored in the Castor database.

Sample-size calculation

Based on the analysis of 95 TAPS twins stored in in our database between 2003-2017, we expect a prolongation of pregnancy of 3 weeks for the group treated with laser therapy (mean gestational age at birth: 32 weeks) compared to the group treated with standard care (mean gestational age at birth: 29 weeks). Based on these expected values, group sample sizes of 22 achieve 80% power to detect a difference of 3 weeks between the null hypothesis that both groups means are 29 weeks of gestational age, and the alternative hypothesis that the mean gestational age of the laser group is 32 weeks, with estimated group standard deviations of 3.1 and 3.5 and with a significance level (alpha) of 0.05 using a two-sided Mann-Whitney U test. With the expected number of TAPS pregnancies referred to at least 5 participating centers, this would mean an inclusion period of 2.5-3 years.

Statistical analyses

Continuous data will be reported as median (interquartile range) and will be compared between groups using the Mann-Whitney U test. Proportions will be reported as n/N (%) and compared between groups using the χ^2 test or the Fisher's exact test where appropriate. Differences with a p-value of less than 0.05 will be regarded as statistically significant. To account for the fact that observations between co-twins are not independent, outcome for management groups will be compared using the Generalized Estimated Equation (GEE) module.

Discussion

According to previous literature, TAPS is associated with high rates of perinatal mortality and short- and long-term morbidity, mainly in TAPS donors.[12, 17, 18] A strong risk factor for adverse outcome is gestational age at birth. In order to improve the outcome for TAPS twins, it is of paramount importance to determine the most optimal management strategy for this condition. Fetoscopic laser surgery is the only treatment that is aimed at treating the cause of TAPS and prolonging pregnancy. In the TAPS Trial, a multicenter open-label randomized controlled trial, we will investigate the possible beneficial effect of laser treatment on gestational age at birth, perinatal mortality and neonatal morbidity, and long-term outcome.

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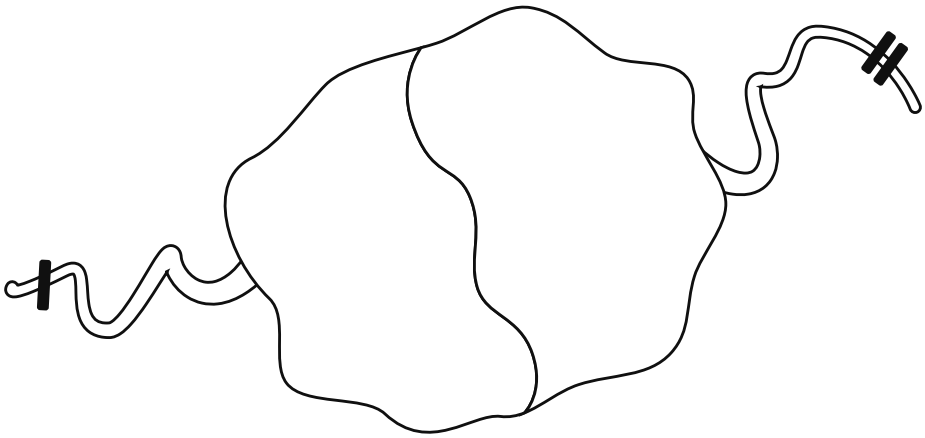
PART 7

postnatal diagnosis



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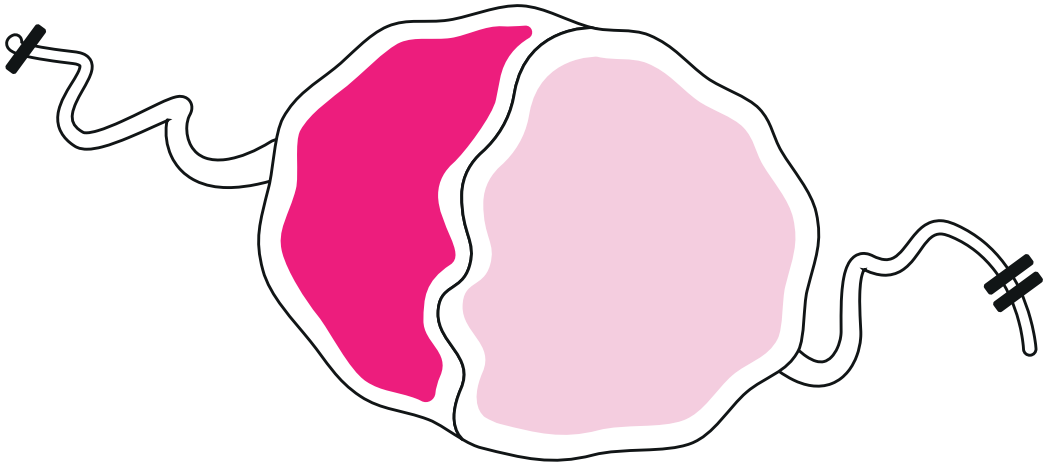
Fetal Diagnosis and Therapy, 2016; 40: 123-127

Chapter 7

Color difference in placentas with twin anemia polycythemia sequence: an additional diagnostic criterion?



Abstract

Objective

To determine the color intensity difference between the two placental shares in monochorionic placentas with twin anemia polycythemia sequence (TAPS).

Methods

We evaluated all digital pictures of TAPS placentas examined at our center and compared them to a control group of uncomplicated monochorionic placentas. We determined the color intensity of the individual placental shares of the maternal side of the placenta using an image-processing program and calculated the color difference ratio (CDR).

Results

Digital pictures of 19 TAPS and 19 uncomplicated monochorionic placentas were included in this study. The TAPS group consisted of 12 spontaneous TAPS placentas (63%) and 7 post-laser TAPS placentas (37%). The median CDR in the group with TAPS was significantly higher than in the control group, 2.73 (range 1.73-6.36) versus 1.09 (range 1.00-1.35), respectively ($p < 0.01$). A CDR value > 1.5 was found in all TAPS placentas and in none of the control-group placentas. We found a positive correlation between CDR and inter-twin hemoglobin difference ($R = 0.83$, $p < 0.01$), which was mainly present in the TAPS group ($R = 0.66$, $p < 0.01$) and not in the control group ($R = 0.04$, $p = 0.87$)

Conclusion

TAPS placentas have a significantly higher CDR compared to uncomplicated monochorionic twin placentas. Large inter-twin hemoglobin differences are associated with higher CDR.

Introduction

Twin anemia-polycythemia sequence (TAPS) is a recently described form of chronic feto-fetal transfusion, characterized by large inter-twin hemoglobin differences, without signs of twin-twin transfusion syndrome (TTTS).[1] TAPS may occur spontaneously in 3-5% of monochorionic pregnancies (spontaneous TAPS)[2], or after laser surgery for TTTS in 2-13% of TTTS cases (post-laser TAPS).[3, 4] The pathogenesis of TAPS is based on the presence of few, minuscule arterio-venous anastomoses allowing a slow transfusion of blood from the donor to the recipient and leading gradually to discordant hemoglobin levels.[1] The postnatal criteria for TAPS are based on inter-twin hemoglobin difference > 8 g/dL, and at least one of the following: reticulocyte count ratio > 1.7 and/or presence of few minuscule placental vascular anastomoses determined through color dye injection.[5] However, the reticulocyte count ratio is not always measured and placental injection is difficult to perform. This prompted us to search for an alternative diagnostic criterion that could be more practical and readily available for all practitioners involved in the care of monochorionic twins throughout the world. Anecdotal reports have shown that the maternal side of TAPS placentas is also characterized by a striking difference in color between the pale placental share of the anemic donor and the plethoric share of the recipient.[6, 7] The difference in thickness and echodensity has also been noted antenatally during ultrasound examination.[1]

The objective of this study is to determine the color difference between the two placental shares in a cohort of TAPS placentas compared to a control group of uncomplicated monochorionic placentas and to discuss the potential value of this new and alternative diagnostic criterion.

Methods

All monochorionic placentas examined at our center (Leiden University Medical Center) are routinely injected with colored dye and photographed according to a previously published protocol.[7] In the last 5 years, an additional evaluation was added to the protocol and included a routine picture of the maternal side of the placenta. For this study, we included all TAPS placentas evaluated at our center in which a clear digital picture of the maternal side of the placenta was recorded. All pictures were taken under the same (light) conditions. Each

TAPS placenta (spontaneous TAPS and post-laser TAPS) was compared with a consecutive control placenta from uncomplicated monochorionic twin pregnancies, in which a digital picture of the maternal side was taken. TAPS was diagnosed using the previously described postnatal criteria: an inter-twin hemoglobin difference > 8g/dL and at least a reticulocyte count > 1.7 or small vascular anastomoses (diameter < 1mm) at the placental surface.[5]

The following obstetric and neonatal data were retrieved from our database, including gender, gestational age at delivery, type of delivery, hemoglobin levels and reticulocyte count at birth. For the purpose of this study, we excluded placentas with insufficient picture quality and placentas with incomplete data.

The primary outcome of this study was the color difference ratio (CDR) between the two placental shares of the maternal side of each placenta from TAPS or uncomplicated monochorionic twins. The CDR was measured using a freely available image-processing program called ImageJ version 1.48 (http://imagej.nih.gov/ij/download.html). The digital picture of the placenta was first converted to a red spectrum channel in order to measure the intensity of the red (placenta) color. Measurement areas of the placenta share of each twin were selected manually. Two color-intensity histograms of the different placental shares were then obtained. The x-axis of the color-intensity histogram displays a continuous red color scale (with values ranging from 0-255), whereas the y-axis displays the number of pixels corresponding with that particular shade of red. A peak on the color-intensity histogram reflects the mode, which represents the color that is most present in the area selected. CDR is determined by dividing the mode of the color-intensity histogram with the higher peak by the mode of the color-intensity histogram with lower peak. A step-by-step tutorial on measuring CDR can be watched at https://www.youtube.com/watch?v=_OSd6utv2Bw. An example of CDR measurement and matching color intensity histograms is depicted in Figure 1.

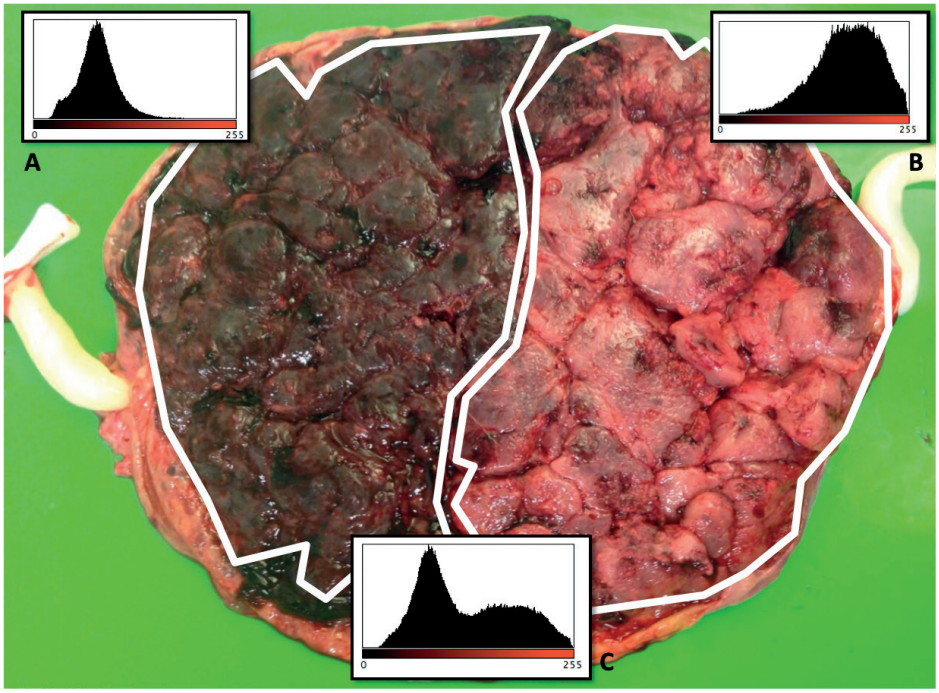


Figure 1. Maternal side of a TAPS placenta showing the difference in color between the plethoric share of the recipient (left side of the placenta) and anemic share of the donor (right side). Histograms show the color intensity from the two placental shares (A is recipient, B is donor) and from the entire placenta (C).

Statistics

Continuous variables were compared using an independent sample *t*-test. The Spearman rank correlation test was used to study the association between the inter-twin hemoglobin differences and CDR in the TAPS group and control group. A p-value less than 0.05 was considered to indicate statistical significance. Statistical analysis was performed using IBM SPSS Statistics v20.0 (SPSS, Inc., an IBM company, Chicago, IL, USA).

Results

A total of 22 TAPS placentas were eligible for this study. We excluded 3 placentas due to an insufficient picture quality (n=2) and incomplete data (n=1). A total of 19 TAPS placentas and 19 uncomplicated monochorionic placentas were analyzed.

The TAPS group consisted of 12 spontaneous TAPS placentas (63%) and 7 post-laser TAPS placentas (37%). The median gestational age at delivery was 33 weeks (interquartile range (IQR) 30-35 weeks) in the TAPS group and 36 (IQR 33-37) in the control group. Further details on the baseline characteristics are shown in Table 1.

Table 1 Baseline characteristics in monochorionic twin pairs with spontaneous TAPS and uncomplicated monochorionic twin pairs

	TAPS group (N=19)	Control group (N=19)
Female	7/19 (37)	9/19 (47)
Gestational age at delivery, weeks	33 (30-35)	36 (33-37)
Cesarean delivery	11/19 (58)	8/19 (42)
Birth weight, g	1790 (1500-2350)	2222 (1575-2750)

Data are displayed as median (IQR) or n/N (%)/ TAPS, twin anemia polycythemia sequence

Details on the CDR and hematological characteristics in the TAPS group and the control group are shown in Table 2. An inter-twin Hb difference > 8 g/dL was found in all TAPS twin pairs (median 12.7; range 10.0-21.8) and in none of the uncomplicated monochorionic twin pairs (median 1.6; range 0.2-7.7). Reticulocyte count ratio was measured in 84% (16/19) of the TAPS twin pairs (median 2.8; range 1.0-6.1) and in 100% (19/19) of the uncomplicated monochorionic twin pairs (median 1.1; range 0.7-1.6). The median CDR in the group with and without TAPS was 2.73 (range 1.73-6.36) and 1.09 (range 1.00-1.35), respectively (p<0.01).

Table 2 Hematological differences and placenta share color difference between monochorionic twin pairs with TAPS and uncomplicated monochorionic twin pairs

	TAPS group (N=19)	Control group (N=19)	P values
Inter-twin Hb difference, g/dL	12.7 (11.5-17.5)	1.6 (0.6-5.7)	<0.01
Reticulocyte count ratio	3.8 (2.6-4.9)	1.1 (1.0-1.2)	<0.01
CDR	2.74 (2.05-4.13)	1.09 (1.01-1.21)	<0.01

Data are shown as median (IQR); Hb: hemoglobin

Figure 2 shows the CDRs in both TAPS and uncomplicated monochorionic twin placentas. All TAPS placentas had a CDR value > 1.5, whereas none of the CDR values of the control group was > 1.5. CDR measurements and inter-twin Hb difference values were positively correlated (Spearman correlation

coefficient = 0.83, p < 0.01). This positive correlation was mainly present in the TAPS group (R=0.66, p<0.01), but not in the control group (R = 0.04, p = 0.87).

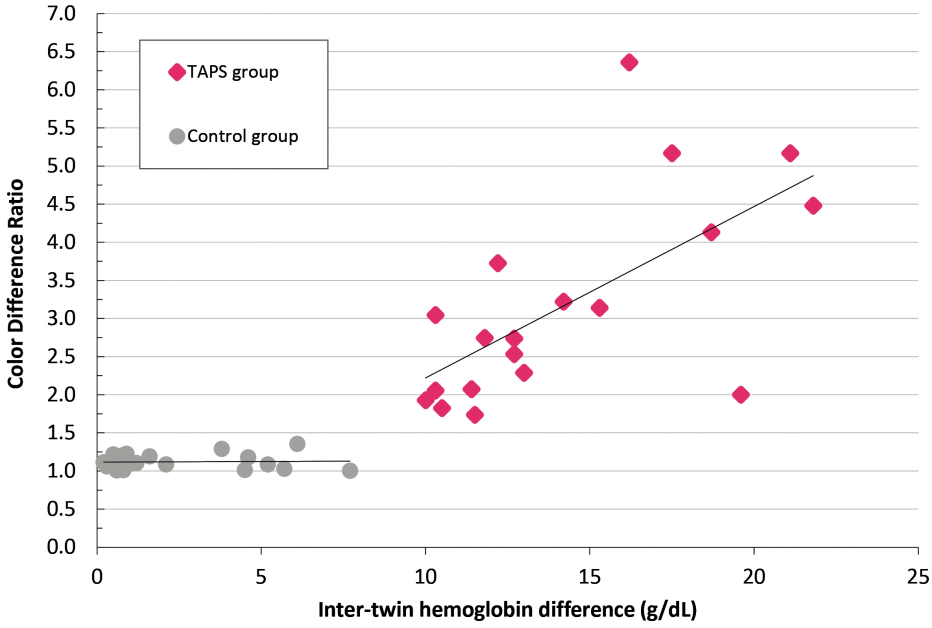


Figure 2. Color difference ratio versus inter-twin hemoglobin difference in the TAPS group and control group of uncomplicated monochorionic twin placentas. Correlation between CDR and inter-twin hemoglobin difference in the TAPS group and control group is R=0.66 (p=0.002) and R=0.04 (p=0.873), respectively.

Discussion

This study shows that TAPS placentas are characterized by a higher color difference ratio (CDR) compared to uncomplicated monochorionic twin placentas. In analogy with the color of the neonates at birth, the placental share of the recipient twin in TAPS is typically dark and plethoric and the placenta share of the donor twin is pale and anemic. In this first pilot study of 38 placentas, all TAPS placentas had a CDR > 1.5 whereas the CDR in the control group of uncomplicated monochorionic placentas was always < 1.5, suggesting that a cut-off of 1.5 could be useful to differentiate both groups. In addition, we found a positive correlation between CDR and inter-twin hemoglobin difference, suggesting a strong association between larger inter-twin hemoglobin difference and higher CDR. However, this correlation was

mainly present in the TAPS group and not in the control group of monochorionic twins. Whether measurements of CDR in placentas can be added as a new diagnostic criterion to the list of postnatal diagnostic criteria for TAPS requires further investigation.

The current postnatal diagnostic criteria for TAPS are based on the presence of an inter-twin hemoglobin difference > 8 g/dL and at least one of the following: a reticulocyte count ratio > 1.7 and/or the presence of small anastomoses (diameter < 1mm) on the placental surface.[5] However, reticulocyte count is not always measured and placental injection is difficult to perform and therefore only done by a few specialized centers. In addition, evaluation of the fetal side of the placenta after color dye injection may sometimes be hampered by leakage of color dye or damage of the placental vessels preventing accurate evaluation of the vascular anastomoses. As shown in this study, measurements of the color difference between the two placenta shares using a freely available image-processing program, ImageJ, could prove to be of additional value in the diagnosis of TAPS. These measurements made using a digital picture of the placenta were quick and easy to perform. Some important technical specifications are required to perform accurate measurements, such as ensuring that blood clots on the maternal side of the placentas are washed out before taking the picture and avoiding direct light shining on the placentas to prevent reflections.

Whether this method can eventually be added to the list of postnatal criteria requires further investigations in larger series of placentas with and without TAPS to determine the sensitivity and specificity of the test. Importantly, CDR measurements should also be investigated in placentas with acute TTTS to determine whether this method can help distinguish TAPS cases from cases with acute peripartum TTTS. In both situations, large inter-twin hemoglobin differences are present at birth.[5] However, inter-twin transfusion in TAPS is a chronic process leading to chronic anemia in the donor (reflected by the presence of increased reticulocyte count) and chronic polycythemia in the recipient. In contrast, inter-twin transfusion in acute peripartum TTTS is an acute event leading to acute anemia in the donor and hypovolemic shock in case of massive blood loss, and acute polycythemia in the recipient.[8] Neonatal management in both situations requires a different approach with acute interventions in acute TTTS (including acute blood transfusion in the donor)

in contrast to a more conservative approach in case of chronic transfusion in TAPS. Whether the examination of the maternal side of the placenta will help perinatologists in the future to make a quick distinction between acute peripartum TTTS and TAPS remains to be established.

In conclusion, this new and promising method shows that TAPS placentas have a large color difference compared to placentas from uncomplicated monochorionic twins. However, our data should be interpreted with care due to the retrospective nature of the study and the relatively small sample size. Further investigations are needed to determine the sensitivity and specificity of this test and to examine whether measurement of color difference would also help distinguishing between TAPS and acute peripartum TTTS.

Acknowledgements

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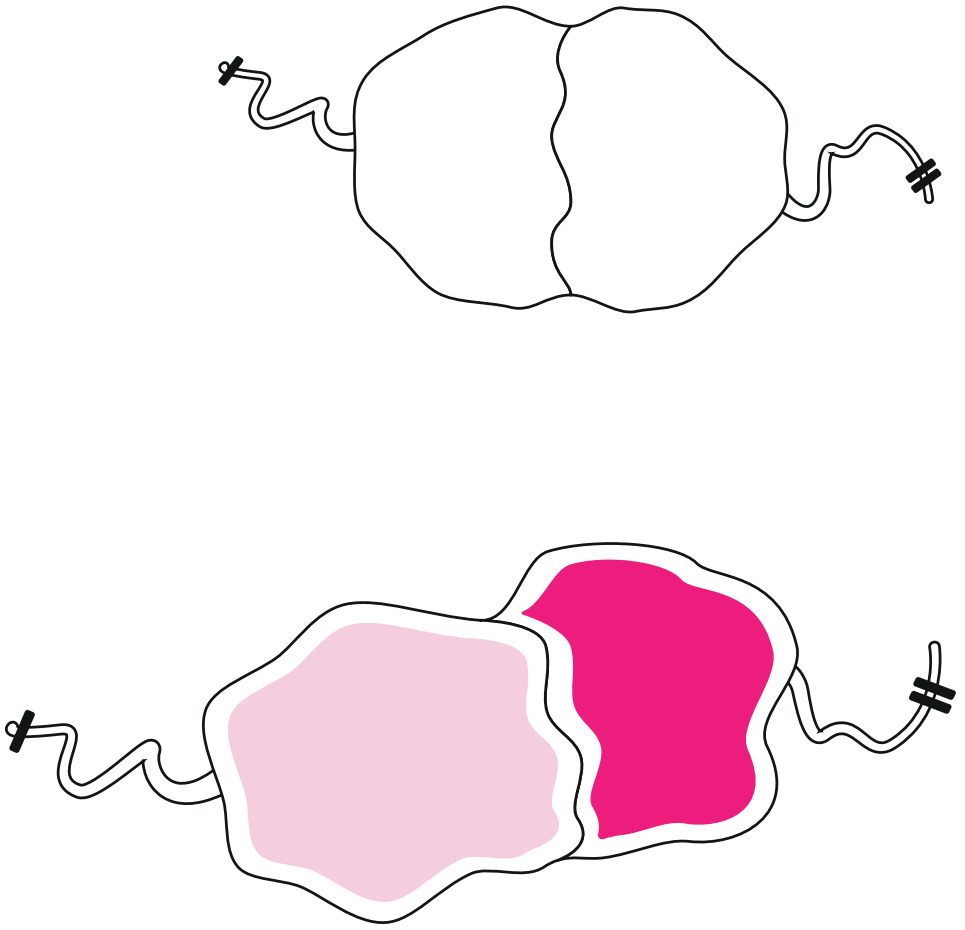

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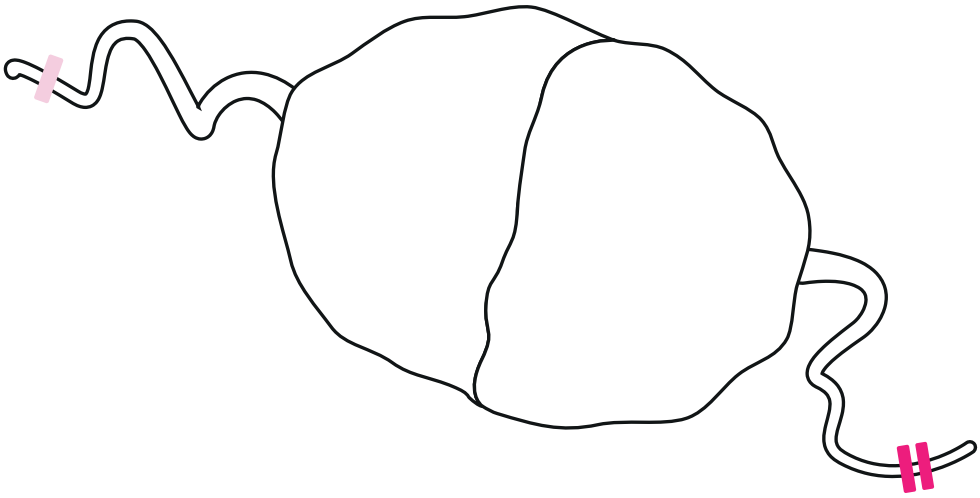
Placenta, 2017; 57: 189-193

Chapter 8

Can color difference on the maternal side of the placenta distinguish between acute peripartum twin-twin transfusion syndrome and twin anemia polycythemia sequence?



Abstract

Objective

To investigate the color difference between two placental shares in monochorionic placentas with acute peripartum twin–twin transfusion syndrome (TTTS) and twin anemia polycythemia sequence (TAPS).

Methods

We evaluated all digital pictures of TAPS, acute peripartum TTTS and a control group of uncomplicated monochorionic placentas examined at our center. We determined the color intensity of the individual placental share on the maternal side of each monochorionic placenta using an image–processing program and calculated the color difference ratio (CDR).

Results

Digital pictures of 5 acute peripartum TTTS, 25 TAPS and 54 control group placentas were included in this study. The median CDR in acute peripartum TTTS was significantly lower compared to TAPS placentas, 1.20 (inter-quartile range (IQR) 1.05–1.20) and 2.50 (IQR 1.85–3.34), respectively ($p < 0.01$), and was comparable to the control group (CDR 1.11, IQR 1.05-1.22).

Conclusion

TAPS placentas have a higher CDR compared to acute peripartum TTTS placentas. Examining color difference on the maternal side of the placenta might help distinguish between acute peripartum TTTS and TAPS.

Introduction

Monochorionic twins share a single placenta and are connected to each other through vascular anastomoses, allowing inter-twin blood transfusion. Unbalanced net inter-twin blood transfusion can lead to various disorders, including chronic twin–twin transfusion syndrome (TTTS), acute peripartum TTTS and twin anemia polycythemia sequence (TAPS).

Chronic TTTS, the most well-known form of TTTS, is characterized by the development of oligohydramnios in the donor and polyhydramnios in the recipient. This chronic form of TTTS occurs in 10% of monochorionic twin pregnancies and is most often diagnosed during the second trimester.[1] Acute peripartum TTTS results from a rapid and large inter-twin blood transfusion from donor to recipient through large anastomoses during delivery,[2, 3] and complicates 2.5% of the monochorionic twin pregnancies.[4]

In contrast to acute peripartum TTTS, TAPS is characterized by a chronic and slow blood transfusion from donor to recipient through miniscule vascular anastomoses during the course of pregnancy, causing the donor to become anemic and the recipient to become polycythemic, without discordances in amniotic fluid[5]. TAPS may occur spontaneously (spontaneous TAPS) in 2–5% of the monochorionic twin pregnancies or after laser surgery for chronic TTTS (post-laser TAPS) in 3–16% of the chronic TTTS cases.[6-9]

Distinction at birth between acute peripartum and TAPS may be difficult. In both cases, twins show a striking difference in skin color (a pale anemic donor and a plethoric polycythemic recipient twin) and a large difference in hemoglobin (Hb) levels (> 8 g/dL).[4, 5] Nevertheless, the required therapeutic approach is different in acute peripartum TTTS and TAPS. Therefore, distinction between the two conditions is of utmost importance. Measurement of the reticulocyte count ratio and injection of the placenta with color dye are two methods currently used to differentiate between acute peripartum TTTS and TAPS.[10-12] However, reticulocyte count is not always measured and placental injection is a complex and time-consuming procedure and is therefore only performed in specialized medical centers.

A previous study showed that measuring the color difference ratio (CDR) between the two placental shares of the maternal side can provide additional diagnostic information,[13] as TAPS placentas are characterized by a large CDR.

To date however, there are no reports on color differences in placentas from acute peripartum TTTS.

The aim of this study is to examine the CDR on the maternal side of acute peripartum TTTS placentas and to investigate whether this tool can help distinguish between acute peripartum TTTS and TAPS.

Methods

All monochorionic diamniotic twin placentas with a clear digital picture of the maternal side evaluated at our center between 2002–2016 were included in this retrospective study, and subdivided into three groups: 1.) acute peripartum TTTS placentas 2.) TAPS placentas (spontaneous and post-laser) and 3.) a control group of uncomplicated monochorionic twin placentas. Some of the cases we included were already used in a previous study.[13]

For the purpose of this study, digital pictures with insufficient quality or with incomplete Hb values were excluded. The quality of the picture was considered insufficient in case of unequal light exposure, low resolution, excessive light reflection or the presence of blood clots on the placenta.

TAPS was diagnosed using the following criteria: an inter-twin Hb difference > 8 g/dL and a reticulocyte count ratio > 1.7 or the presence of only miniscule anastomoses (diameter < 1 mm) detected through placental injection.[14] Diagnosis of acute peripartum TTTS was based on the presence of an inter-twin Hb difference > 8 g/dL and no signs of TAPS or chronic TTTS (according to the internationally accepted standardized antenatal ultrasound criteria for TTTS.[15]

The following obstetric and neonatal data were retrieved from our database: gestational age at birth, antenatal intervention, mode of delivery, sex, birth weight, Hb levels and reticulocyte count at birth and the presence of anastomoses. The presence of anastomoses was examined through placental color dye injection. The primary outcome of this study was the color difference ratio (CDR) between the different shares of the maternal side of the placenta. CDR was calculated using an image processing program called Image J version 1.57. A step-by-step tutorial on calculating the CDR using Image J created by our institution can be viewed at: https://www.youtube.com/watch?v=_OSd6utv2Bw

Data are reported as medians and interquartile rages (IQR). Since the size of the groups was small and data were not normally distributed, non-parametric tests were used. A Kruskal–Wallis test was applied to compare the results of the three different groups. To study the association between the inter-twin Hb differences and CDR the Spearman rank correlate on test was used. A p-value < 0.05 was considered to indicate statistical significance. Statistical analysis was performed using IBM Statistics v23.0 (SPSS, Inc., an IBM company, Chicago, IL, USA).

Results

A total of 108 pictures of the maternal side were considered eligible for this retrospective study. We excluded 1 acute peripartum TTTS case due to an insufficient picture quality (blood clots and unequal light exposure). In the control group 23 cases were excluded because of insufficient picture quality (n = 2) and missing Hb values (n = 21). In total, 5 acute peripartum TTTS, 25 TAPS, and 54 control group placentas were analyzed. The TAPS group consisted of 14 spontaneous TAPS (56%) placentas and 11 post-laser TAPS placentas (44%). Figure 1 provides an overview of the selection of the study population.

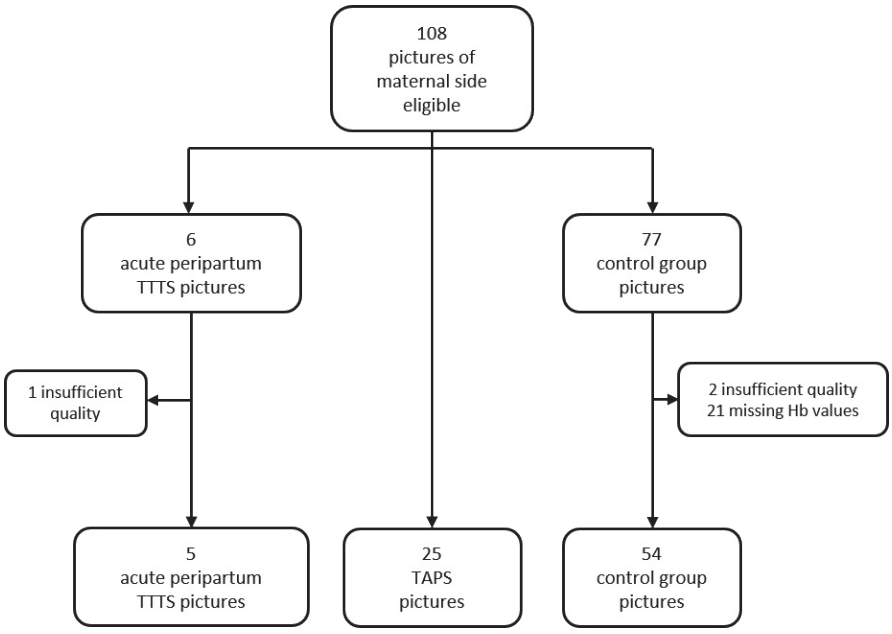


Figure 1. Description of the study population

The median gestational age in the acute peripartum TTTS group was 37 weeks (IQR 33–37 weeks), 32 weeks (IQR 30–35 weeks) in the TAPS group and 35 weeks (IQR 32–36 weeks) in the control group. Baseline characteristics of the three groups are presented in Table 1.

Table 1. Baseline characteristics

	Control group (n = 54)	**Acute peripartum TTTS (n =5)**	**TAPS (n = 25)**
Female	30/53 (57)	1/5 (20)	10/24 (42)
Gestational age at birth (weeks)	35 (32–36)	37 (34–37)	32 (30–35)
Cesarean Delivery	61/108 (57)	4/10 (40)	28/50 (56)
Birth weight (g)	2104(1610–2424)	2665 (1987–2850)	1722 (1223–2125)
Birthweight discordance	11 (5-21)	2 (0-8)	18 (10-31)
Post-natal TAPS stage			
Stage 1			6 (24)
Stage 2			8 (32)
Stage 3			4 (16)
Stage 4			4 (16)
Stage 5			3 (16)
Spontaneous TAPS			14 (56)
Antenatal diagnosis			9 (64)
Antenatal treatment (with IUT)			3 (21)
Post-laser TAPS			11 (44)
Antenatal diagnosis			8 (73)
Antenatal treatment (with IUT)			4 (36)

Data are median (IQR), n/N(%) or n (%)

Table 2 shows hematological characteristics and the CDR for the three different groups. Reticulocyte count ratio was measured in 60% (3/5) of the acute peripartum TTTS cases, 92% (23/25) of the TAPS cases and 41% (22/54) of the cases in the control group. In cases in which reticulocyte count ratio was not available, placental injection was used to fulfill the required diagnostic criteria for TAPS or acute peripartum TTTS. In agreement with the postnatal diagnostic criteria for TAPS, all TAPS cases had an inter-twin Hb difference > 8 g/dL, median 12.7 (IQR 10.5–18.1), and a reticulocyte count ratio > 1.7, median 3.7 (IQR 2.5–5.0). In the acute peripartum TTTS group, all 5 cases showed an inter-twin Hb difference > 8.0 g/dL and the median reticulocyte count ratio was 1.1

(IQR 1.0-1). In the control group, all 54 cases had an inter-twin Hb difference < 8 g/dL.

Table 2. Hematological differences and placental color differences (CDR)

	Control group (n = 54)	Acute peripartum TTTS (n = 5)	TAPS (n = 25)	P-values
Inter-twin Hb difference (g/dL)	1.4 (0.6–3.8)	10,2 (8.6–13.1)	12.7 (10.5–18.1)	< 0.01
Reticulocyte count ratio	1.05 (1.00–1.14)	1.1 (1.0-*)	3.7 (2.5–5.0)	< 0.01
Color difference ratio (CDR)	1.11 (1.05–1.22)	1.20 (1.05–1.20)	2.50 (1.85–3.45)	< 0.01

Data are median (IQR) or mean ± SD
*Since there were only three reticulocyte count ratios available for the acute peripartum TTTS group, the upper quartile could not be measured (reticulocyte count ratios were: 1.0, 1.1 and 1.4)

The median CDR in the acute peripartum TTTS group was 1.20 (IQR 1.05–1.20) compared to the median of 2.50 (IQR: 1.85–3.45) in the TAPS group and a median of 1.11 (1.05–1.22) in the control group (p < 0.01). There was no significant difference in CDR between spontaneous TAPS cases and post-laser TAPS cases (p = 0.149).

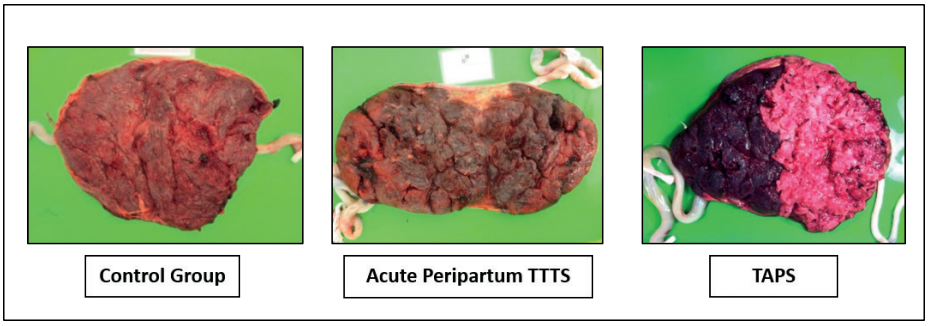


Figure 2. Maternal side of a control group placenta, an acute peripartum TTTS placenta and a TAPS placenta, illustrating the color difference in TAPS cases.

Figure 2 shows the maternal side of a control group placenta, acute peripartum TTTS placenta and a TAPS placenta. In Figure 3, pictures of the maternal side of the placenta are shown for ascending CDR values to illustrate the increasing difference in color intensity.

* Upper quartile could not be measured since only three reticulocyte count ratios (1.0, 1.1 and 1.4) were available.

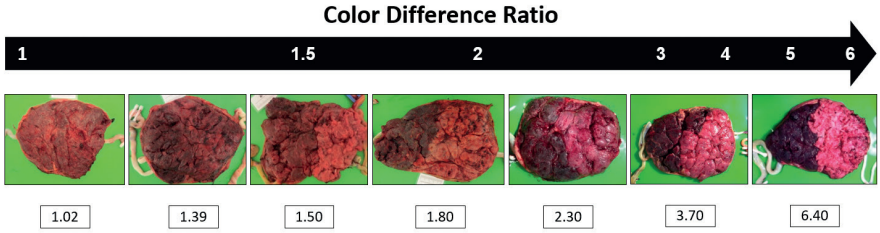


Figure 3. Pictures of the maternal side for ascending CDR values showing the increasing difference in color intensity between the two maternal shares.

In Figure 4, the relation between CDR and inter-twin Hb difference for the acute peripartum TTTS, TAPS and the control group is depicted. All the CDR values of the TAPS group were larger than 1.5, except for one post-laser TAPS case (CDR = 1.3). In this case an inter-twin Hb difference of 9.3 g/dL was found, corresponding with TAPS stage 1.[11] All control group cases showed a CDR < 1.5, with the exception of one case (CDR = 1.5). This placenta belonged to a twin with selective intra-uterine growth restriction (sIUGR), with an inter-twin Hb difference of 3.8 g/dL and a high reticulocyte count ratio (3.7). In all acute peripartum TTTS cases a CDR < 1.5 was found. Median CDR in the spontaneous TAPS group was 2.6 (IQR 2.0–4.7) and 2.1 (IQR 1.5–3.0) in the post-laser TAPS group ($p = 0.14$). As shown in Figure 4, there was no correlation between CDR and inter-twin Hb difference in the control group ($R = 0.18$, $p = 0.098$) or the acute peripartum TTTS group ($R = 0.63$, $p = 0.253$). In the TAPS group, there was a positive correlation between inter-twin Hb difference and CDR ($R = 0.58$, $p < 0.01$).

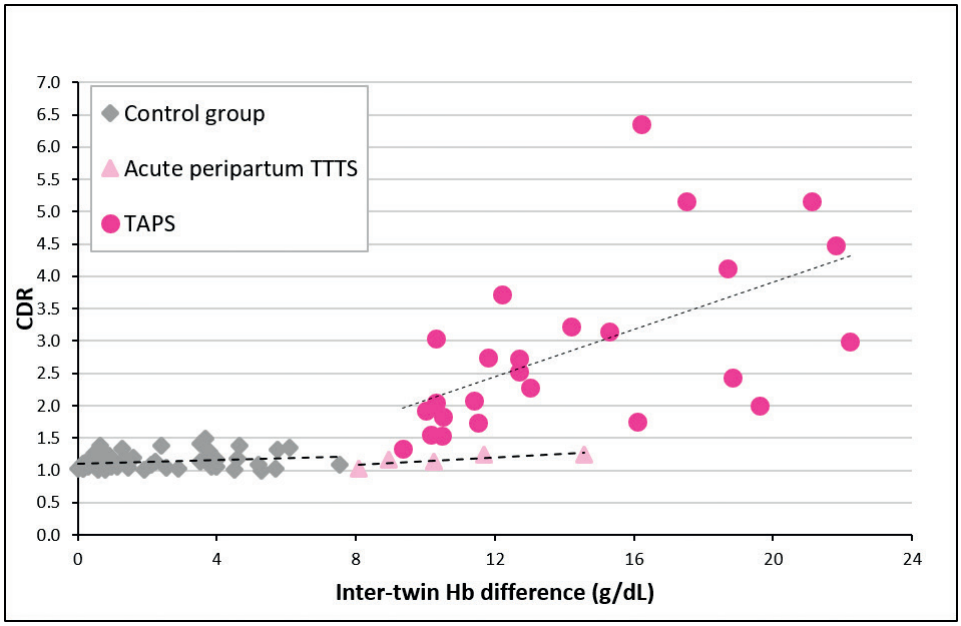


Figure 4. CDR versus inter-twin Hb difference in the control group, acute peripartum TTTS group and TAPS group. Correlation between CDR and inter-twin Hb difference in the control group, acute peripartum TTTS group and TAPS group is R = 0.18 (p = 0.098), R = 0.63, (p = 0.316) and R = 0.58 (p < 0.01), respectively.

Discussion

This is the first study reporting on color differences on the maternal side of acute peripartum TTTS placentas. We found a significantly lower median CDR in acute peripartum TTTS placentas compared to TAPS placentas ($p < 0.01$). Furthermore, all acute peripartum TTTS placentas had a CDR < 1.5, whereas almost every TAPS placenta (except one) had a CDR higher than 1.5.

Our study suggests that inspection of the maternal side of the placenta may help distinguish between acute peripartum TTTS and TAPS. Although twins with acute peripartum TTTS and TAPS present similarly at birth (highly discordant Hb values and the combination of a polycythemic plethoric recipient twin and an anemic pale donor twin), the two clinical conditions are different and require distinct neonatal management. Acute peripartum TTTS is characterized by an acute blood flow through large anastomoses during delivery leading to acute anemia and hypovolemic shock (in case of massive blood loss) in the donor and acute polycythemia in the recipient.[4] In contrast, TAPS is a result of slow and chronic blood transfusion through miniscule vascular anastomoses

(diameter < 1 mm) during the course of pregnancy, leading to chronic anemia in the donor and chronic polycythemia in the recipient.[5] Consequently, neonatal management in the donor twin of either the acute peripartum TTTS group or the TAPS group, calls for a different approach. Donors with acute peripartum TTTS suffer from acute blood loss and may thus need an acute blood transfusion within the first few hours after birth.[4] In contrast, since TAPS donors are diagnosed with a chronic form of TTTS, they would benefit from a more conservative therapeutic approach, with slower blood transfusion or, in case of sufficient erythropoiesis, even no blood transfusion at all.[16] In TAPS donors, acute blood transfusion is not only contra-indicated, it may even cause hemodynamic complications as these neonates are not hypovolemic. Therefore, a quick distinction between acute peripartum TTTS and TAPS shortly within one hour of birth could be helpful.

At the moment, there are two different methods available to distinguish between acute peripartum TTTS and TAPS. The first one includes calculating the reticulocyte count ratio by dividing the reticulocyte count of the donor by the reticulocyte count of the recipient.[12] In TAPS donors, the reticulocyte count is significantly increased, reflecting a constant high demand for erythrocytes due to chronic blood loss. Since blood transfusion in acute peripartum TTTS occurs rapidly, this compensating mechanism does not have time to take place, and therefore the reticulocyte count in the anemic twin is not increased.[4] Thus, an inter-twin reticulocyte count ratio > 1.7 is only indicative for the diagnosis of TAPS. The second criterion regards the size of the connecting vascular anastomoses detected through placental color dye injection.[14] In TAPS, only a few miniscule vascular anastomoses (diameter < 1 mm) at the placental surface are present.[11] On the contrary, in acute peripartum TTTS anastomoses with a large diameter are crucial to allow a rapid and large transfusion of blood.

However, reticulocyte count is not always measured and placental injection is a complex and time-consuming procedure and thus only performed by specialized medical centers. An additional quick and easy diagnostic tool could therefore be useful. In a previous study, we discovered that the maternal side of the placenta might contain important additional diagnostic information, by showing that TAPS placentas are characterized by a striking color difference on the maternal side.[13] The current study was set up to investigate whether this color difference was also present in acute peripartum TTTS cases. We conjectured

that there was no color difference on the maternal side in acute peripartum TTTS placentas, since blood transfusion only occurs acutely. Our results show indeed a significantly lower CDR in acute peripartum TTTS placentas compared to TAPS placentas. This finding underlines the idea that the visual examination of the maternal side of the placenta shortly after birth is an easy and quick way to distinguish between acute peripartum TTTS and TAPS.

The exact mechanism responsible for coloration (or discoloration) of the placenta remains unknown. Possibly, the level of erythrocytes and the time erythrocytes are present in the placenta play an important role. In the TAPS group, we found a positive correlation between inter-twin Hb difference and CDR, suggesting that the more discordant the hemoglobin values are, the more likely the placental mass was to change in color intensity. Furthermore, acute peripartum TTTS cases (in which a high inter-twin Hb difference is also seen) did not show color differences on the maternal side, supporting the idea that not only the degree of hemoglobin discordance, but also the time the placenta is exposed the low or high counts of erythrocytes is of influence. Unfortunately, placental histology is not routinely performed in our center. Histologic investigation of TAPS and uncomplicated monochorionic placentas could be of additional value in further unveiling the underlying mechanisms for coloration of the placental mass.

Our data should be interpreted with care due to the retrospective character of this study and the low sample size, especially in the acute peripartum TTTS group. Additional (prospective) studies with a larger group of acute peripartum TTTS placentas are required to help confirm the benefits of visual examination of the maternal side of the placenta for differentiation between TAPS and acute peripartum TTTS.

In conclusion, this study shows that there is a significant difference in CDR between acute peripartum TTTS placentas and TAPS placentas. TAPS placentas were characterized by a striking color difference whereas acute peripartum TTTS placentas showed no color difference on the maternal side. We strongly encourage caretakers in the obstetrical and neonatal field to examine the maternal side when a pale and plethoric monochorionic twin pair is delivered and obstetrical data is lacking or inconclusive. In contrast to reticulocyte count measurement and placental injection, visual inspection of color difference on the maternal side of the placenta can be performed on-site, shortly after

delivery of the placenta. Although the clinical presentation of the twins should be leading in determining the optimal neonatal management, the outcome of this study shows that color difference on the maternal side of the placenta could serve as an additional visual diagnostic tool, thus leading to better supported decision making regarding optimal neonatal care.

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PART 8

short-term outcome

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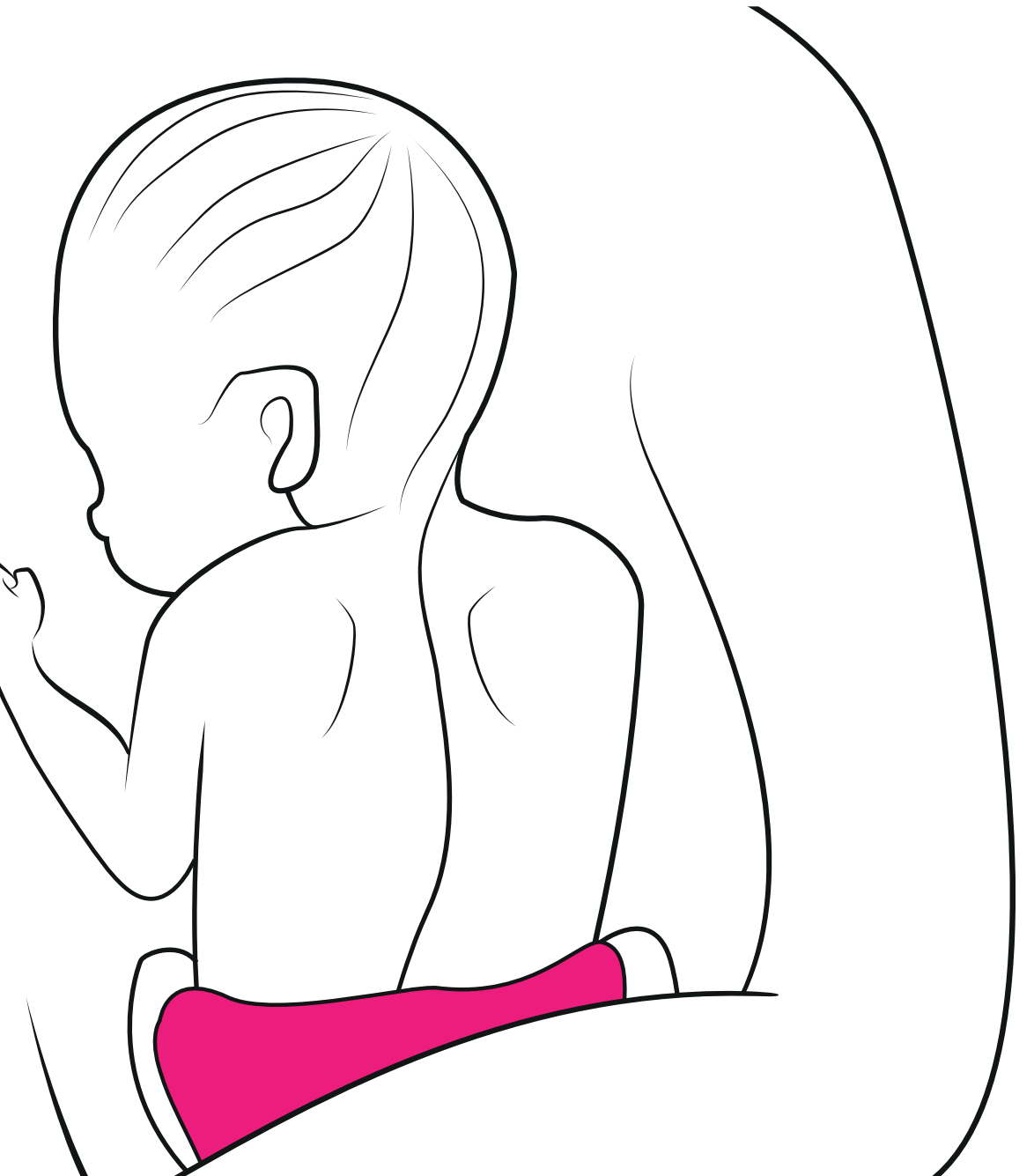
Chapter 9

Spontaneous twin anemia polycythemia sequence: management and outcome in an international cohort of 249 cases



Abstract

Objective

To investigate the management and outcome in spontaneous twin anemia polycythemia sequence (TAPS).

Methods

Data of the international TAPS Registry, collected between 2014-2019, were used for this study. The primary outcomes were perinatal mortality and severe neonatal morbidity. Secondary outcomes included a risk factor analysis for perinatal mortality and severe neonatal morbidity.

Results

A total of 249 spontaneous TAPS cases were included in this study, of which 219 (88%) were diagnosed antenatally and 30 (12%) postnatally. TAPS was diagnosed antenatally at a median gestational age (GA) of 23.7 weeks (IQR: 19.7-28.8; range: 15.1-35.3). Antenatal management included laser surgery in 39% (86/219), expectant management in 23% (51/219), delivery in 16% (34/219), intrauterine transfusion (with partial exchange transfusion) in 12% (26/219), selective feticide in 8% (18/219) and termination of pregnancy in 1% (3/219). Median gestational age at birth was 32.3 weeks (IQR: 30.1-34.9, range: 18.7-39.6). Perinatal mortality rate was 15% (72/493) for the total group; 22% (54/243) for donors and 7% (18/242) for recipients ($p < 0.001$). Severe neonatal morbidity occurred in 33% (141/432) of TAPS twins, being similar for donors (32%; 63/196) and recipients (33%; 75/228), $p= 0.628$. Independent risk factors for spontaneous perinatal mortality were donor status (OR = 3.8, 95%CI 1.9-7.5, $p < 0.001$), antenatal TAPS stage (stage 2: OR = 6.3, 95%CI 1.4-27.8, $p = 0.016$; stage 3: OR 9.6, 95%CI 2.1-45.5, $p = 0.005$; stage 4: OR 20.9, 95%CI 3.0-146.4, $p = 0.002$) and GA at birth (OR = 0.8, 95%CI 0.7-0.9, $p = 0.001$). Independent risk factors for severe neonatal morbidity were antenatal TAPS stage 4 (OR = 7.9, 95%CI 1.4-43.3, $p = 0.018$) and GA at birth (OR = 1.7, 95%CI 1.5-2.1, $p < 0.001$).

Conclusions

Spontaneous TAPS can develop at any time in pregnancy, from the beginning of the second trimester to the end of the third. Management for TAPS varies considerably, with laser surgery being the most frequent intervention. Perinatal mortality and severe neonatal morbidity were high, the former especially in donor twins.

Introduction

Twin anemia polycythemia sequence (TAPS) is a chronic form of unbalanced feto-fetal transfusion through minuscule placental anastomoses in monochorionic twins, leading to anemia in the TAPS donor and polycythemia in the TAPS recipient.[1] In contrast to twin-twin transfusion syndrome (TTTS), TAPS develops in the absence of twin oligohydramnios-polyhydramnios sequence (TOPS). TAPS can occur spontaneously in up to 5% of monochorionic twins.[2] The optimal antenatal treatment for TAPS has yet to be determined, but options include expectant management, preterm delivery, intrauterine transfusion (IUT) with or without a partial exchange transfusion (PET), fetoscopic laser surgery and selective feticide.[3, 4] Perinatal outcome in TAPS may vary between isolated hemoglobin differences to severe cerebral injury and perinatal death.[5, 6] Due to the low incidence of TAPS, studies investigating perinatal outcome are scarce with current data based on small cohort studies. Limited knowledge on optimal management and short- and long-term outcome restricts adequate parental counseling and informed decision-making. To improve our knowledge on TAPS, we set up the TAPS Registry, a large international collaboration aimed at collecting data on diagnosis, treatment and outcome in TAPS twins.

In the current study, the data from this TAPS Registry were used to (1) examine characteristics of diagnosis, management and outcome in twins with spontaneous TAPS, (2) to compare perinatal outcome between donors and recipients and (3) to investigate potential risk factors for adverse perinatal outcome.

Methods

The TAPS Registry was established in 2014 and utilised a web-based registry for anonymous data collection (www.tapsregistry.org). Fetal therapy centers across the world were invited to participate. Participating centers were supplied with personal credentials to enter data of their TAPS cases into the online registry. Between 2014 and 2019, 17 specialized fetal therapy centers contributed to data collection (see Appendix 1).

Monochorionic twin pregnancies diagnosed with spontaneous TAPS were eligible for this study. Cases with post-laser TAPS (TAPS after incomplete laser for TTTS) were excluded from this study and are described in a separate study.[7]

The antenatal diagnosis for TAPS was based on discordant fetal middle cerebral artery peak systolic velocity (MCA-PSV) measures, with an increased MCA-PSV being defined as > 1.5 Multiples of the Median (MoM) in the TAPS donor, suggestive of fetal anemia, combined with a decreased MCA-PSV measure (< 1.0 MoM) in the TAPS recipient, suggestive of fetal polycythemia, and without signs of TOPS.[8] TAPS was diagnosed postnatally in cases with an inter-twin hemoglobin difference > 8.0 g/dL in conjunction with least one of the following: a reticulocyte count ratio > 1.7 or the presence of only minuscule vascular anastomoses (diameter < 1 mm) detected through color dye injection of the placenta.[9, 10]

The following data were retrieved from local medical records: gravidity, parity, location of the placenta, moment of diagnosis (antenatal or postnatal), gestational age (GA) at diagnosis, TAPS stage at diagnosis and the presence of additional ultrasound findings including starry-sky liver in the recipient and/or difference in placental echogenicity, and mode of delivery. The severity of antenatal TAPS was assessed in agreement with the previously published staging system by Slaghekke et al.[11] For antenatal management for TAPS, the type of management was recorded: expectant management, delivery (defined as a delivery within 7 days after diagnosis), IUT (± PET), fetoscopic laser surgery, selective feticide, termination of pregnancy (TOP). Additionally, information on placental color dye injection was collected, including the type (arterio-venous (AV), veno-arterial (VA), arterio-arterial (AA), veno-venous (VV)), number and size of (residual) anastomoses. For perinatal outcome, the following parameters were obtained: donor/recipient status, birth weight, hemoglobin and reticulocyte values, treatment with blood transfusion or partial exchange transfusion on day one, presence of severe neonatal morbidities and/or severe cerebral injury and occurrence of perinatal mortality.

The primary outcomes were perinatal mortality and severe neonatal morbidity. Perinatal mortality was defined as fetal demise or neonatal death within 28 days after birth. As a part of fetal demise is intentional in the context of selective feticide or TOP, a distinction is made between spontaneous fetal demise and intended fetal demise. Severe neonatal morbidity was a composite measure and defined as the presence of at least one of the following, detected within 28 days after birth or before discharge to home: respiratory distress syndrome requiring mechanical ventilation and surfactant, patent ductus

arteriosus requiring treatment, necrotizing enterocolitis ≥ stage 2,[12] retinopathy of prematurity ≥ stage 3,[13] amniotic band syndrome, ischemic limb injury or severe cerebral injury. Severe cerebral injury was diagnosed in case of one of the following abnormalities were detected on cerebral imaging: intraventricular hemorrhage ≥ stage 3,[14] ventricular dilatation (including post-hemorrhagic ventricular dilatation)[15], cystic periventricular leukomalacia ≥ grade 2,[16] porencephalic or parenchymal cysts, arterial infarction or other severe cerebral lesions associated with adverse outcome.

Secondary outcomes included diagnosis- and therapy-related characteristics, hematological and placental characteristics, and a risk factor analysis for spontaneous perinatal mortality and severe neonatal morbidity. For the risk factor analysis for spontaneous mortality, cases with intentional fetal demise due to selective feticide or TOP were excluded. Since TAPS cases can be managed according to different strategies in the same pregnancy, management group assignment was based on the first treatment strategy that was performed. The following parameters were investigated in the univariate risk analysis for spontaneous perinatal mortality: GA at diagnosis TAPS, antenatal TAPS stage, TAPS donor/recipient status, type of antenatal management and GA at birth (weeks). For antenatal TAPS stage, the highest antenatal TAPS stage that was seen during pregnancy was selected. In case of TAPS stage 5, the highest TAPS stage before stage 5 was used. For the risk factor analysis for severe neonatal morbidity, two more parameters were added: severe growth restriction defined as birth weight < 3rd centile and the presence of postnatal TAPS.

The following additional parameters were determined: inter-twin hemoglobin difference (highest hemoglobin value – lowest hemoglobin value), reticulocyte count ratio (highest reticulocyte value (‰)/ lowest reticulocyte value (‰)), the presence of severe or mild growth restriction (defined as a birth weight < 3rd or < 10th centile respectively, according to Hoftiezer[17]), postnatal TAPS stage (according to Slaghekke[11]) and the configuration of anastomosis type per TAPS placenta.

Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). Data are reported as means ± standard deviation (SD) or as medians and/ or interquartile ranges (IQR) or ranges (minimum-maximum), as appropriate. A p-value < 0.05 was considered to indicate statistical significance. Differences between donors and recipients were calculated using the paired t-test for

normally distributed continuous outcomes and the Generalized Estimated Equation module for categorical outcomes. Potential risk factors were checked for correlation using Spearman's Rank test (R). Correlation coefficient R > (-)0.7 was considered to indicate a strong relationship between the factors. Potential risk factors for perinatal mortality and severe neonatal morbidity were studied in a univariate logistic regression model. A multivariate logistic regression model was applied to the variables that showed significant association in the univariate analysis. Results are expressed as odds ratios (OR) with 95% confidence intervals (CI).

Results

Out of the 422 TAPS cases entered in the TAPS Registry, 249 (59%) were spontaneous TAPS and included in this study, while 173 (41%) were post-laser TAPS and excluded from the study. The number of spontaneous TAPS cases per fetal therapy center is shown in Appendix 1. TAPS was diagnosed antenatally in 88% (219/249) of the group and postnatally in 12% (30/249). Further baseline characteristics of the population are presented in Table 1.

Table 1. Baseline characteristics of spontaneous TAPS twins

	Spontaneous TAPS (N = 249 pregnancies, 498 fetuses)
Gravidity	2 (1-3)
Parity	1 (0-1)
Antenatal diagnosis of TAPS	219/249 (88)
Location of placenta†	
Anterior	127/236 (54)
Posterior	104/236 (44)
Other	5/236 (2)

Data are presented as n/N (%) or median (IQR).
† In 13 cases, position of the placenta was unknown. Other types of placental position included: partly anterior and partly posterior (n=2), lateral left (n=2) and lateral right (n=1).

The median GA at diagnosis was 23.7 weeks (IQR: 19.7-28.8) with a wide range from 15.1 weeks to 35.3 weeks (Figure 1). In antenatally detected TAPS, 39% (86/219) was treated with laser surgery, 24% (52/219) was managed expectantly, 16% (34/219) had a delivery, 13% (26/219) received IUT (± PET), 8% (18/219) was

treated with selective feticide and in 1% (3/219) a TOP was performed. Table 2 further details diagnosis- and management-related characteristics.

Table 2. Diagnosis- and management related characteristics

	Spontaneous TAPS (N = 249 pregnancies, 498 fetuses)
GA at diagnosis (weeks)	23.7 (19.7-28.8; 15.1-35.3)
TAPS stage at diagnosis	
1	80/219 (37)
2	91/219 (42)
3	38/219 (17)
4	10/219 (5)
5	0/219 (0)
Highest TAPS stage during pregnancy	
1	64/219 (29)
2	88/219 (40)
3	52/219 (24)
4	12/219 (6)
5	3/219 (1)
Presence of additional ultrasound markers†	
Starry sky liver (recipient)	93/200 (47)
Difference in placental echogenicity	96/220 (44)
Antenatal management	
Expectant management	51/219 (23)
Delivery‡	34/219 (16)
IUT (± PET)	26/219 (12)
Laser surgery	86/219 (39)
Selective feticide	18/219 (9)
Termination of pregnancy	3/219 (1)
Female§	251/468 (53)
Cesarean¶	330/488 (68)

Data are median (IQR) or n/N(%)
†The presence of a starry sky liver and difference in placental echogenicity was assessed in 200 and 220 cases, respectively.‡ 1 case that had a delivery at 27 weeks based on TAPS stage 3 was a monoamniotic twin § In 30 fetuses, gender is unknown. One case was a male-female pair. ¶ In 10 fetuses mode of delivery is unknown. TAPS, twin anemia polycythemia; GA, gestational age; IUT, intrauterine transfusion; PET, partial exchange transfusion

Color-dye injection of the placenta was performed in 44% (109/249) of the cases (Table 3). In total, 24% (26/109) of injected placentas belonged to TAPS cases treated with laser surgery and 76% (83/109) belonged to TAPS cases that were not treated with laser surgery. In placentas not treated with laser, the median total number of anastomoses was 3 (1-6) and 84% (70/83) of the placentas demonstrated AV and/or VA anastomoses. AA and VV anastomoses

were detected in 19% (16/83) and 7% (6/83) of the group, respectively. In three TAPS cases, the placenta demonstrated only one AA or VV anastomoses at the vascular equator. Seven placentas did not show any anastomoses after placental injection: three cases had spontaneous resolution of TAPS during pregnancy, one case had normal hemoglobin values despite an antenatal diagnosis of TAPS, and the three remaining cases presented with severe postnatal TAPS (≥ stage 4) and a reticulocyte count ratio > 1.7. In total, 94% (74/76) of the placentas with anastomoses, showed only minuscule anastomoses (diameter < 1mm). Residual anastomoses were detected in 11% (3/26) of placentas treated with laser. In all three cases, the residual anastomoses were small and the twins had evidence of antenatal and postnatal TAPS.

Table 3. Characteristics of spontaneous TAPS placentas (not treated with laser)

	Injected TAPS placentas (N = 83)
Total number of anastomoses	3 (1-6)
Number of AV anastomoses	2 (1-3)
Number of VA anastomoses	1 (0-2)
Number of AA anastomoses	0 (0-0)
Number of VV anastomoses	0 (0-0)
Presence of anastomoses	
Presence of AV/VA anastomoses	70/83 (84)
Presence of AA anastomoses	16/83 (19)
Presence of VV anastomoses	6/83 (7)
Type of anastomoses per placenta	
No anastomoses	7/83(8)
AV (one direction)	21/83 (25)
AVs (both directions)	34/83 (41)
AV/VA and AA	13/83 (16)
AV/VA and VV	4/83 (5)
Only AA	2/83 (2)
Only VV	1/83 (1)
AV/VA, AA and VV	1/83 (1)
All anastomoses diameter < 1 mm†	74/76 (97)

Data are median (IQR) or n/N(%)
† Reported only in cases with anastomoses, the 10 cases without anastomoses were excluded.
TAPS, twin anemia polycythemia; TTTS, twin-twin transfusion syndrome; AV, arterio-venous; VA, veno-arterial; AA, arterio-arterial; VV, veno-venous; mm, millimetre

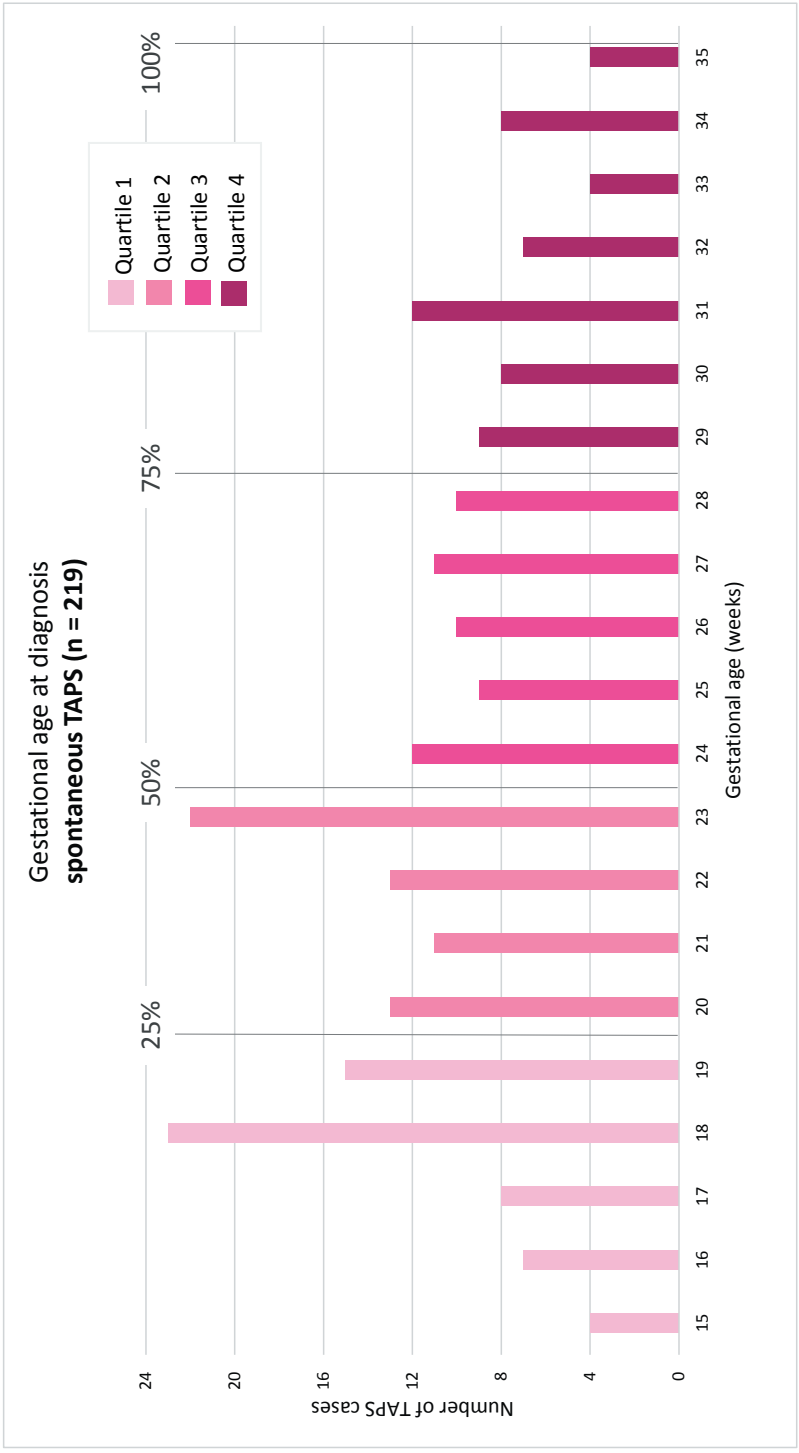


Figure 1. Gestational age at diagnosis in twins with spontaneous twin anemia polycythemia sequence

Table 4 shows perinatal outcome for the total group of TAPS twins, and for donors and recipients separately. The median gestational at birth for TAPS twins was 32.3 weeks (IQR: 30.1-34.9; range: 18.7-39.6). Donors had significantly lower birth weights than recipients, 1483g ± 566g vs. 1765g ± 620g, p < 0.001. In addition, donors were more often severely growth restricted than recipients 49% (98/200) vs. 11% (26/228), p < 0.001. Fetal demise occurred in 11% (54/494) of the group, either spontaneously in 5% (24/494) or intended in 6% (30/494). Donors had a higher risk for fetal demise than recipients, both for spontaneous fetal demise (8%; (19/242) vs. 2% (5/241); p = 0.002) and intended fetal demise (10%; (24/242) vs 3% (6/242) p < 0.001). Overall perinatal mortality (including intended demise) occurred in 15% (72/493) of TAPS twins, in 22% (54/243) of the donors and 7% (18/242) of the recipients, respectively (p < 0.001). Spontaneous perinatal mortality was observed in 9% (42/493) of the group, in 12% (30/243) of donors and in 5% (12/242) of recipients (p < 0.001). The rate of neonatal mortality was 4% (18/439) and was comparable between donors and recipients, 6% (11/200) vs. 3% (7/231), respectively (p = 0.159). Severe neonatal morbidity was diagnosed in 33% (141/432) of live-born TAPS twins, and was similar for donors (32%; 63/196) and recipients (33%; 75/228), p = 0.628. Severe cerebral injury was identified in 4% (15/432) of live-born TAPS twins, in 2% (4/196) of donors and 5% (11/228) of recipients (p = 0.11). Type of severe cerebral injury included intraventricular hemorrhage ≥ grade 3 (n = 9), cystic periventricular leukomalacia ≥ grade 2 (n = 4), (post-hemorrhagic) ventricular dilatation (n = 2), arterial infarction (n = 2) and other severe lesions associated with adverse outcome (n = 3). Amniotic band syndrome and ischemic limb injury did not occur in this cohort of spontaneous TAPS twins. In twins that were diagnosed with TAPS at birth (43%; 108/249) –and did not have a successful laser or spontaneous resolution– inter-twin hemoglobin difference was 14.3 mmol/L (IQR: 11.7-17.8) and reticulocyte count ratio was 3.9 (IQR: 2.5-5.3). Donors needed a blood transfusion at birth in 76% (81/108) and recipients a PET in 51% (51/108). In twins diagnosed with TAPS at birth, 17% (18/108) had postnatal TAPS stage 1, 28% (30/108) stage 2, 24% (26/108) stage 3, 19% (21/108) stage 4, and 12% (13/108) stage 5.

Table 4. Perinatal outcome for spontaneous twin anemia polycythemia sequence

	Spontaneous TAPS (N = 249 pregnancies, 498 fetuses)	TAPS donors† (N = 244 fetuses)	TAPS recipients† (N = 244 fetuses)	p-value
GA at birth (weeks)	32.3 (30.1-34.9; 18.7-39.6)	-	-	-
Fetal demise‡	54/494 (11)	43/243 (18)	11/243 (5)	< 0.001
Spontaneous	24/494 (5)	19/243 (8)	5/243 (2)	0.002
Intended	30/494 (6)	24/243 (10)	6/243 (3)	< 0.001
Neonatal mortality§	18/439 (4)	11/200 (6)	7/231 (3)	0.161
Perinatal mortality (overall)§	72/493 (15)	54/243 (22)	18/242 (7)	< 0.001
Perinatal mortality (spontaneous)§	42/493 (9)	30/243 (12)	12/242 (5)	< 0.001
Severe neonatal morbidity¶	141/432 (33)	63/196 (32)	74/228 (33)	0.652
Respiratory distress syndrome	118/432 (27)	51/196 (26)	64/228 (28)	0.413
Patent ductus arteriosus	34/432 (8)	15/196 (8)	19/228 (8)	0.671
Necrotizing enterocolitis	15/432 (4)	7/196 (4)	8/228 (4)	0.905
Retinopathy of prematurity	7/432 (2)	3/196 (2)	4/228 (2)	0.778
Severe cerebral injury	15/432 (4)	4/196 (2)	11/228 (5)	0.109
Birth weight (gram)¥	1645 ± 609	1483 ± 566	1765 ± 620	< 0.001
Severe growth restriction (bw < p3)¥	126/434 (29)	98/200 (49)	26/228 (11)	< 0.001
Mild growth restriction (bw < p10)¥	211/434 (49)	135/200 (68)	71/228 (31)	< 0.001

Data are presented as mean ± (SD) medians (IQR) or n/N (%).
† in 5/249 cases, the donor-recipient status was unknown
‡ 4 missing values
§ 5 missing values (same as '‡' plus 1 missing value from a liveborn recipient with unknown neonatal mortality information).
¶ 12 missing values (same as '§', plus 4 cases with unknown neonatal morbidity info and 3 cases that died shortly after birth)
¥ 9 missing values (as in '‡' plus 5 cases with unknown birth weights)
TAPS, twin anemia polycythemia sequence; GA, gestational age; bw, birth weight

Univariate risk factor analysis demonstrated that spontaneous perinatal mortality was significantly associated with donor status (OR = 3.0, 95% CI 1.7-5.4, p < 0.001) and antenatal TAPS stage (stage 2: OR = 7.2, 95%CI 1.5-32.2, p = 0.009; stage 3: OR = 11.3, 95%CI 2.5-50.5, p = 0.002; stage 4: OR = 32.5 95%CI 5.7-146.4, p < 0.001) and GA at birth (OR = 0.8, 95%CI 0.7-0.9, p < 0.001). Antenatal TAPS stage, donor status and GA at birth were not correlated (antenatal TAPS stage – donor status (R < 0.000, p = 0.998), antenatal TAPS stage – GA at birth (R < 0.001 p = 1.000), GA at birth – donor status (R < 0.000 p =0.997)), so all parameters were included in multivariate analysis. Multivariate analysis demonstrated that donor status (OR = 3.8, 95%CI 1.9-7.5, p < 0.001) and antenatal TAPS stage (stage 2: OR = 6.3, 95%CI 1.4-27.8, p = 0.016; stage 3: OR 9.6, 95%CI 2.1-45.5, p = 0.005; stage 4: OR 20.9, 95%CI 3.0-146.4, p = 0.002) and GA at birth (OR = 0.8, 95%CI 0.7-0.9, p = 0.001) were independent risk factors for spontaneous perinatal mortality.

Univariate risk factor analysis revealed that antenatal TAPS stage 4 (OR = 4.4, 95%CI 1.2-16.0, p = 0.026), antenatal management with delivery (OR = 2.3 95%CI 1.0-5.6, p = 0.046), GA at birth (OR = 1.7, 95%CI 1.5-1.9, p < 0.001) and the presence of postnatal TAPS (OR = 1.9, 95%CI 1.0-3.3, p = 0.039) were significant risk factors for severe neonatal morbidity. There was no strong correlation between the four parameters (GA at birth – postnatal TAPS (R < 0.001, p = 1.000), antenatal TAPS stage – postnatal TAPS (R = -0.155, p = 0.006), antenatal management (R = -0.493, p < 0.001), GA at birth – antenatal TAPS stage (R = -0.209, p < 0.001), GA at birth – antenatal management (R = 0.154, p = 0.002), antenatal management – antenatal TAPS stage (R = 0.307, p < 0.001), so all were included in multivariate analysis. In the multivariate analysis, antenatal TAPS stage 4 (OR = 7.9, 95%CI 1.4-43.3, p = 0.018), GA at birth (OR = 1.7, 95%CI 1.5-2.1, p < 0.001) independently associated with severe neonatal morbidity. More details on univariate and multivariate risk analysis for perinatal mortality and severe neonatal morbidity are presented in Table 2a and 2b of Appendix 2.

Appendix 1. Spontaneous TAPS cases per center.

Center	Country	Spontaneous TAPS Cases
Leiden University Medical Center	The Netherlands	70
Leuven University Hospital	Belgium	30
Necker-Enfants Malades Hospital Paris	France	23
Hospital Universitari Vall d'Hebron Barcelona	Spain	16
University Medical Center Hamburg-Eppendorf	Germany	15
Center Medico-Chirurgical Obstetrical Strasbourg	France	13
Medical University of Graz	Austria	13
Mount Sinai Hospital Toronto	Canada	12
Children's Hospital V. Buzzi Milan	Italy	11
University of Texas McGovern Medical School at Houston	United States of America	10
Saint George's Hospital London	United Kingdom	9
Mater Hospital Brisbane	Australia	8
Brugmann University Hospital	Belgium	7
Yale New Haven Hospital	United States of America	6
Karolinska University Hospital Stockholm	Sweden	3
V.I. Kulakov National Medical Research Center of Obstetrics, Gynecology and Perinatology Moscow	Russia	2
Birmingham Women's and Children's NHS Foundation Trust	United Kingdom	1

APPENDIX 2 a Univariate and multivariate risk analysis for spontaneous perinatal mortality in spontaneous twin anemia polycythemia sequence.

	Death† (n = 42/463)	**Alive† (n = 421/463)**	**Univariate analysis OR (95% CI)**	**SE**	**P**	**Multivariate analysis OR (95% CI)**	**SE**	**P**
GA at diagnosis TAPS	22.7 ± 4.8	24.7 ± 5.4	0.9 (0.8-1.0)	0.05	0.124			
Antenatal TAPS stage								
1	2/126 (2)	124/126 (98)	-*					
2	17/162 (11)	145/162 (89)	7.2 (1.5-32.2)	0.8	**0.009**	6.3 (1.4-27.8)	0.8	**0.016**
3	14/91 (15)	77/91 (85)	11.3 (2.5-50.5)	0.8	**0.002**	9.6 (2.1-45.5)	0.8	**0.005**
4	8/15 (35)	18/15 (65)	32.5 (5.7-186.7)	0.9	**< 0.001**	20.9 (3.0-146.4)	1.0	**0.002**
Recipient‡	12/236 (5)	224/236 (95)	-*					
Donor‡	30/219 (14)	189/219 (86)	3.0 (1.7-5.4)	0.3	**< 0.001**	3.8 (1.9-7.5)	0.3	**< 0.001**
Antenatal therapy								
Expectant management	12/101 (10)	89/101 (88)	-*					
Delivery	5/68 (7)	63/68 (93)	0.6 (0.2-1.8)	0.6	0.334			
IUT (± PET)	2/52 (4)	50/52 (96)	0.3 (0.1-1.4)	0.9	0.118			
Laser surgery	21/163 (13)	142/163 (87)	1.1 (0.5-2.5)	0.4	0.865			
Selective feticide (co-twin)	2/17 (11)	17/19 (89)	0.9 (0.2-4.3)	0.8	0.855			
GA at birth	29.5 ± 4.7	32.6 ± 2.9	0.8 (0.7-0.9)	0.1	**< 0.001**	0.8 (0.7-0.9)	0.1	**0.001**

Values are odds ratios (OR) (95% confidence intervals (CI)), standard error (SE) and p-value. *was set as a reference. † 30 cases were excluded as mortality occurred in context of selective feticide or termination of pregnancy, from the 648 cases 5 cases have missing values ‡ In 5 cases, donor-recipient status was unknown

GA, gestational age; TAPS, twin anemia polycythemia sequence; IUT, intrauterine transfusion; PET, partial exchange transfusion

APPENDIX. 2b Univariate and multivariate risk analysis for severe neonatal morbidity in spontaneous twin anemia polycythemia sequence.

	SNM† (N=141/432)	No SNM† (N= 291/432)	Univariate analysis OR (95% CI)	SE	P	Multivariate analysis OR (95% CI)	SE	P
GA at diagnosis TAPS	25.4 ± 5.2	24.5 ± 5.6	1.0 (0.9-1.0)	0.02	0.300	-	-	-
Antenatal TAPS stage								
1	40/123 (33)	83/123 (67)	-*					
2	44/148 (30)	104/148 (70)	0.9 (0.5-1.7)	0.3	0.651	0.7 (0.3-1.6)	0.4	0.414
3	31/82 (38)	51/82 (62)	1.1 (0.6-2.4)	0.4	0.749	1.0 (0.4-3.0)	0.5	0.953
4	14/19 (74)	5/19 (26)	4.4 (1.2-16.0)	0.7	**0.026**	7.9 (1.4-43.3)	0.8	**0.018**
Recipient‡	74/226 (33)	153/226 (67)	-*					
Donor‡	63/196 (32)	133/196 (68)	1.1 (0.8-1.3)	0.1	0.628	-	-	-
Antenatal therapy								
Expectant management	26/93 (28)	67/93 (72)	-*					
Delivery	32/68 (47)	35/68 (53)	2.3 (1.0-5.6)	0.4	0.046	0.5 (0.1-1.5)	0.5	0.252
IUT (± PET)	22/50 (44)	28/50 (56)	1.9 (0.8-4.6)	0.5	0.150	1.3 (0.4-4.0)	0.6	0.695
Laser surgery	44/145 (31)	108/145 (69)	1.2 (0.5-2.4)	0.4	0.661	1.6 (0.6-4.9)	0.6	0.370
Selective feticide	4/17 (24)	13/17 (76)	0.8 (0.2-2.8)	0.6	0.710	- §		
GA at birth	30.1 ± 2.7	33.6 ± 2.3	1.7 (1.5-1.9)	0.1	**< 0.001**	1.7 (1.5-2.1)	0.1	**< 0.001**
Severe growth restriction, no	99/304 (33)	205/304 (67)	-*					
Severe growth restriction, yes	41/122 (34)	81/122 (66)	1.0 (0.7-1.5)	0.2	0.842	-	-	-
Postnatal TAPS, no	40/156 (26)	116/156 (74)	-*					
Postnatal TAPS, yes	81/211 (38)	130/211 (62)	1.9 (1.0-3.3)	0.3	**0.039**	2.1 (0.9-5.0)	0.4	0.068

Values are odds ratios (OR) (95% confidence intervals (CI)), standard error (SE) and p-value. *was set as a reference † 12 neonates with missing neonatal outcome. ‡In 5 cases, donor-recipient status was unknown. §Group too small to calculate OR in multivariate analysis.
SNM, severe neonatal morbidity; GA, gestational age; TAPS, twin anemia polycythemia sequence; IUT, intrauterine transfusion; PET, partial exchange transfusion

Discussion

This is the first large international study investigating management and outcome in spontaneous TAPS. We found that TAPS can develop across a wide range of gestation. Management in all 17 centers varied considerably, with fetoscopic laser surgery being the most frequent intervention. In this cohort, perinatal outcome was poor, particularly due to a high perinatal mortality rate in donor twins. These findings stress the need for increased awareness by clinicians concerning the severity of TAPS. Adaptation of guidelines to ensure early diagnosis is needed, as well as prospective, well-controlled studies to determine the most optimal diagnostic criteria and management strategy.

Until now, information regarding time of onset of TAPS was scarce and mostly based on small cohort studies. To develop adequate TAPS screening guidelines, using routine MCA-PSV Doppler measurements, knowledge concerning the time of onset of TAPS is essential. This study shows that TAPS can develop from 15 weeks to 35 weeks' gestation. As three of the four cases detected at 15 weeks were stage 2 or higher, it is likely that TAPS manifested even earlier. Currently, there is no consensus on when to start with MCA-PSV surveillances to check for the presence of TAPS. The ISUOG twin guideline recommends bi-weekly MCA-PSV screening starting from 20 weeks' gestation, especially in cases treated with laser surgery for TTTS.[18] The Society for Maternal and Fetal Medicine does not recommend MCA-PSV screening at all, due to the lack of evidence that routine screening improves perinatal outcome in TAPS.[19] This study showed that an advanced antenatal TAPS stage was a significant risk factor for perinatal mortality and severe neonatal morbidity. We can therefore speculate that a timely detection allowing antenatal intervention could improve outcome. Based on the mounting evidence of serious effects of TAPS[20], we suggest that to improve early detection and possibly outcome, routine MCA-PSV examination should be included in the standard bi-weekly work-up starting in the early second trimester.

Due to international collaboration, we were able to report on the largest group of spontaneous TAPS placentas. In line with previous studies, we found that the vast majority (94%) of TAPS placentas showed only minuscule anastomoses.[1] Our data also demonstrate that AA anastomoses do not prevent the development of TAPS, as they were observed in 19% of TAPS placentas. This rate is slightly higher than the 11% reported before.[21] Moreover, this study was the first to show

that VV anastomoses are rare, but not absent in TAPS placentas.[22] Interestingly, there were three TAPS placentas that showed only one minuscule AA or VV anastomosis. It is postulated that, these bidirectional anastomoses act like an AV anastomosis (allowing unidirectional flow) under certain circumstances. Of note, there were seven placentas from TAPS cases showing no anastomoses after color dye injection. In three cases, TAPS resolved during pregnancy, likely as a result of spontaneous thrombosis in an AV anastomosis.[23] The other four placentas belonged to severe postnatal TAPS cases, which also presented with high reticulocyte ratios, suggestive of a true chronic unbalanced feto-fetal transfusion. Possibly, deep-hidden anastomoses were responsible for the unbalanced blood flow.[24] Alternatively, minuscule anastomoses may not have been seen due to suboptimal color-dye injection, which is known to be technically challenging in TAPS placentas.[25]

Studies investigating perinatal outcome in TAPS are scarce, and the majority combines outcome of spontaneous and post-laser TAPS twins. In a recent long-term outcome, study fetal demise occurred in 3% and neonatal mortality in 2% of spontaneous TAPS twins, which is roughly comparable to the 5% and 4% in this study, respectively.[20] The long-term follow-up study also showed that spontaneous TAPS donors have a four-fold higher odd of neurodevelopmental impairment than recipients, and show high rates of cognitive impairment and deafness. The current study demonstrated that TAPS donors do not only have a more detrimental outcome on the long-term, but are also at increased risk for mortality antenatally. Additionally, almost half of the donors in our cohort were severely growth-restricted, in contrast to 12% of recipients. Growth restriction in TAPS is not related to unequal placental sharing, but is likely a result of chronic anemia and hypoalbuminemia.[26] In this cohort, GA at birth was a strong predictor for both perinatal mortality and severe neonatal morbidity, showing that prolonging pregnancy is crucial to improve outcome in spontaneous TAPS twins.

This study shows that perinatal mortality is lower in spontaneous TAPS than in post-laser TAPS, 15% vs. 25%, respectively.[7] Aside from the fact that spontaneous TAPS did not experience TTTS, a possible alternative explanation for this difference can be found in the type of placental anastomoses. Most spontaneous TAPS placentas demonstrated AV anastomoses in both directions, allowing compensating blood flow from recipient to donor. In post-laser TAPS,

most placentas showed AV anastomoses in one direction.[7] Alternatively, antenatal treatment might have been of influence. Although management in spontaneous TAPS is diverse, a significant proportion of TAPS twins was treated with laser surgery, an intervention aimed at permanently blocking unbalanced inter-twin blood transfusion, thereby preventing further deterioration of the disease. A detailed comparison of differences in perinatal outcome between various treatment strategies is presented in a separate study.[27]

Caution is needed when interpreting the findings of our study, due to the limitations associated with registry investigations. Notably, this study depended on local registrations of tertiary fetal therapy centers. Therefore, our outcome could be biased towards severe cases of TAPS, as they are more likely to be referred by peripheral clinics. Furthermore, this study did not compare the outcome of spontaneous TAPS twins to a cohort of uncomplicated monochorionic twins. Nonetheless, this large international study presents new important information which has potential implications for the future care of monochorionic twins.

To conclude, spontaneous TAPS can occur across a wide gestational-age range, is managed heterogeneously, and is associated with high rates of adverse perinatal outcome, particularly in donor twins. Since perinatal outcome is greatly dependent on TAPS stage, timely detection allowing consideration of antenatal treatment is of utmost importance. To adequately investigate the best treatment for TAPS, an international randomized controlled trial is needed.[28]

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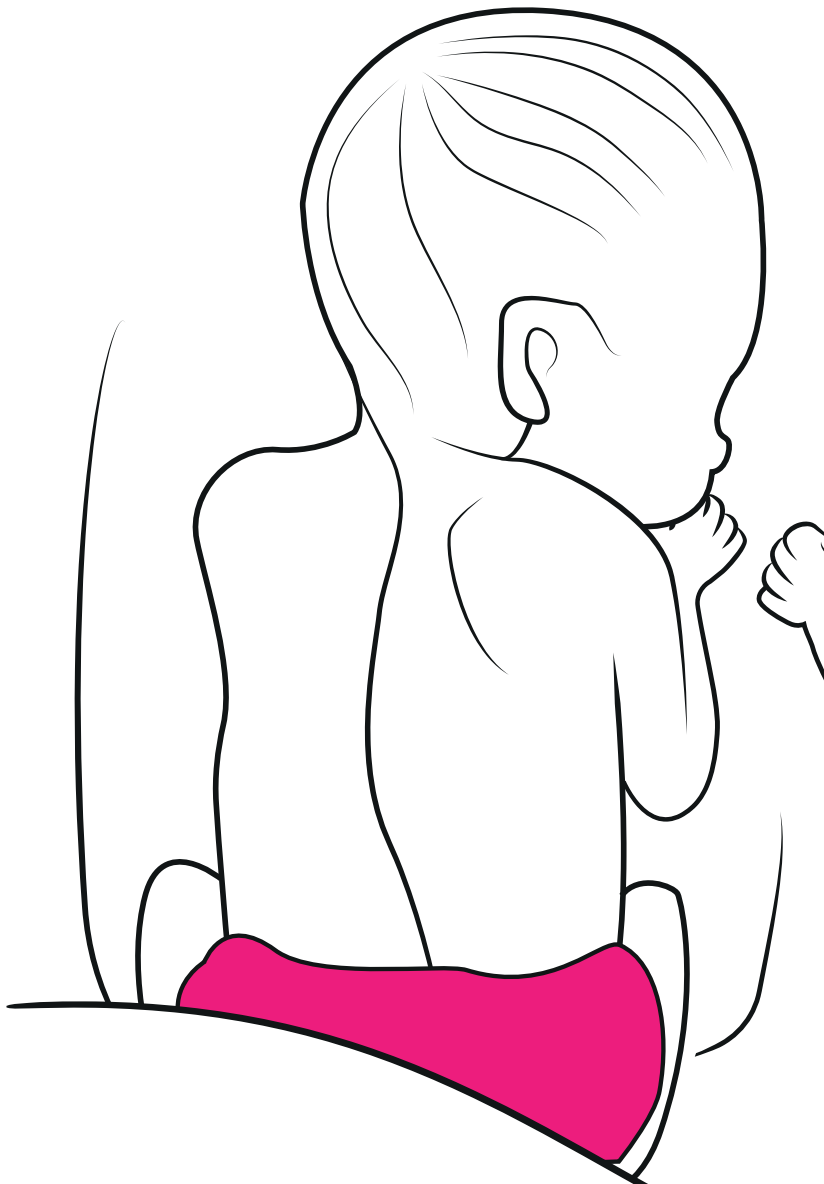

Chapter 10

Post-laser twin anemia polycythemia sequence: management and outcome in an international cohort of 164 cases

Abstract

Objective

to investigate the management and outcome in post-laser twin anemia polycythemia sequence (TAPS)

Methods

Data of the international TAPS Registry, collected between 2014-2019, were used for this study. The primary outcomes were perinatal mortality and severe neonatal morbidity. Secondary outcomes included a risk-factor analysis for perinatal mortality and severe neonatal morbidity.

Results

A total of 164 post-laser TAPS cases were included, of which 92% (151/164) were diagnosed antenatally and 8% (13/164) postnatally. Median number of days between laser for TTTS and detection of TAPS was 14 (IQR: 7-28, range: 1-119). Antenatal management included expectant management in 43% (62/151), intrauterine transfusion with or without partial exchange transfusion in 29% (44/151), repeated laser surgery in 15% (24/151), selective feticide in 7% (11/151), delivery in 6% (9/151), and termination of pregnancy in 1% (1/151). Median gestational age (GA) at birth was 31.7 weeks (IQR: 28.6-33.7; range: 19.0-41.3). Perinatal mortality rate was 25% (83/327) for the total group, 37% (61/164) for donors and 14% (22/163) for recipients ($p < 0.001$). Severe neonatal morbidity was detected in 40% (105/263) of the cohort, and was similar for donors (43%; 51/118) and recipients (37%; 54/145), $p= 0.568$. Independent risk factors for spontaneous perinatal mortality were antenatal TAPS stage 4 (OR = 3.4, 95%CI 1.4-26.0, $p = 0.015$), TAPS donor status (OR = 4.2, 95%CI 2.1-8.3, $p < 0.001$) and GA at birth (OR = 0.8, 95%CI 0.7-0.9, $p = 0.001$). Severe neonatal morbidity was significantly associated with GA at birth (OR = 1.5, 95%CI 1.3-1.7, $p < 0.001$).

Conclusion

Post-laser TAPS most often occurs within one month after laser for TTTS, but can develop up to 17 weeks after initial surgery. Management was mostly expectant, but varied greatly amongst different centers highlighting the lack of consensus on the optimal treatment. Perinatal outcome was poor, particularly due to the high rate of perinatal mortality in donor twins.

Introduction

Twin anemia polycythemia sequence (TAPS) can arise from chronic unbalanced feto-fetal transfusion through minuscule placental anastomoses in monochorionic twins, leading to anemia in the TAPS donor and polycythemia in the TAPS recipient.[1] Unlike twin-twin transfusion syndrome (TTTS), TAPS develops in the absence of twin oligohydramnios-polyhydramnios sequence. The iatrogenic form of TAPS, post-laser TAPS, can develop in 2-16% after laser surgery for TTTS due to the presence of minuscule residual anastomoses.[2-4] Post-laser TAPS can be prevented by using the Solomon technique. With this approach, the entire placental vascular equator is laser photocoagulated, thereby blocking all anastomoses, even the miniscule ones that may not be visualized.[5] Although the rate of residual anastomoses is significantly reduced by the implementation of the Solomon technique, post-laser TAPS can still occur.[6, 7]Options to manage post-laser TAPS include expectant management, preterm delivery, intrauterine transfusion (IUT) with or without a partial exchange transfusion (PET), fetoscopic laser surgery and selective feticide.[8] The best treatment has not been established. Technical feasibility of a second intrauterine intervention might be limited due to complications that have arisen from the initial laser procedure for TTTS, such as amnion-chorion separation or preterm premature rupture of the membranes (PPROM). For informed decision making regarding the preferred intervention, it is crucial to have insight into perinatal outcome of post-laser TAPS twins. Due to the rarity of the complication, perinatal outcome is insufficiently investigated and available information is based on small cohort studies. To expand our knowledge on TAPS, we set up the TAPS Registry, an international collaboration aimed at collecting data on diagnosis, treatment and outcome in TAPS twins.

In the current study, the data from this TAPS Registry were used to (1) characterize diagnosis, treatment modalities, and outcome in post-laser TAPS twins (2) to compare perinatal outcome between TAPS donors and recipients, and (3) to investigate potential risk factors for adverse perinatal outcome.

Methods

The TAPS Registry, established in 2014, was web-based registry for anonymous data collection (www.tapsregistry.org). Fetal therapy centers across the world

were invited to participate. Participating centers were provided with personal credentials to enter data of their TAPS cases into the online registry. Between 2014 and 2019, 17 specialized fetal therapy centers contributed to data collection (see Appendix 1).

All monochorionic twin pregnancies diagnosed with post-laser TAPS were considered eligible for this study. Pregnancies with spontaneous TAPS were excluded and are described in a separate study[9].

Antenatal diagnosis of TAPS was based on discordant MCA-PSV measures, with an increased MCA-PSV (> 1.5 Multiples of the Median (MoM)) in the TAPS donor, indicative of fetal anemia, combined with a decreased MCA-PSV measure (< 1.0 MoM) in the TAPS recipient, indicative of fetal polycythemia.[10] Postnatal diagnosis was reached by the presence of an inter-twin hemoglobin difference > 8.0 g/dL combined with at least one of the following: a reticulocyte count ratio > 1.7 or the presence of only minuscule vascular anastomoses (diameter < 1 mm) detected through color dye injection of the placenta.[11, 12] Cases that were diagnosed with TAPS within one week after laser surgery for TTTS were excluded from the study, unless TAPS persisted. We did this as a large MCA-PSV discrepancy shortly after laser is likely to be a result of hemodynamic re-equilibration, and is not based on TAPS.[13]

The following information was obtained from local medical records: gravidity, parity, location of the placenta, moment of diagnosis (antenatal or postnatal), gestational age (GA) at diagnosis, TAPS stage at diagnosis and the presence of additional ultrasound findings including starry-sky liver in the recipient and/or difference in placental echogenicity. The severity of antenatal TAPS was determined according to the previously published staging system by Slaghekke et al.[14] For post-laser TAPS specifically, the following data regarding preceding TTTS were collected: Quintero stage, GA at laser, laser technique, operator's opinion on completeness of the laser procedure and TTTS-donor-recipient role. In addition, the antenatal management for TAPS was recorded, including expectant management, delivery (defined as a delivery within 7 days after diagnosis), IUT (± PET), fetoscopic laser surgery, selective feticide and termination of pregnancy (TOP). Also, information on placental color dye injection was collected, including classification of the type (arterio-venous (AV), veno-arterial (VA), arterio-arterial (AA), veno-venous (VV)), number and size of anastomoses. Perinatal outcome measures included: TAPS donor/recipient

status, birth weight, hemoglobin and reticulocyte values, treatment with blood transfusion or partial exchange transfusion on day one, the presence of severe neonatal morbidity and/or severe cerebral injury and the occurrence of perinatal mortality.

Primary outcomes were perinatal mortality and severe neonatal morbidity. Perinatal mortality was defined as fetal demise or neonatal death within 28 days after birth. In the context of selective feticide or TOP, a distinction was made between spontaneous fetal demise and intended fetal demise. Severe neonatal morbidity was defined as the presence of at least one of the following, diagnosed within 28 days after birth or prior to discharge: respiratory distress syndrome requiring mechanical ventilation and surfactant, patent ductus arteriosus requiring treatment, necrotizing enterocolitis ≥ stage 2[15], retinopathy of prematurity ≥ stage 3,[16] amniotic band syndrome, ischemic limb injury or severe cerebral injury. Severe cerebral injury was diagnosed in case of one of the following abnormalities was identified on cerebral imaging: intraventricular hemorrhage ≥ stage 3,[17] ventricular dilatation (including post-hemorrhagic ventricular dilatation),[18] cystic periventricular leukomalacia ≥ grade 2,[19] porencephalic or parenchymal cysts, arterial infarction or other severe cerebral lesions associated with adverse outcome.

Secondary outcomes included diagnosis- and therapy-related characteristics, hematological and placental characteristics, and a risk-factor analysis for spontaneous perinatal mortality and severe neonatal morbidity. Cases with intended fetal demise in context of selective feticide or termination of pregnancy were excluded for the risk-factor analysis for spontaneous perinatal mortality. Since TAPS cases may be managed according to different strategies in one pregnancy, management-group assignment was based on the first treatment that was performed. The following parameters were investigated in the univariate risk analysis: GA at diagnosis of TAPS, GA at laser for TTTS, days between laser for TTTS and development of TAPS, Quintero stage, antenatal TAPS stage, persistence of TTTS-TAPS donor-recipient status, type of antenatal management and GA at birth (weeks). For antenatal TAPS stage, the highest recorded antenatal TAPS stage was selected. In case of TAPS stage 5, the highest TAPS stage before stage 5 was used. For the risk factor analysis for severe neonatal morbidity, two more parameters were added: severe growth

restriction defined as birth weight < 3rd centile and the presence of postnatal TAPS.

The following additional outcomes were determined: inter-twin hemoglobin difference (highest hemoglobin value – lowest hemoglobin value), reticulocyte count ratio (highest reticulocyte value (‰)/ lowest reticulocyte value (‰)), the presence of severe growth restriction (defined as a birth weight < 3rd centile according to Hoftiezer[20]), postnatal TAPS stage (according to Slaghekke[8]) and the configuration of anastomosis type per TAPS placenta.

Statistical analyses were carried out using SPSS version 25.0 (IBM, Armonk, NY, USA). Data are presented as mean ± SD or as median and interquartile range (IQR) and/or range (minimum-maximum), as appropriate. A p-value < 0.05 was considered statistically significant. Differences between donors and recipients were calculated using the paired t-test for normally distributed continuous outcomes. To account for the fact that observations between co-twins are not independent, the Generalized Estimated Equation module was executed for analyses per fetus or neonate. Potential risk factors were checked for correlation using Spearman's Rank test (R). A correlation coefficient R > (-) 0.7 was considered to indicate a strong relationship between the factors. Potential risk factors for perinatal mortality and severe neonatal morbidity were assessed in a univariate logistic regression model. A multivariate logistic regression model was applied to the variables that showed significant association in the univariate analysis. Results are expressed as odds ratios (OR) with 95% confidence intervals (CI).

Results

Of the 422 TAPS cases entered into the TAPS Registry, 249 (59%) were spontaneous TAPS and were excluded from the study. The remaining 173 (41%) were post-laser TAPS and eligible for the study. In eight post-laser TAPS cases, TAPS was diagnosed within one week after laser for TTTS. As TAPS did not persist one week after laser surgery for TTTS, these cases were excluded. One case was excluded based on TAPS being diagnosed at stage 5. A total of 164 post-laser TAPS cases were included in the analysis for the current study.

Table 1. Baseline characteristics for post-laser twin anemia polycythemia sequence

	Post-laser TAPS (N = 164 pregnancies, 328 fetuses)
Gravidity	2 (1-3)
Parity	0 (0-1)
Antenatal diagnosis of TAPS	151/164 (92)
Location of placenta	
Anterior	72/164 (44)
Posterior	86/164 (52)
Other†	6/164 (4)
TTTS stage‡	
Q1	20/159 (13)
Q2	75/159 (47)
Q3	59/159 (37)
Q4	5/159 (3)
Solomon technique for TTTS laser	60/164 (37)
GA at laser for TTTS	20.6 (18.0-23.0)
Time between laser and post-laser TAPS (days)	14 (7-28)
Persistence of TTTS-TAPS donor-recipient role§	73/161 (45)
Laser for TTTS complete (surgeon's opinion)¶	126/156 (81)

Data are presented as n/N (%) or median (IQR).
†Other types of placental position included: partly anterior and partly posterior (n=1) and partly fundal (n=1) lateral left (n=2) and lateral right (n=2). ‡5 missing values §3 missing values ¶ 8 missing values
TAPS, twin anemia polycythemia sequence; TTTS, twin-twin transfusion syndrome; Q, Quintero; GA, gestational age

TAPS was diagnosed antenatally in 92% (151/164) and only postnatally in 8% (13/164) of the group (Table 1). Laser for TTTS (prior to post-laser TAPS) was performed using the Solomon technique in 37% (60/164) and the selective technique in 63% (104/164). The operating surgeon assumed that the laser for TTTS was complete in 81% (126/156) of post-laser TAPS cases, in 74% (43/58) of cases treated with the Solomon technique and in 85% (83/98) of cases treated with the selective technique. Reasons for incomplete laser were: poor visibility (n = 11), fetal position (n = 7), placental position (n = 5), bleeding (n = 2), velamentous anastomoses (n = 1), vomiting of patient (n = 1) and not specified (n = 3). In half of the antenatally detected TAPS cases, TAPS developed within 14 days (IQR: 7-28; range 1-119) after laser treatment for TTTS. Figure 1. depicts time between laser for TTTS and the diagnosis of post-laser TAPS for all antenatally diagnosed cases. In 45% (73/161) of cases, TTTS donors became TAPS donors.

Antenatal management included expectant management in 43% (62/151), IUT (± PET) in 29% (44/151), laser surgery in 15% (24/151), selective feticide in 7% (11/151), delivery in 6% (9/151), and TOP in 1% (1/151) (Table 2).

Table 2. Diagnosis- and management-related characteristics for post-laser twin anemia polycythemia sequence

	Post-laser TAPS (N = 164 pregnancies, 328 fetuses)
GA at diagnosis	23.1 (20.4-25.9)
TAPS stage at diagnosis	
1	43/151 (28)
2	69/151 (48)
3	27/151 (17)
4	12/151 (8)
Highest TAPS stage	
1	22/151 (15)
2	62/151(40)
3	26/151 (16)
4	22/151 (14)
5	19/151 (15)
Presence of additional ultrasound markers†	
Starry sky liver (recipient)	56/141 (40)
Difference in placental echogenicity	35/151 (23)
Antenatal management	
Expectant management	62/151 (43)
Delivery	9/151 (6)
IUT (± PET)	44/151 (29)
Laser surgery	24/151 (15)
Selective feticide	11/151 (7)
Termination of pregnancy	1/151 (1)
Female‡	162/308 (53)
Cesarean§	206/326 (63)

Data are median (IQR) or n/N(%)
†In 10 cases, the presence of a starry-sky liver was not assessed. ‡20 missing values §2 missing values.
TAPS, twin anemia polycythemia; GA, gestational age; IUT, intrauterine transfusion; PET, partial exchange transfusion

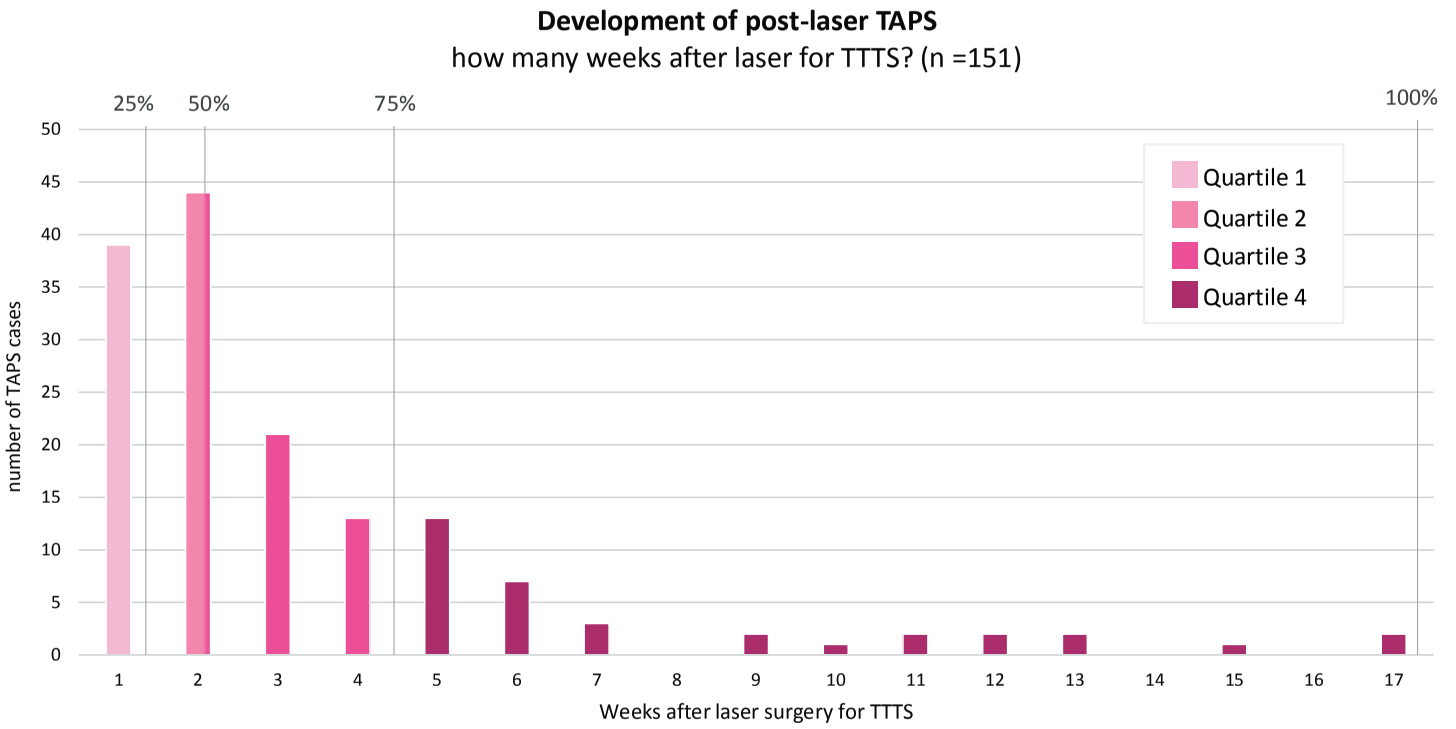

Figure 1. Weeks between laser for twin-twin transfusion syndrome and diagnosis of post-laser twin anemia polycythemia sequence

In total, 51% (84/164) of post-laser TAPS placentas were injected with color dye (Table 3), of which ten cases were treated with laser reintervention, and 74 cases were not treated with laser reintervention. In placentas not treated with laser reintervention, the median total number of anastomoses was 1 (IQR: 0-1) and 55% (40/74) had only AV anastomoses running in one direction. AA and VV anastomoses were detected in 8% (6/74) and 7% (5/74) of the group, respectively. In ten TAPS placentas no residual anastomoses were found after color dye injection: five placentas belonged to cases with spontaneous resolution of TAPS after expectant management or treatment with IUT (±PET), and the other five belonged to cases with TAPS confirmed both antenatally and postnatally. Residual anastomoses were found in 40% (4/10) of post-laser TAPS cases treated with laser reintervention. Three cases with residual anastomoses had only one minuscule AV anastomosis, and the fourth had one single minuscule AA anastomosis. Out of the four cases with residual anastomoses, two cases had confirmed postnatal TAPS, one case had a double fetal demise after reintervention with laser and one case showed an inter-twin hemoglobin difference of 8 g/dL (borderline TAPS) and had a low reticulocyte count ratio, but required a blood transfusion and partial exchange transfusion on day one.

Table 4 provides information on the perinatal outcome of post-laser TAPS twins, separated for TAPS donors and recipients. Median GA at birth was 31.7 weeks (IQR: 28.6-33.7, range 19.0-41.3). TAPS donors had significantly lower mean birth weights than TAPS recipients, 1346g ± 525g vs. 1533g ± 588g $p < 0.001$. Fetal demise occurred in 17% (56/327) of the group, either spontaneously in 10% (33/327) or intentionally in 7% (23/327). TAPS donors had a higher risk of fetal demise than TAPS recipients, both for spontaneous fetal demise (15% (25/164) vs. 5% (8/163); $p < 0.001$) and intended fetal demise, 11% (18/164) vs 3% (5/163); $p = 0.007$. The rate of neonatal mortality was 10% (27/271) and was higher in TAPS donors than in TAPS recipients, 15% (18/121) vs 6% (9/150), respectively ($p = 0.008$). Overall perinatal mortality (including intended demise) occurred in 25% (83/327) of TAPS twins, in 37% (61/164) of TAPS donors and 14% (22/163) of TAPS recipients, respectively ($p < 0.001$). Severe neonatal morbidity was diagnosed in 40% (105/263) of liveborn twins, and was similar for TAPS donors (43%; 51/118) and TAPS recipients (37%; 54/145), $p = 0.568$. Severe cerebral injury was identified in 11% (28/263) of liveborn twins, in 11% (13/118) of TAPS donors and 10% (15/145) of TAPS recipients ($p = 0.916$).

Table 3. Characteristics for placentas not treated with laser reintervention for post-laser twin anemia polycythemia sequence

	Injected post-laser TAPS placentas (N = 74)
Total number of anastomoses	1 (1-2)
Number of AV anastomoses	1 (0-1)
Number of VA anastomoses	0 (0-1)
Number of AA anastomoses	0 (0-0)
Number of VV anastomoses	0 (0-0)
Presence of anastomoses	
Presence of AV/VA anastomoses	58/74 (80)
Presence of AA anastomoses	6/74 (8)
Presence of VV anastomoses	5/74 (7)
Type of anastomoses per placenta	
No anastomoses	10/74 (14)
AV (one direction)	40/74 (54)
AVs (both directions)	14/74 (19)
AV/VA and AA	3/74 (4)
AV/VA and VV	2/74 (3)
Only AA	2/74 (3)
Only VV	2/74 (3)
AV/VA, AA and VV	1/74 (1)
All anastomoses diameter < 1 mm	62/64 (97)

Data are median (IQR) or n/N(%)
TAPS, twin anemia polycythemia; TTTS, twin-twin transfusion syndrome; AV, arterio-venous; VA, veno-arterial; AA, arterio-arterial; VV, veno-venous

Severe cerebral injury was diagnosed based on intraventricular hemorrhage ≥ grade 3 (n = 15), cystic periventricular leukomalacia ≥ grade 2 (n = 5), (post-hemorrhagic) ventricular dilatation (n = 6), porencephalic or parenchymal cysts (n = 3), arterial infarction (n = 1) and other severe lesions associated with adverse outcome (n = 1). Ischemic limb injury did not occur in this cohort of post-laser TAPS twins.

In cases that were diagnosed with TAPS at birth (72%; 76/106), the median inter-twin hemoglobin difference was 12.6 g/dL (IQR: 10.3-15.1, range: 8.2-21.7), and median reticulocyte count ratio was 2.7 (IQR: 2.3-3.9). At birth, 68% (52/76) of donors needed a blood transfusion to treat anemia and 49% (37/76) of recipients needed a PET to treat polycythemia. In total, 33% (25/76) had stage 1 postnatal TAPS, 34% (26/76) stage 2, 20% (15/76) stage 3, 11% (8/76) stage 4 and 1% (1/76) had stage 5 postnatal TAPS.

Table 4. Perinatal outcome in post-laser twin anemia polycythemia sequence.

	Post-laser TAPS (N = 164 pregnancies; 328 fetuses)	TAPS donors (N = 164 fetuses)	TAPS recipients (N = 164 fetuses)†	p-value
GA at birth	31.7 (28.6-33.7; 19.0-41.3)			
Fetal demise†	56/327 (17)	43/164 (26)	13/163 (8)	<0.001
Spontaneous	33/327 (10)	25/164 (15)	8/163 (5)	<0.001
Intended	23/327 (7)	18/164 (11)	5/163 (3)	0.007
Neonatal mortality	27/271 (10)	18/121 (15)	9/150 (6)	0.008
Perinatal mortality (overall)†	83/327 (25)	61/164 (37)	22/163 (14)	<0.001
Perinatal mortality (spontaneous)†	60/327 (18)	43/164 (26)	17/163 (10)	<0.001
Severe neonatal morbidity‡	105/263 (40)	51/118 (43)	54/145 (37)	0.568
Respiratory distress syndrome	88/263(34)	42/118 (36)	46/145 (32)	0.945
Patent ductus arteriosus	20/263 (8)	11/118 (9)	9/145 (6)	0.278
Necrotizing enterocolitis	6/263 (2)	4/118 (3)	2/145 (1)	0.275
Retinopathy of prematurity	9/263 (3)	6/118 (5)	3/145 (2)	0.165
Amniotic band syndrome	3/263 (1)	2/118 (2)	1/145 (1)	0.451
Severe cerebral injury	28/263 (11)	13/118 (11)	15/145 (10)	0.916
Birth weight (g)§	1390 ± 567	1346 ± 525	1533 ± 588	<0.001
Severe growth restriction (bw < p3)§	62/270 (23)	31/121 (26)	31/149 (21)	0.267
Mild growth restriction (bw < p10)§	122/270 (45)	60/121 (50)	61/149 (41)	0.061

Data are presented as means ± SD, medians (IQR), n/N (%)
† 1 missing value (1 post-laser TAPS recipient with unknown perinatal outcome)
‡ 9 missing values (same as '†' plus 4 cases with unknown neonatal morbidity info and 4 cases that died shortly after birth). § 2 missing values (same as '†' plus one case with missing birth weight)
TAPS, twin anemia polycythemia sequence; GA, gestational age; bw, birth weight

Univariate risk factor analysis showed that spontaneous perinatal mortality was significantly associated with Quintero stage 3 (OR = 3.6, 95%CI 1.1-12.0, p = 0.039), antenatal TAPS stage 4 (OR = 6.1, 95%CI 1.8-20.4, p = 0.003), TAPS donor status (OR = 3.7, 95%CI 2.2-6.3, p < 0.001), and GA at birth (OR = 0.8, 95%CI 0.7-1.0, p = 0.006). There was no strong correlation between the risk factors (Quintero stage – TAPS donor status (R < 0.001, p = 1.000); Quintero stage – antenatal TAPS stage (R = 0.078, p = 0.187); TAPS donor status-antenatal TAPS stage (R = -0.005, p = 0.931); Quintero stage – GA at birth (R = -0.022, p = 0.701); TAPS donor status – GA at birth (R < 0.001, p = 0.998); antenatal TAPS stage – GA at birth (R = -0.005, p = 0.931)), so all parameters were included in the multivariate analysis. In the multivariate analysis, antenatal TAPS stage 4 (OR = 6.1, 95%CI 1.4-26.0, p = 0.015), TAPS donor status (OR = 4.2, 95%CI 2.1-8.3,

p < 0.001) and GA at birth (OR = 0.8, 95%CI 0.7-0.9, p = 0.001) were identified as independent risk factors for spontaneous perinatal mortality. Univariate risk factor analysis showed that severe neonatal morbidity was significantly associated with GA at birth (OR = 1.5, 95%CI 1.3-1.8, p < 0.001) and Quintero stage 2 (OR = 3.2 95%CI 1.1-9.7, p = 0.038). Both parameters were included in multivariate risk factor analysis as no correlation was found between the two (R = -0.022, p = 0.701). Multivariate risk factor analysis revealed that only GA at birth was an independent risk factor for severe neonatal morbidity (OR = 1.5, 95%CI 1.3-1.7, p < 0.001). More details on the risk analyses for perinatal mortality and severe neonatal morbidity are presented in Table 2a and 2b of Appendix 2.

Appendix 1. Participating fetal therapy centers and their number of contributed post-laser TAPS cases

Center	Country	Post-laser TAPS Cases
Leiden University Medical Center	The Netherlands	66
Children's Hospital V. Buzzi Milan	Italy	17
Necker-Enfants Malades Hospital Paris	France	16
Leuven University Hospital	Belgium	13
Center Medico-Chirurgical Obstetrical Strasbourg	France	10
Mount Sinai Hospital Toronto	Canada	10
Saint George's Hospital London	United Kingdom	5
Birmingham Women's and Children's NHS Foundation Trust	United Kingdom	5
Medical University of Graz	Austria	3
Mater Hospital Brisbane	Australia	4
University of Texas McGovern Medical School at Houston	United States of America	4
V.I. Kulakov National Medical Research Center of Obstetrics, Gynecology and Perinatology Moscow	Russia	4
Karolinska University Hospital Stockholm	Sweden	3
Hospital Universitari Vall d'Hebron Barcelona	Spain	2
Brugmann University Hospital	Belgium	1
University Medical Center Hamburg-Eppendorf	Germany	1
Yale New Haven Hospital	United States of America	0

Appendix 2a. Univariate and multivariate risk analysis for spontaneous perinatal mortality in post-laser twin anemia polycythemia sequence

	Death† (n = 60/304)	**Alive† (n = 244/304)**	**Univariate analysis OR (95% CI)**	**SE**	**P**	**Multivariate analysis OR (95% CI)**	**SE**	**P**
GA at diagnosis TAPS	23.3 ± 3.3	23.7 ± 3.7	1.0 (0.9-1.1)	0.1	0.623			
GA at laser TTTS	20.1 ± 2.4	20.8 ± 3.3	0.9 (0.8-1.0)	0.1	0.233			
Days between TTTS laser and TAPS	20.9 ± 18.4	21.8 ± 23.1	1.0 (1.0-1.0)	0.0	0.829			
Quintero stage for TTTS ‡								
1	3/35 (9)	32/35 (91)	-*			-*		
2	27/140 (19)	113/140 (81)	2.5 (0.7-8.5)	0.6	0.139	1.7 (0.4-6.9)	0.7	0.439
3	27/111 (24)	84/111(76)	3.6 (1.1-12.0)	0.6	**0.039**	3.5 (0.9-13.2)	0.7	0.069
4	2/10 (20)	8/10 (80)	2.8 (0.2-32.4)	1.3	0.418	3.4 (0.3-33.6)	1.2	0.298
Antenatal TAPS stage								
1	6/47 (13)	41/47 (87)				-*		
2	12/122 (10)	110/122 (90)	0.9 (0.3-3.2)	0.6	0.864	0.8 (0.2-3.2)	0.7	0.790
3	14/56 (25)	50/56 (75)	2.6 (0.7-9.1)	0.6	0.136	2.6 (0.6-10.3)	0.7	0.182
4	20/46 (44)	26/46 (55)	6.1 (1.8-20.4)	0.6	**0.003**	6.1 (1.4-26.0)	0.7	**0.015**
TAPS Recipient	17/158 (11)	146/158 (89)	-*			-*		
TAPS Donor	43/146 (30)	103/146 (70)	3.7 (2.2-6.3)	0.3	**<0.001**	4.2 (2.1-8.3)	0.3	**<0.001**
Persistence of TTTS-TAPS donor-role, no §	25/162 (15)	137/162 (85)	-*					
Persistence of TTTS-TAPS donor-role, yes §	34/137 (25)	103/137 (77)	1.8 (1.0-3.5)	0.3	0.061			
Antenatal therapy ¶								
Expectant management	27/124 (22)	97/124 (78)	-*					
Delivery	4/18 (22)	14/18 (78)	1.4 (0.3-4.2)	0.6	0.622			
IUT (± PET)	16/81 (20)	72/81 (82)	0.9 (0.4-1.9)	0.4	0.893			
Laser surgery	9/44 (21)	38/44 (79)	1.0 (0.3-2.8)	0.5	0.942			
Selective feticide (co-twin)	0/11 (0)	11/11 (100)	0.3 (0.0-2.9)	1.1	0.330			
GA at birth	29.5 ± 5.0	31.6 ± 3.1	0.8 (0.7-1.0)	0.1	**0.006**	0.8 (0.7-0.9)	0.1	**0.001**

Values are odds ratios (OR) (95% confidence intervals (CI)), standard error (SE) and p-value. *was set to zero as reference † 23/327 cases were excluded based on selective feticide or termination of pregnancy ‡ 5 cases had missing Quintero-stage value.§ In 3 cases persistence of TTS-TAPS donor-role was unknown. ¶ SPPS is not able to calculate OR for groups in which the event (spontaneous mortality) does not occur, therefore a correction for non-occurring events is applied, with this correction an unaffected child is changed to an affected child, for all groups. TAPS, twin anemia polycythemia sequence; GA, gestational age; TTTS, twin-twin transfusion syndrome; IUT, intrauterine transfusion; PET, partial exchange transfusion

Appendix 2b. Univariate and multivariate risk analysis for severe neonatal morbidity in post-laser twin anemia polycythemia sequence

	SNM† (139/429)	No SNM† (290/429)	Univariate analysis OR (95% CI)	SE	P	Multivariate analysis OR (95% CI)	SE	P
GA at diagnosis TAPS	24.5 ± 3.1	23.4 ± 4.0	0.9 (0.8-1.0)	0.1	0.090			
GA at laser TTTS	21.1 ± 2.6	20.6 ± 3.6	0.9 (0.8-1.0)	0.1	0.261			
Days between TTTS laser and TAPS	22.3 ± 21.9	22.1 ± 23.9	1.0 (1.0-1.0)	0.0	0.987			
Quintero stage for TTTS								
1	8/34 (24)	26/34 (76)	-*			-*		
2	64/123 (52)	59/123 (48)	3.2 (1.1-9.7)	0.6	**0.038**	2.8 (0.9-9.0)	0.6	0.066
3	28/89 (32)	61/89 (68)	1.3 (0.4-4.2)	0.6	0.617	1.3 (0.4-3.9)	0.6	0.690
4	3/10 (30)	3/10 (70)	1.3 (0.2-9.3)	1.0	0.782	1.1 (0.1-10.0)	1.1	0.959
Antenatal TAPS stage	18/45 (40)	30/45 (60)	-*					
1	41/112 (37)	71/112 (63)	1.0 (0.4-2.5)	0.5	0.970			
2	16/45 (36)	29/45 (64)	0.7 (0. 2-2.2)	0.6	0.570			
3	17/32 (53)	15/32 (47)	1.6 (0.5-5.0)	0.6	0.381			
4								
TAPS Recipient	54/145(37)	91/145 (63)	-*					
TAPS Donor	51/118 (43)	67/118 (57)	1.1 (0.8-1.5)	0.2	0.568			
Persistence of TTTS-TAPS donor-role, no	47/139 (34)	92/139 (66)	-*					
Persistence of TTTS-TAPS donor-role, yes	58/120 (48)	62/120 (52)	1.6 (0.9-3.0)	0.3	0.116			
Antenatal therapy								
Expectant management	34/100 (34)	66/100 (66)	-*					
Delivery	9/17 (53)	8/17 (47)	2.1 (0.6-8.1)	0.7	0.270			
IUT (± PET)	34/72 (47)	38/72 (53)	1.7 (0.8-3.7)	0.4	0.158			
Laser surgery	12/37 (32)	25/37 (68)	1.1 (0.4-2.9)	0.5	0.851			
Selective feticide	3/11 (27)	8/11 (73)	0.7 (0.2-3.2)	0.7	0.720			
GA at birth	29.4 ± 2.6	32.9 ± 2.8	1.5 (1.3-1.7)	0.1	**<0.001**	1.5 (1.3-1.7)	0.1	**<0.001**
Severe growth restriction, no	81/200 (41)	119/200 (60)	-*					
Severe growth restriction, yes	24/62 (39)	38/62 (61)	1.3 (0.8-2.2)	0.3	0.351			
Postnatal TAPS, no	23/58 (40)	35/58 (60)	-*					
Postnatal TAPS, yes	64/151 (42)	87/151(58)	1.1 (0.5-2.5)	0.4	0.800			

Values are odds ratios (OR) (95% confidence intervals (CI)), standard error (SE) and p-value. *was set to zero as reference. † 9 missing values (6 cases with unknown neonatal outcome, and 3 neonates that died shortly after birth) SNM, severe neonatal morbidity; TAPS, twin anemia polycythemia sequence; GA, gestational age; IUT, intrauterine transfusion; PET, partial exchange transfusion

Discussion

This is the first large international study investigating management and outcome in post-laser TAPS twins. Our study shows that post-laser TAPS generally develops within one month after laser for TTTS, but can be detected up to 17 weeks after laser intervention. Management for post-laser TAPS was mostly expectant, but varied considerably, highlighting the lack of consensus for optimal treatment. In this cohort perinatal outcome was poor, particularly due to high perinatal mortality rates in TAPS donors. This study provides important information for clinicians involved in the care for TTTS twins treated with laser surgery and might contribute to a better understanding of post-laser TAPS.

This is the first study that gives a clear overview of the time of onset of post-laser TAPS and demonstrates a wide range in timing of presentation. This variation might be attributed to two factors. First, reversal of the donor-recipient role could result in a slower development of post-laser TAPS. Possibly, former TTTS recipients that become TAPS donors may be protected against anemia for a longer period of time due to the excess of blood they received during TTTS. In contrast, former TTTS donors that become TAPS donors might suffer sooner from anemia due to their relatively hypovolemic state. Alternatively, TAPS might develop later in cases with compensating blood supply, allowed by VA, AA or VV anastomoses. Our results show that half of the post-laser TAPS cases presented within two weeks after laser surgery for TTTS. Although all cases in this study had signs of ongoing TAPS after the first week, spontaneous normalization of a large MCA-PSV discordancy after laser surgery has also been reported.[13, 21] Importantly, a large MCA-PSV difference shortly after laser might in some cases be the result of fetal hemodynamic reequilibration after intervention, rather than the onset of post-laser TAPS due to the presence of a patent anastomosis. Consequently, intervening directly within 1-2 weeks after laser surgery in these cases could lead to unnecessary treatment since there is no ongoing transfusion. Therefore, close follow-up ultrasound examination to identify persistence or progression of MCA-PSV discrepancy after the first weeks after laser surgery is recommended to confirm the diagnosis of post-laser TAPS and prevent unnecessary intervention and exposure to iatrogenic risks.

Our study showed that 81% of the surgeons initially thought that the laser for TTTS was complete. This ‘low index of suspicion’ causes TAPS to often occur

unexpectedly and shows that operator-reported completeness cannot be relied upon. Interestingly, approximately a third of post-laser TAPS twins was treated for TTTS with the Solomon technique. This illustrates that, although the Solomon technique has proved to decrease the incidence of post-laser TAPS[2], clinicians should remain vigilant for the development of this complication even after a complete Solomon line was thought to be achieved. In agreement with the current recommendations of the ISUOG twin guideline[22], we strongly underline the importance of strict routine MCA-PSV Doppler follow-up examination in TTTS twins treated with laser surgery during the entire pregnancy to check for the presence of post-laser TAPS.

We found high rates of perinatal mortality, particularly in the TAPS donor, reflecting the detrimental impact of fetal anemia on perinatal survival. Remarkably, TAPS donors only showed increased risk for perinatal mortality. After birth, donors and recipients have similar rates of severe neonatal morbidity, suggesting that neonatal health is more strongly related to the degree of prematurity than TAPS donor-recipient status. Importantly, this study showed that GA at birth is a strong risk factor for both perinatal mortality and severe neonatal morbidity in post-laser TAPS twins. Compared to post-laser TAPS survivors that were previously investigated in the Solomon trial[23], we report similar rates of severe neonatal morbidity (38% vs 39%), and higher rates of perinatal mortality (18% vs. 26%). Of note, long-term outcome was not investigated in this study and therefore a difference in long-term neurodevelopmental impairment between donors and recipients cannot be precluded.

Post-laser TAPS twins show an overall more detrimental outcome than spontaneous TAPS twins[9]. The exact cause for this difference in outcome is not entirely clear, but it is likely to be multifactorial. The first and most obvious explanation is that post-laser TAPS twins have already experienced TTTS, a severe condition on itself.[24] Therefore, their fetal condition might already be compromised when they start developing TAPS. Given the fact that half of the post-laser TAPS cases occur within the first two weeks after laser, twins have had only limited time to recover, making them more prone to decompensation. A second explanation could be found in the angioarchitecture of placentas of post-laser TAPS twins. This study represents the biggest cohort of injected post-laser TAPS placentas and confirms previous findings that post-laser TAPS

placentas often show only one or a few placental anastomoses.[25] Additionally, we found that most cases had only unidirectional AV anastomoses, without compensating flow from VA, AA, or VV anastomoses in the opposite direction. This might lead to an accelerated deterioration of TAPS, resulting more rapidly in abnormal Doppler blood flows, hydrops and fetal death. Interestingly, a minority of post-laser TAPS placentas did not show residual anastomoses, in spite of the presence of confirmed postnatal TAPS. This could be explained by suboptimal color dye or by the presence of deep-hidden anastomoses.[4, 26] Third, the choice of antenatal management might have also influenced the condition of post-laser TAPS twins. Our results show that treatment for post-laser TAPS is diverse, but that the majority of the group was managed expectantly or received IUT (± PET); two treatment strategies that are not definitive in nature and allow the condition to progress. Possibly, laser was considered more challenging or not feasible in cases that already underwent laser, due to expected reduced visibility, membrane separation, iatrogenic monoamnionicity, PPROM, or because of the same reasons that caused the laser to be incomplete in the first place, such as the position of the placenta. Illustratively, we found a high rate (40%) of residual anastomoses in twins treated with laser reintervention. A detailed evaluation of differences in perinatal outcome between the various management strategies will be presented in a separate study.[27, 28]

As with all registries, this study is fully dependent on local registrations of post-laser TAPS cases. In many countries, TTTS cases are sent back to the referring hospital after laser procedure, leaving care in less experienced hands. As the diagnosis of post-laser TAPS is only reached by adequate MCA-PSV Doppler examination, hemoglobin and reticulocyte measures and placental-injection studies, it is likely that some post-laser TAPS cases have been missed. Nonetheless, this study represents the largest cohort of post-laser TAPS twins to date and is able to provide valuable insights into management and outcome in post-laser TAPS.

To conclude, post-laser TAPS can occur at any time after laser for TTTS, is managed heterogeneously, and is associated with poor outcome, particularly in donor twins. Our findings necessitate further research into the best treatment option for TAPS. To adequately investigate the best treatment for TAPS, an international randomized controlled trial is needed.

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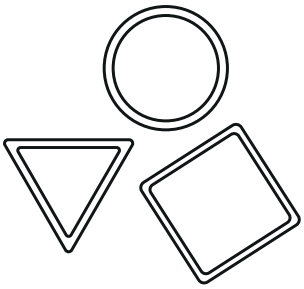
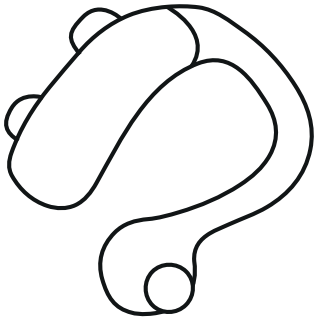
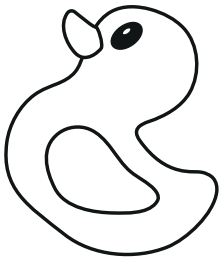
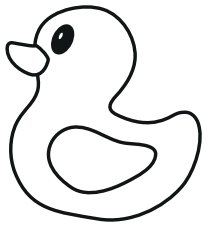
PART 9

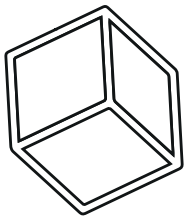
long-term outcome

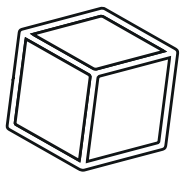












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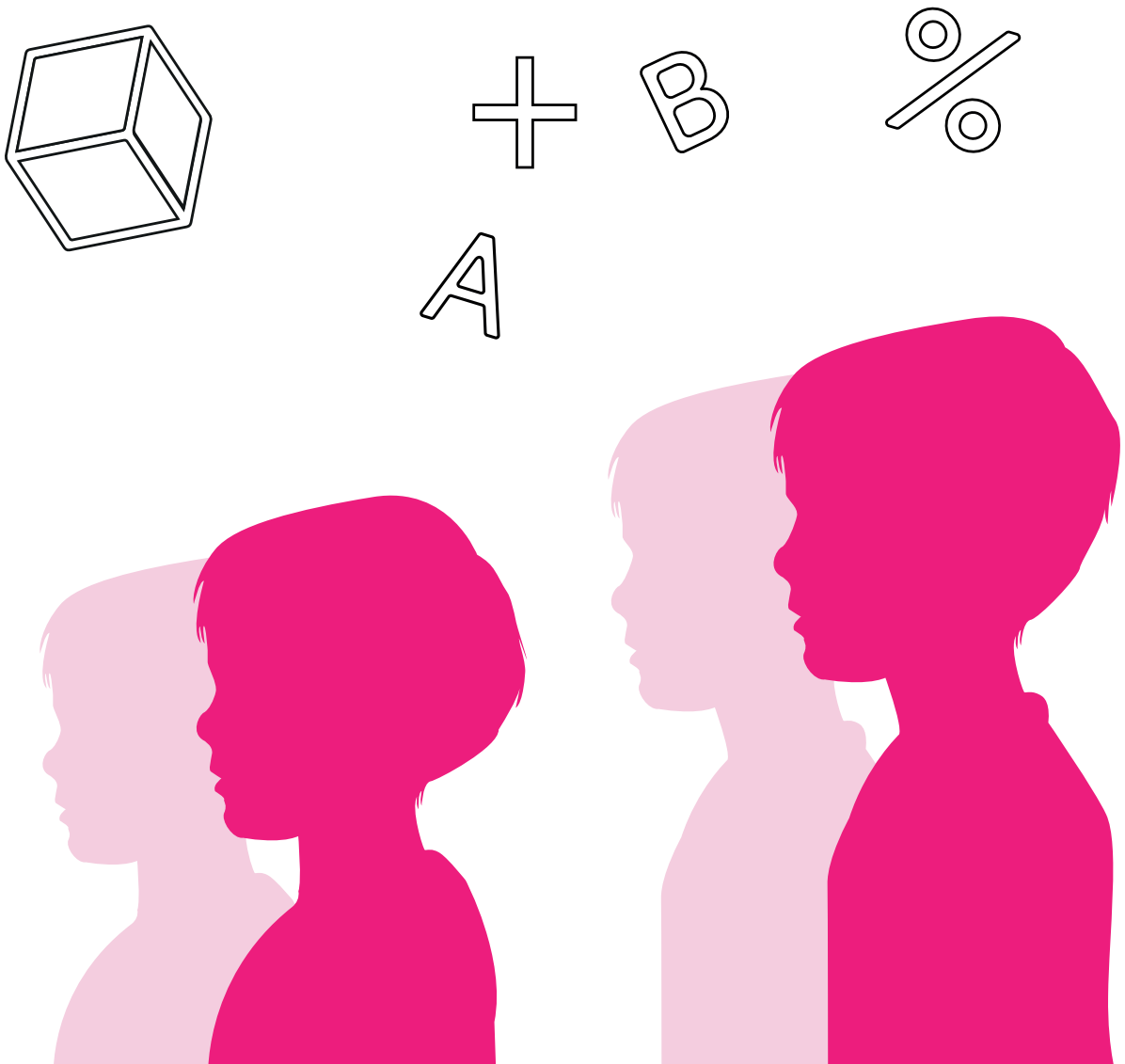
Chapter 11

High risk of long-term neurodevelopmental impairment in donor twins with spontaneous twin anemia polycythemia sequence



Abstract

Objective

The aim of this study was to evaluate the long-term neurodevelopmental and behavioural outcome in survivors of twin anemia polycythemia sequence (TAPS).

Methods

This was a retrospective study of a consecutive cohort of spontaneous TAPS survivors delivered between2005 and 2017 at the Leiden University Medical Center, The Netherlands. Neurological, motor, cognitive, and behavioural development was assessed at median age of 4 years. The primary outcome was neurodevelopmental impairment (NDI), which was a composite outcome of cerebral palsy, deafness, blindness and motor and/or cognitive delay. NDI was subdivided into two grades of severity: mild-to-moderate and severe NDI. Outcome was compared between surviving donor and recipient twins. Logistic regression analysis was used to assess risk factors for NDI

Results

Fourty-nine twin pregnancies complicated by spontaneous TAPS were eligible for inclusion. The perinatal survival rate was 83% (81/98) of twins. Neurodevelopmental assessment was performed in 91% (74/81) of surviving twins. NDI occurred in 31% (22/74) of the TAPS survivors, and was found more often in donors (44%; 15/34) than in recipients (18%; 7/40) (OR 4.1, 95%CI 1.8-9.1, p = 0.001). Severe NDI was detected in 9% (7/74) of survivors and was higher in donors compared with recipients, 18% (6/34) versus 3% (1/40), although the difference did not reach statistical significance (p = 0.056). Donors demonstrated lower cognitive scores compared with recipients (p = 0.011). Bilateral deafness was identified in 15% (5/34) of the donors compared with 0% (0/40) of recipients (p = 0.056). Parental concern regarding development was reported more often for donor than for recipient twins (p = 0.001). On multivariate analysis, independent risk factors for NDI were gestational age at birth (OR = 0.7, 95%CI 0.5-0.9, p = 0.003) and severe anemia (OR = 6.4, 95%CI 2.4-17.0, P < 0.001).

Conclusion

Surviving donor twins of pregnancies complicated by spontaneous TAPS have four-fold higher odds of NDI compared with recipient co-twins. TAPS donors have a fourfold higher risk of NDI compared with recipient co-twins and are at increased risk of cognitive delay and deafness.

Introduction

Twin anemia polycythemia sequence (TAPS) is a form of chronic imbalanced foeto-foetal transfusion through minuscule placental anastomoses in monochorionic twin pregnancies, leading to anemia in the donor twin and polycythemia in the recipient twin.[1] Unlike twin-to-twin transfusion syndrome (TTTS), TAPS is not associated with amniotic fluid discordance. TAPS occurs spontaneously in 3-5% of the monochorionic twin pregnancies (spontaneous TAPS) and can develop iatrogenically due to residual anastomoses in 2-16% of pregnancies treated with laser surgery for TTTS (post-laser TAPS). [2, 3] While the optimal antenatal management in TAPS is not known, management options include expectant management, induced preterm delivery, intrauterine blood transfusion (IUT) with or without partial exchange transfusion (PET), fetoscopic laser coagulation of the placental anastomoses and selective feticide.

Short-term outcome in TAPS varies from isolated hemoglobin differences to severe cerebral injury and neonatal death.[4] Due to an increasing number of monochorionic twins being liveborn after a complicated pregnancy, attention is shifting from short-term perinatal outcome to long-term neurodevelopmental outcome, focusing more on survival without impairment and quality of life. Only one previous study in a cohort of post-laser TAPS evaluated the long-term outcome of TAPS and showed that severe neurodevelopmental impairment (NDI) occurs in 9% of survivors.[5] However, in spontaneous TAPS the long-term neurodevelopmental outcome is unknown, which hampers adequate parent counselling. Moreover, knowledge of the long-term outcome is of paramount importance for designing future RCT's to determine the best treatment option for TAPS.

The aims of the current study were to evaluate the long-term neuro-developmental and behavioural outcomes in a large cohort of children of pregnancies complicated by spontaneous TAPS, to compare outcome between donors and recipients, and to identify potential risk factors for NDI.

Methods

All consecutive monochorionic twins with spontaneous TAPS evaluated at our center between 2005 and 2017 were eligible for this study. The Leiden University Medical Centre (LUMC) is the national referral centre for complicated twin

pregnancies and fetal therapy. The study was approved by the institutional ethics review board and all parents gave written informed consent for their children to participate. Monochorionic twin pregnancies identified as having TAPS either antenatally and/or postnatally were eligible for inclusion. Antenatal diagnosis was based on the recently updated ultrasound Doppler criteria for TAPS.[6] In brief, TAPS was diagnosed in presence of change u delta middle cerebral artery peak systolic velocity (MCA-PSV) > 0.5 multiples of the median (MoM), which is suggestive of the imbalanced chronic fetofetal transfusion leading to fetal anemia and polycythemia. Postnatal diagnosis was based on a large (> 8 g/dL) inter-twin difference in hemoglobin with at least one of the following criteria: reticulocyte count ratio > 1.7 and the presence of only minuscule (diameter < 1mm) anastomoses at the placental surface detected by color dye injection.[7] TAPS was classified antenatally and postnatally from stages 1 to 5, in accordance with the previously published staging systems for TAPS.[6, 8]

The following perinatal data were retrieved from our databases: gestational age at diagnosis, antenatal TAPS stage, antenatal treatment, gestational age at birth, sex, birth weight, small-for-gestational age (SGA: birth weight < 10th percentile) or fetal growth restriction (FGR; birthweight < 3rd percentile, according to the charts of Hoftiezer et al.[9], severe fetal anemia, hemoglobin and reticulocyte values at birth, need for blood transfusion or partial exchange transfusion on day 1 after delivery, severe neonatal morbidity, severe cerebral injury, and perinatal death. Severe fetal anemia was defined as the need for IUT, fetal MCA-PSV value > 1.7 MoM or a blood transfusion at birth. The definition of severe neonatal morbidity is based on the presence of at least one of the following conditions: respiratory distress syndrome requiring mechanical ventilation or surfactant, patent ductus arteriosus requiring medical therapy or surgical closure, necrotizing enterocolitis ≥ grade two[10], or severe cerebral injury. Severe cerebral injury was diagnosed in case of the presence of one of the following abnormalities detected on cerebral imaging: intraventricular haemorrhage ≥ grade three[11] cystic periventricular leukomalacia ≥ grade two[12], ventricular dilatation ≥ 97th percentile[13], porencephalic cysts, or arterial or venous infarction.

A follow-up appointment was scheduled at a minimum age of 24 months and consisted of a neurologic and cognitive assessment and a behavioural questionnaire. Cognitive development was assessed using three standardized

psychometric age-appropriate tests, providing cognitive scores with a normal distribution with a mean of 100 and a SD of 15. For children aged 2-3 years, the Dutch version of the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III-NL)[14] was used. When children were aged between three and six years the Wechsler Preschool and Primary Scale of Intelligence third Edition (WPPSI-III-NL) was used.[15] For children aged 7 years or older, the Wechsler Intelligence Scale for Children third edition (WISC-III-NL)[16], was used. To investigate behavioural problems, parents completed the Child's Behaviour Checklist (CBCL) for 1.5-5 years or 6-18 years, as appropriate.[17, 18] In cases in which the child presented with hearing loss, vision loss, or cerebral palsy, additional medical information from our center or peripheral hospitals was requested to determine the grade of severity of the impairment. Maternal educational level was recorded and divided into three levels. A score of one was given when the mother's education was low (primary school), a score of two for intermediate level (secondary school and intermediate vocational school), and a score of three for higher levels (higher vocational school and university).

The primary outcome of this study was NDI, which was defined as a composite outcome consisting of four different domains: motor and/or cognitive impairment, vision loss, hearing loss, and cerebral palsy. NDI was subdivided into two grades of severity: mild-to-moderate and severe NDI. For mild-to-moderate NDI at least one of the following criteria needed to be fulfilled: mild cognitive or motor delay (IQ score < 85 (-1SD)), vision loss, hearing loss, or cerebral palsy (Gross Motor Functioning Classification System (GMFCS)[19], Level 1). Severe NDI was diagnosed in case of at least one of the following: severe cognitive or motor delay (IQ score < 70 (-2SD)), bilateral blindness, bilateral deafness (requiring amplification), or severe cerebral palsy (GMFCS Level ≥ 2). The incidence of NDI was compared between TAPS donors and recipients. The secondary outcomes included behavioural problems and a risk factor analysis for NDI. The presence of behavioural problems was defined as a T-score ≥ 64 in one of the following broadband scales: total problems, internalizing problems (anxious/depressed, withdrawn, somatic complaints), or externalizing problems (rule-breaking, aggressive behaviour). A specific item of the CBCL open field regarding parental concerns about their child's development, was included as a separate secondary outcome. The following risk factors were analysed for NDI: management strategy, donor status, severe fetal anemia, FGR and maternal educational level.

Statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Data are reported as mean ± SD or as median and interquartile ranges (IQR), as appropriate. A P-value < 0.05 was considered to indicate statistical significance. To compare outcomes between TAPS donors and recipients, a paired sampled T-test and generalized estimating equations was performed for continuous or categorical outcomes, respectively. As a generalized estimating equations cannot be used in case of non-occurring events in one of the groups, an adjustment to the data was applied in which an unaffected child was changed into an affected child, for both groups. This correction generates more conservative P-values. Potential risk factors were checked for correlation using Pearson's square (*r*). An *r*-value of > 0.7 or < -0.7 was considered to indicate a strong relationship between the factors. Potential risk factors for NDI were assessed in a univariate logistic regression model. A multivariate logistic regression model was applied on the variables that showed significant association in the univariate analysis. Results are expressed as odds ratios (OR) with 95% CI.

Results

Between 2005 and 2017, 49 monochorionic twin pregnancies were diagnosed with spontaneous TAPS at the LUMC. Demise occurred in 15 fetuses, which was in all cases related to or preceded by an intervention, including selective feticide (n = 8), laser surgery (n = 3), or termination of pregnancy (n = 4). Neonatal mortality occurred in two infants, yielding a total population of 81 TAPS survivors eligible for long-term follow up. Seven (9%) children were lost to follow-up due to declined consent (n= 3) or loss of contact information (n = 4). In total, long-term follow-up assessment was performed in 91% (74/81) of TAPS survivors, including 34 donors and 40 recipients, from 41 TAPS pregnancies. The derivation of the study population is summarized in Figure 1.

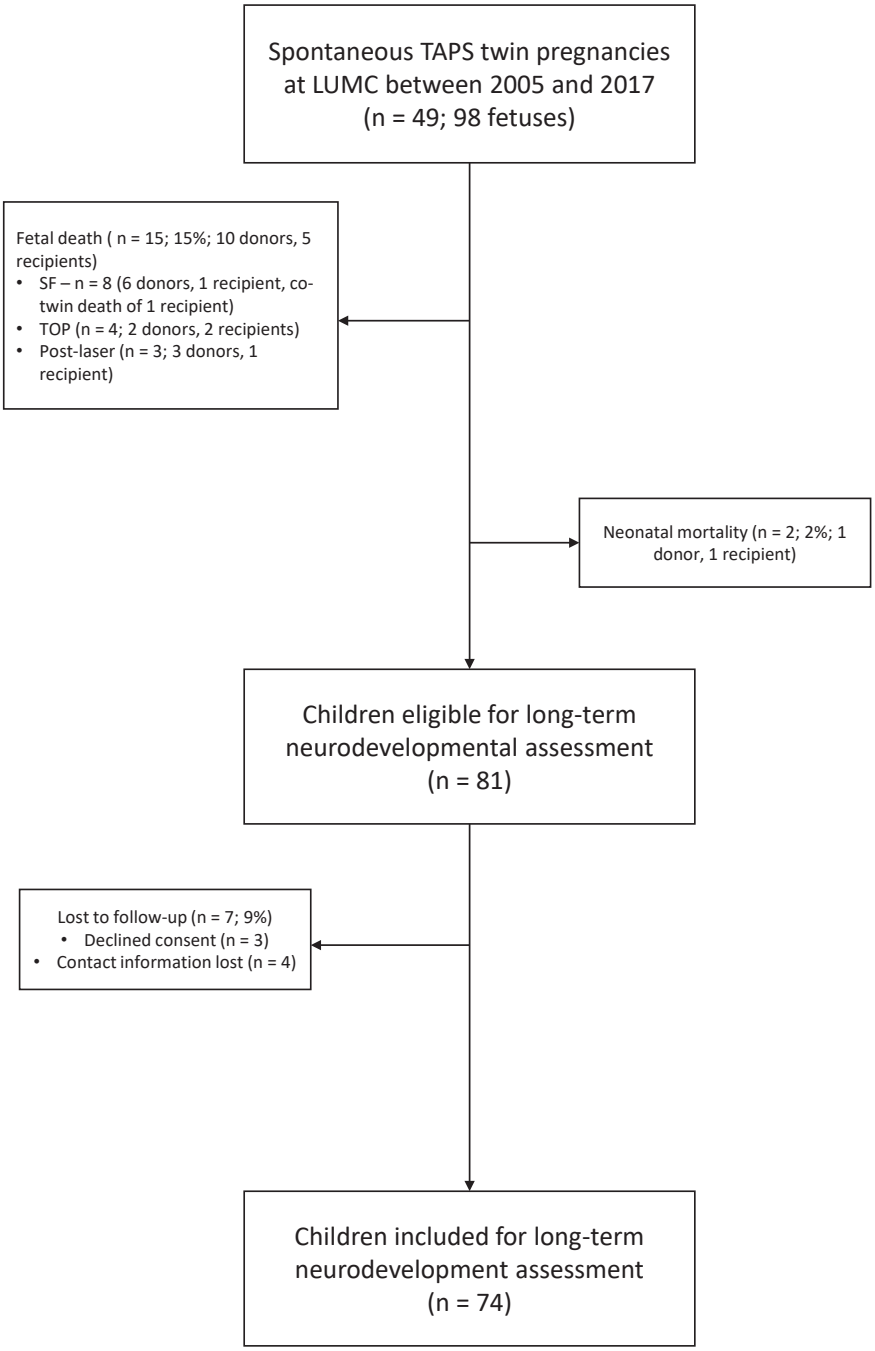


Figure 1. Derivation of the study population. TAPS. Twin anemia polycythemia sequence, LUMC: Leiden University Medical Centre, SF: selective feticide, TOP: termination of pregnancy.

Pregnancy characteristics and neonatal outcome in the total population are shown in Table 1 and 2, respectively. TAPS was detected antenatally in 71% (29/41) of the cases, with TAPS stage ranging from 1 to 3. Fetal therapy was performed in 59% of this group and consisted of IUT/PET (14% (4/29)), laser surgery (28% (8/29)), and selective feticide (17%, (5/29)). Twelve (41%) TAPS pregnancies were managed expectantly and in two (17%), spontaneous resolution occurred after 3 and 6 weeks, respectively. In 50% (6/12) of the cases managed expectantly, preterm delivery was induced due to fetal distress or progression of the disease. In 29% (12/41) of the total population, TAPS was not detected antenatally and diagnosed postnatally. Overall, median gestational age at birth was 33.0 weeks (IQR: 30.1-35.7).

Table 1. Characteristics for 41 pregnancies complicated by twin anemia polycythemia sequence (TAPS)

	TAPS pregnancies (N = 41)
Antenatal diagnosis	29/41 (71)
Gestational age at diagnosis (weeks)	20.4 (18.0-27.0)
Antenatal TAPS stage	
Stage 1	5/29 (17)
Stage 2	11/29 (38)
Stage 3	13/29 (45)
Stage 4	-
Stage 5	-
Management	
Expectant	12/29(41)
IUT (with PET)	4/29(14)
Laser Surgery	8/29 (28)
Selective Foeticide	5/29 (17)
Gestational age at birth (weeks)	33.0 (30.1-35.7)
Postnatal diagnosis (only)	12/41 (29)
Postnatal TAPS stage	
Stage 1	3/27 (11)
Stage 2	10/27 (37)
Stage 3	5/27 (19)
Stage 4	5/27 (19)
Stage 5	4/27 (14)
Maternal education	
Low	4/41 (10)
Intermediate	13/41 (32)
High	24/41 (58)

Data are presented as median (IQR) or n/N (%)
TAPS: twin anemia polycythemia sequence, IUT: intrauterine transfusion, PET: partial exchange transfusion

Donors and recipients differed significantly in birth weight (p < 0.001). In addition, 53% of the donors were affected by FGR compared with 8% of the recipients (p < 0.001). There was no difference in rate of severe neonatal morbidity between donors and recipients. Within 1 day after delivery, TAPS donors received a blood transfusion for anemia in 65% of cases, whereas 38% of recipients were treated with a partial exchange transfusion for polycythemia.

Table 2. Neonatal outcome in 74 survivors of 41 pregnancies complicated by spontaneous twin anemia polycythemia sequence, overall and according to donor or recipient status

	Total (N = 74)	**Donors (N = 34)**	**Recipients (N = 40)**	**p-value**
Female sex	37/74 (50)	17/34 (50)	20/40 (50)	1.000
Birth weight (g)	1835 (1295-2238)	1733 (1160-1980)	2042 (1396-2424)	**< 0.001**
SGA	32/74 (43)	24/34 (71)	8/40 (20)	**< 0.001**
FGR	21/74 (28)	18/34 (53)	3/40 (8)	**< 0.001**
Severe neonatal morbidity				
Respiratory distress	21/74 (28)	8/34 (24)	13/40 (33)	0.116
syndrome	21/74 (28)	8/34 (24)	13/40 (33)	0.116
Patent ductus arteriosus	5/74 (7)	3/34 (9)	2/40 (5)	0.218
Necrotizing enterocolitis	2/74 (3)	1/34 (3)	1/40 (3)	0.905
Severe cerebral injury	1/74 (1)	0/34 (0)	1/40 (3)	0.668
Severe fetal anemia	30/74 (41)	30/34 (88)	0/40 (0)	-
Blood transfusion*	22/74 (30)	22/34 (65)	0/40 (0)	-
Partial exchange transfusion*	15/74 (20)	0/34 (0)	15/40 (38)	-

Data are presented as n/N (%) or median (interquartile range). *Within 1 day after delivery. FGR, fetal growth restriction (birth weight < 3rd percentile); SGA, small-for-gestational age (birth weight < 10th percentile)

Long-term neurodevelopmental outcome in the 74 TAPS survivors was assessed at a median age of 4 years (IQR: 2-6). Twenty-seven (37%) children were tested with a Bayley-III-NL, 33 (45%) children using WPPSI-III-NL and in 14 (19%) children, assessment was performed using WISC-III-NL. Table 3 shows long-term outcome. NDI was detected in 30% of the total group, affecting 15/34 (44%) donors and 7/40 (18%) recipients (p = 0.001). Donors had 4.1 (95%CI, 1.8-9.1)-fold higher risk for NDI compared to their recipient co-twins (p = 0.001). The incidence of severe NDI was 9% (7/74), occurring in 6/34 (18%) donors and in 1/40 recipient (3%) (p = 0.056). Further details on the cases with severe NDI are displayed in Table 4. Mild-to-moderate NDI was found in 20% (15/74) of all children, occuring in 26% (9/34) of donors and 15% (6/40) of recipients (p = 0.093). In addition,

donors had significantly lower cognitive scores compared with recipients (95 vs. 101; p = 0.001). Bilateral deafness was observed in 5/34 (15%) TAPS donors, which was in all cases due to auditory neuropathy spectrum disorder (ANSD), while none of the recipients had deafness (p = 0.056). Behavioural problems were reported in 10% (7/72) of the total group, with no difference between TAPS donors and recipients. Parents concern regarding development was reported more often for donor than for recipient twins (p = 0.001).

Table 3. Long-term outcome in 74 spontaneous TAPS survivors

	Total (N =74)	**Donors (N = 34)**	**Recipients (N = 40)**	**P-value**
Cognitive score	97 (87-105)	95 (87-105)	101 (90-106)	**0.011**
Cognitive delay				
Mild (score < 1 SD)	19/74 (26)	12/34 (35)	7/40 (18)	**0.006**
Severe (score < 2 SD)	2/74 (3)	2/34 (6)	0/40 (0)	0.265
Motor delay				
Mild (score < 1 SD)	1/26 (4)	1/12 (8)	0/14 (1)	0.471
Severe (score < 2 SD)	0/26 (0)	0/12 (0)	0/14 (0)	-
Bilateral blindness	1/74 (1)	0/34 (0)	1/40 (3)	0.657
Bilateral deafness	5/74 (7)	5/34 (15)	0/40 (0)	**0.056**
Cerebral Palsy (GMFCS Level 1)	2/74 (3)	2/34 (6)	0/40 (0)	0.265
NDI	22/74 (30)	15/34 (44)	7/40 (18)	**0.001**
Mild-to-moderate	15/74 (20)	9/34 (26)	6/40 (15)	0.093
Severe	7/74 (9)	6/34 (18)	1/40 (3)	**0.056**
NDI-free survival	52/82 (63)	19/41 (46)	33/41 (80)	**< 0.001**
Behavioural problems†				
Total	7/72 (10)	3/33 (9)	4/39 (10)	0.435
Internalizing	6/72 (8)	2/33 (6)	4/39 (10)	0.264
Externalizing	8/72 (11)	4/33 (12)	4/39 (10)	0.417
Parental concern regarding child's development	33/72 (46)	20/33 (61)	13/39 (33)	**0.001**

Data are presented as median (interquartile range) or n/N(%).*26 children had complete motor assessment with Bayley (BSID III) † Child Behaviour Checklist was not completed for one twin pair
GMFCS: Gross Motor Function Classification System, NDI: neurodevelopmental impairment

Table 4. Characteristics of seven survivors of pregnancies complicated by spontaneous twin anemia polycythemia sequence (TAPS) that had severe neurodevelopmental impairment

TAPS donor / recipient	GA at diagnosis (weeks)	Antenatal TAPS stage	Antenatal Treatment	GA at birth (weeks)	Neonatal morbidity	Hb at birth (g/dL);	Δ Hb (g/dL)	Postnatal TAPS stage	Long-term outcome
Recipient	15^{+2}	Stage 2	Expectant management	25^{+2}	RDS PDA IVH stage 2 ROP stage 3	21.6	NA†	NA	Bilateral blindness Mild cognitive delay Internalizing and externalizing behavioural problems
Donor	26^{+5}	Stage 1	Expectant management	36^{+2}	FGR	9.5	14.2	3	Severe cognitive delay Externalizing behavioural problems
Donor	28^{+2}	Stage 3	IUT, induced delivery	29^{+1}	RDS, PDAPDA	4.8	15.6	3	Bilateral deafness (ANSD) CP (GMFCS Level 1) Severe cognitive delay
Donor	30^{+1}	Stage 1	Induced delivery	30^{+1}	RDS, FGR	3.1	16.3	3	Bilateral deafness (ANSD) Mild cognitive delay
Donor	25^{+9}	Stage 1	Expectant management, Induced delivery	28^{+6}	RDS	8.1;	13.5	2	Bilateral deafness (ANSD)
Donor	28^{+3}	Stage 2	Laser surgery	28^{+4}	RDS	4.0	22.2	5	Bilateral deafness (ANSD)
Donor	Postnatal	-	-	35^{+5}	FGR	6.4	16.1	3	Bilateral deafness (ANSD) CP (GMFCS Level 1) Externalizing behavioural problems

*Difference in hemoglobin (Hb) level between twin and cotwin. †Neonatal mortality occurred within 1 day after birth in donor cotwin due to low birth weight (430 g, < 3rd centile) so Hb level was not available and Hb could not be calculated. ANSD, auditory neuropathy spectrum disorder; CP, cerebral palsy; FGR, fetal growth restriction; GA, gestational age; GMFCS, Gross Motor Function Classification System; IUT, intrauterine transfusion; IVH, intraventricular hemorrhage; NA, not available; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity.

Table 5. Univariate and multivariate logistic regression analysis of potential risk factors for neurodevelopmental impairment in 74 surviving twins of 41 pregnancies complicated by spontaneous twin anemia polycythemia sequence

			Univariate analysis			Multivariate analysis		
	NDI (n = 22)	**No NDI (n = 52)**	**OR (95% CI)**	**SE**	**P**	**OR (95% CI)**	**SE**	**P**
Management								
Postnatal diagnosis	5/24 (21)	19/24 (79)	-	-	-			
Expectant management	11/22 (50)	11/22 (50)	3.8 (0.9-15.3)	0.7	0.061			
IUT (with or without PET)	3/8 (38)	5/8 (62)	2.3 (0.3-15.0)	1.0	0.177			
Laser surgery	2/15 (13)	13/15 (87)	0.6 (0.1-4.0)	1.0	0.577			
Selective feticide	1/5 (20)	4/5 (80)	1.0 (0.1-10.9)	1.2	0.967			
Severe anemia	16/30 (53)	14/30 (47)	6.2 (2.6-14.8)	0.4	**<0.001**	6.4 (2.4-17.0)	0.5	**<0.001**
Donor*	15/34 (44)	19/34 (56)	4.1 (1.8-9.1)	0.4	**0.001**			
GA at birth (completed weeks)	31 ± 3.4	33 ± 2.8	0.8 (0.6-1.0)	0.1	**0.024**	0.7 (0.5-0.9)	0.1	**0.003**
FGR	10/21 (48)	11/21 (52)	3.1 (1.1-8.4)	0.5	**0.030**	2.1 (0.7-6.9)	0.6	0.211
Level of education mother			0.8 (0.3-2.2)	0.5	0.802			
Low	1/4 (25)	3/4 (75)						
Intermediate	5/13 (38)	8/13 (62)						
High	6/24 (25)	18/24 (75)						

Data are given as n/N (%) or mean ± SD, unless stated otherwise. *Donor status was excluded from multivariate analysis due to strong correlation with severe anemia (R = 0.84, P < 0.001). †Odds ratio (OR) for maternal education level based on score in which 1 = low (primary school), 2 = intermediate (secondary school and intermediate vocational school) and 3 = high (high vocational school and university). FGR, fetal growth restriction (birth weight < 3rd centile); GA, gestational age; IUT, intrauterine transfusion; NDI, neurodevelopmental impairment; PET, partial exchange transfusion; SE, standard error.

Univariate logistic regression analysis of potential risk factors for NDI showed a significant association with severe fetal anemia (OR = 6.2, 95%CI 2.6-14.8, p < 0.001), donor status (OR = 4.1, 95%CI 1.8-9.1, p = 0.001), FGR (OR = 3.1, 95%CI 1.1-8.4, p = 0.030) and gestational age at birth (OR = 0.8, 95%CI 0.6-1.0, p = 0.024). All significant risk factors were included in the multivariate analysis. As severe fetal anemia was correlated strongly with donor status (r = 0.84, p < 0.001), donor status was excluded from the multivariate analysis. There was no strong significant association between other risk factors. Multivariate analysis demonstrated that severe anemia (OR = 6.4, 95%CI 2.4-17.0, p < 0.001) and gestational age at birth (OR = 0.7, 95%CI 0.5-0.9, P = 0.003) were independent risk factors for NDI. So, for each incremental week of gestation, the risk of NDI decreases by 30%, and children with severe fetal anemia have a 6.4-fold increased risk for NDI (Table 5).

Discussion

This is the first study to investigate long-term neurodevelopmental outcome in surviving infants of pregnancies complicated by spontaneous TAPS. TAPS donors had a fourfold higher risk of NDI compared with TAPS recipients. We also observed an unexpected high risk of bilateral deafness (15%) in TAPS donors, with no cases observed in recipients. In addition, there was a higher rate of mild cognitive delay and lower cognitive scores in TAPS donors compared with TAPS recipients.

The reason for the large discrepancy in long-term outcome between TAPS donors and recipients is unknown. To date, in the vast majority of long-term follow-up in TTTS or post-laser TAPS cohorts, no difference has been reported between donors and recipients.[5, 20] In these cohorts, gestational age at birth was the main predictor of adverse outcome. As twins, including those with TAPS, are born at the same gestational age, we need to look for other factors to explain the differences between donor and recipient. Given that TAPS twins are monochorionic and therefore monozygotic, genetic factors cannot play a role. Typically, in TAPS pregnancies, donors and recipients are exposed to different intrauterine environments. In TAPS, chronic erythrocyte loss from the donor into the recipient's circulation, gradually leading to fetal anemia, may result in a chronic hypoxic environment, impairing fetal brain development in the donors over time. Notably, donors with severe fetal anemia had a six-fold increased risk

for NDI. Alternatively, FGR could also be an important contributor to long-term adverse outcome in donors. In our population, FGR affected 53% of donors. FGR is known to be associated with long-term impairment in singletons[21], likely caused by decreased white and grey matter in the hippocampus and frontal lobe.[22, 23] These cerebral areas are responsible for memory, learning skills and executive functioning and therefore play a crucial role in cognitive impairment. In our study, FGR was a significant risk factor for NDI in univariate analysis, but failed to show significance in multivariate analysis, suggesting an association between FGR and other factors. A relation between fetal anemia and FGR in TAPS is not unlikely. Selective FGR (based on birth-weight discordance ≥ 25%) has previously been described in spontaneous TAPS twins and complicated 30% of the population.[24] Interestingly, growth restriction in TAPS is not related to unequal placental sharing; on the contrary, donors often have larger placental territories than recipients. FGR in TAPS donors might therefore be caused by other factors, such as chronic erythrocyte, albumin and/or protein loss through placental anastomoses.[25]

Parental concern regarding the development of their child was reported more often for donor than for recipient twins. It is reassuring for the validity of our findings that the impairment observed by standardized assessment coincides with parental concerns in daily life. The overall incidence of behavioural problems was 10%, which is comparable to that (10%) in children from the general Dutch population.[26]

Surprisingly, TAPS donors were more affected by deafness than TAPS recipients. The limited sample size and statistical adjustment for non-occurring events in paired groups prevented this difference to reach statistical significance, but clinically we found this striking and warranting more attention. Notably, deafness in all five affected donors was based on ANSD. In ANSD, the cochlea and outer hair cells are unaffected, but the inner hair cells, which connect synapses and/or the auditory nerve itself are damaged, resulting in compromised transmission of sound to the brain.[27] The pathogenesis of ANSD is multifactorial, including prematurity and perinatal hypoxia.[28] In theory, the chronic hypoxic state of the anemic foetus could have damaged not only the brain, but the developing auditory nerve system as well. Interestingly, the high incidence of deafness is not reported before in TTTS survivors or in children suffering from chronic fetal anemia based on erythrocyte alloimmunization.[29,30]

Moreover, the incidence of deafness in TAPS donors is higher compared with that in infants admitted to the neonatal intensive care unit (1-3%) [31]. To further explore the pathogenesis behind deafness in spontaneous TAPS, more elaborate studies are needed; for example, using neonatal brain magnetic resonance imaging.

Our study also revealed that spontaneous TAPS survivors showed a more detrimental outcome than did survivors of post-laser TAPS in a previous study.[5] Although the incidence of severe NDI was 9% for both groups, post-laser TAPS twins had overall a better outcome. They showed a lower rate of mild cognitive delay (17% vs. 26%), did not have bilateral deafness, and did not demonstrate differences between donor and recipient.[5] Two different theories may be considered to explain this discrepancy. Firstly, post-laser TAPS donors are less frequently growth restricted than spontaneous TAPS donors. This is because TAPS donors often have been former TTTS recipients, who generelly have a higher birth weight than TTTS donors.[32] Thus, post-laser TAPS donors might be protected by the relatively higher fetal weight when they start to develop anemia. Alternatively, spontaneous TAPS might develop earlier in gestation compared to post-laser TAPS, leading to a more chronic exposure to anemia during pregnancy.

Caution should be taken when interpreting our results due to the retrospective nature of this study. In our cohort, TAPS pregnancies varied in TAPS stage, gestational age at onset and management, making it hard to draw reliable conclusions with regard to the true effect of the natural course of TAPS. Furthermore, the sample size of this group was small, but it is nonetheless the largest group of spontaneous TAPS survivors reported to date.

In conclusion, this study has shown that spontaneous TAPS is characterized by a high impairment rate and that donors have an increased risk of cognitive impairment and deafness. To date, spontaneous TAPS has been thought to be a relatively benign form of feto-fetal transfusion, but these results show that the long-term consequences of this condition should not be underestimated. These findings necessitate further research into the best antenatal therapy for TAPS. Recently, the TAPS Trial has started, a study that will compare laser treatment with standard care for TAPS.[33] Finally, this study reinforces the importance of long-term follow-up for complicated monochorionic twin pregnancies. Although TAPS survivors have few severe neonatal problems, the true impact of this

condition seems to manifest in childhood. Therefore, routine long-term follow-up including screening for hearing loss should be an essential part of care for TAPS twins.

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PART 10

summary and discussion

Summary

This thesis consists of studies that relate to different aspects of TAPS. The thesis opens with a patient journey (part 1), followed by the general introduction (part 2) and an overview of the literature (part 3).

In Part 4 (pathogenesis) we investigated maternal, placental and outcome characteristics of TTTS twins that present with co-existing anemia-polycythemia (AP) prior to laser surgery. Part 5 (antenatal diagnosis) describes the diagnostic accuracy of delta MCA-PSV> 0.5 MoM, and the prevalence of additional ultrasound markers in TAPS pregnancies. In Part 6 (antenatal management) two studies investigating antenatal management for TAPS are presented: the TAPS Registry and the TAPS Trial. The studies in Part 7 (postnatal diagnosis) focus on the diagnostic value of color difference on the maternal side of the TAPS placenta. In Part 8 (short-term outcome) we investigated diagnosis, management and outcome in spontaneous TAPS and in post-laser TAPS separately. The final chapter (Part 9) of this thesis focuses on long-term outcome in spontaneous TAPS twins.

Review

Chapter 1 comprises a review of the literature and summarizes findings and insights from approximately 100 studies published a decade after our initial report on TAPS. In addition, we propose a flowchart for management for TAPS, based on gestational age at diagnosis and stage of the disease.

Pathogenesis

In **chapter 2**, we showed that that AP (anemia-polycythemia; defined as delta MCA-PSV > 0.5) complicates 15% of TTTS pregnancies prior to laser surgery. Twins with TTTS+AP received laser surgery at a later gestational age, indicating a later time of onset of TTTS. Moreover, placentas from TTTS+AP twins demonstrated fewer anastomoses at the vascular equator than placentas from twins with TTTS-only. Interestingly, despite comparable gestational age at birth, twins with TTTS+AP had a more favorable outcome than twins with TTTS-only. The rate of severe neonatal morbidity (composite of respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC) and severe cerebral injury), and RDS were significantly lower in twins with TTTS+AP. In addition, there was a clear trend towards a more detrimental outcome in

terms of neonatal mortality, PDA, NEC and severe cerebral injury in twins that presented with TTTS-only. These differences were also translated into the long-term outcome: disease-free survival (survival without severe long-term impairment) was significantly higher in twins with TTTS+AP than twins with TTTS-only.

Antenatal diagnosis

In Part 3 we investigated two aspects of antenatal diagnosis of TAPS. In **chapter 3**, we assessed the diagnostic accuracy of delta MCA-PSV > 0.5 MoM for the prediction of TAPS and compared it to the currently used fixed cut-off levels of MCA-PSV > 1.5 for the TAPS donor and <1.0 MoM for the TAPS recipient. We found that delta MCA-PSV > 0.5 MoM was characterized by higher rates of sensitivity and specificity compared to the fixed MCA-PSV cut-off levels. Moreover, we demonstrated a significant correlation between delta MCA-PSV and postnatal inter-twin hemoglobin difference. In **chapter 4** we evaluated the prevalence of various additional ultrasound markers in TAPS. We found that placental dichotomy, cardiomegaly in the donor and a starry-sky liver in the recipient were found in 44%, 70% and 66% of TAPS cases, respectively. A total of 86% of TAPS twins demonstrated at least one of these sonographic markers, meaning that 14% presents solely with abnormal MCA-PSV values. The prevalence of all three markers increased with incrementing TAPS stage.

Antenatal management

In **chapter 5** we investigated management choices for TAPS in 17 fetal therapy centers and compared outcome between expectant management, delivery, intrauterine transfusion (IUT) with or without partial exchange transfusion (PET), laser surgery and selective feticide. We found that management varied greatly within and between fetal therapy centers. Perinatal mortality was comparable for the treatment groups. Neonatal morbidity was significantly higher in cases that had an IUT (± PET) or delivery, compared to cases that were treated with expectant management, laser surgery or selective feticide. Pregnancy was significantly more prolonged in cases managed expectantly, treated with laser surgery or with selective feticide. The incidence of postnatal TAPS was significantly lower in the laser surgery group than in expectant management, delivery or IUT (with PET). Differences between the groups should

be interpreted with caution. Treatment groups differed considerably at baseline in terms of antenatal TAPS stage, gestational age at diagnosis and type of TAPS.

In **chapter 6** we present the study protocol of the open-label international multicenter randomized controlled trial 'The TAPS Trial', in which patients pregnant with a monochorionic twin diagnosed with TAPS stage 2 or higher between a gestational age of 20^{+0} - 27^{+6} will be randomized to the laser treatment group of the standard treatment group (expectant management, IUT (with PET), preterm delivery). The primary outcome will be gestational age at birth, secondary outcomes will include perinatal mortality and severe neonatal morbidity, procedure-related complications, hematological complications and long-term neurodevelopmental outcome.

Postnatal diagnosis

In **chapter 7** we quantified the color difference on the maternal side of the placenta and compared it between TAPS placentas and acute-peripartum-TTTS placentas. We used a freely available and easy to use image processing program called ImageJ and determined the color difference ratio (CDR) between the two placental shares. We found that TAPS placentas had a significantly higher CDR (all > 1.5) than placentas from uncomplicated monochorionic twins. Furthermore, we demonstrated a significant correlation between CDR and inter-twin hemoglobin difference. In a second study, presented in **chapter 8**, we investigated whether the CDR could distinguish between TAPS and acute peripartum TTTS, two feto-fetal transfusion disorders that both present with large inter-twin hemoglobin difference and a pale (donor) and plethoric (recipient) baby at birth. The results of our study showed that TAPS placentas were characterized by a large color difference between the placental shares (reflected by a high CDR) whereas acute peripartum-TTTS placentas displayed no color difference on the maternal side (reflected by a low CDR).

Short-term outcome

In Part 6, we present the second and third study we conducted based on the TAPS Registry data. In **chapter 9** we investigated diagnosis, management and outcome in 249 cases of spontaneous TAPS. We found that spontaneous TAPS can develop within a very wide range in pregnancy, from the beginning of the second trimester until the end of the third trimester (15-35 weeks). Spontaneous TAPS was managed heterogeneously, with the majority being

treated with laser surgery. Perinatal mortality was 15% for the total group, with an almost fourfold increased risk for donor twins. Severe neonatal morbidity occurred in 33% of spontaneous TAPS twins, and was comparable for donors and recipients. Aside from donor status, perinatal mortality was strongly dependent on antenatal TAPS stage and gestational age at birth. Risk factors for severe neonatal morbidity were gestational age at birth and antenatal TAPS stage 4. In **chapter 10**, we assessed diagnosis, management and outcome in 164 twins with post-laser TAPS. Our data showed that approximately 75% of post-laser TAPS cases develops within a month after laser for TTTS, but that a quarter of the population shows late onset of the condition, up until 17 weeks after laser. Management for post-laser TAPS was mostly expectant, but varied considerably. Perinatal mortality occurred in 25% of the population, and was strongly predicted by TAPS donor status, antenatal TAPS stage, and gestational age at birth. Severe neonatal morbidity was detected in 40% of liveborn twins with post-laser TAPS and similar for donors and recipients. Gestational age at birth was the only predictor for severe neonatal morbidity in post-laser TAPS.

Long-term outcome

Chapter 11 is the first study evaluating the long-term outcome in spontaneous TAPS survivors. Neurodevelopmental impairment (NDI) was detected in 31%, and was found more often in donors (44%) than in recipients (18%). Severe NDI was identified in 9% of TAPS survivors, and was higher in donors (18%) than in recipients (3%). Moreover, we found an unexpected high rate of bilateral deafness (15%), only in donor twins. Overall, TAPS donors had a fourfold increased risk for NDI and showed significantly higher rates of cognitive delay and hearing problems. In addition, parents reported to have more concerns about the development of their donor twin than of their recipient twin. In multivariate analysis, gestational age at birth and severe anemia appeared to be independent risk factors for NDI. The rate of behavioral problems was 10%, which is comparable to the prevalence of behavioral problems (10%) in children from the general Dutch population. Donors and recipients demonstrated similar rates of behavioral problems.

Conclusion

In conclusion, with this thesis we have further expanded our knowledge on pathophysiology, diagnosis and management and short- and long-term

outcome in TAPS. The optimal management strategy remains to be elucidated and will be investigated in the TAPS Trial.

General discussion and future perspectives

Twin anemia polycythemia sequence (TAPS) is a severe complication in monochorionic twin pregnancies caused by unbalanced feto-fetal transfusion through placental anastomoses leading to anemia in the donor twin and polycythemia in the recipient twin. Robyr at al. were the first to describe the iatrogenic form of TAPS in a cohort of twins treated with laser surgery for twin-twin transfusion syndrome.[1] Shortly thereafter, our research group reported the same condition to occur spontaneously in three cases of monochorionic twins which had no amniotic fluid discordances during pregnancy.[2] To clearly demarcate this new form of unbalanced feto-fetal transfusion from the well-known twin-twin transfusion syndrome (TTTS), we introduced the term 'twin anemia polycythemia sequence', and its acronym TAPS. We were also the first to unravel the pathophysiology based on the typical presence of only minuscule (diameter < 1 mm) anastomoses, detected through color dye injection of the placental vessels. Although this idea was first encountered with disbelieve, in the years that followed more and more evidence emerged reporting on other cases with similar presentations, leading to increased attention and awareness for this new condition. Now, almost fifteen years later, TAPS has become a distinct entity in monochorionic twinning, with its own characteristic pathogenesis, diagnostic criteria, classification systems and outcome. The following paragraphs will discuss the insights we have yielded through our work in the last three years, and will propose perspectives and opportunities for future research.

Pathogenesis

Color dye injection – the foundation of understanding monochorionic twins

As the majority of monochorionic twin problems derives from the intertwined angio-architecture on the shared placenta, thorough placental examination should be the cornerstone for every researcher or clinician investigating this special subgroup of twins. Therefore, routine placental color dye injection is a fundamental part of the academic training for PhD candidates involved in research into monochorionic twins at our clinic, the Leiden University Medical Center. Figure 1 shows the number of injected placentas in the three years

of this PhD (n = 405), that allowed enhanced understanding on the subject, eventually leading to this thesis.

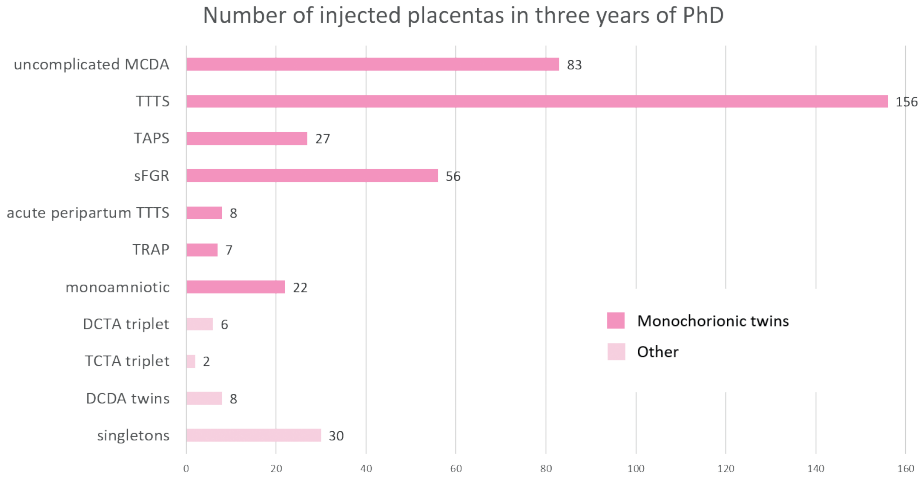

Figure 1. Number of injected monochorionic placentas in three years of PhD divided into type of complications.

Importantly, not only complicated monochorionic twin placentas should be examined; knowledge of the physiologic angioarchitecture of uncomplicated monochorionic twins is crucial to understand the root cause of problems in conditions such as selective fetal growth restriction, TTTS and TAPS. The latter might be the biggest challenge to properly inject with color dye, as the anastomoses are known to be notoriously small and might remain unveiled when the wrong technique is used.[2] To be able to visualize the typical minuscule TAPS anastomoses, the use of a contrasting color dye is crucial.[3] In addition, manual assistance is required to help massage and guide the color dye into the most distant and small vessels. Figure 2 shows an injected TAPS placenta in which the responsible anastomosis was only detected after extensive placental massaging. Notably, injection with milk or air would not have led to visualization of this anastomosis, and therefore, the injection substance is of crucial matter in identifying TAPS.

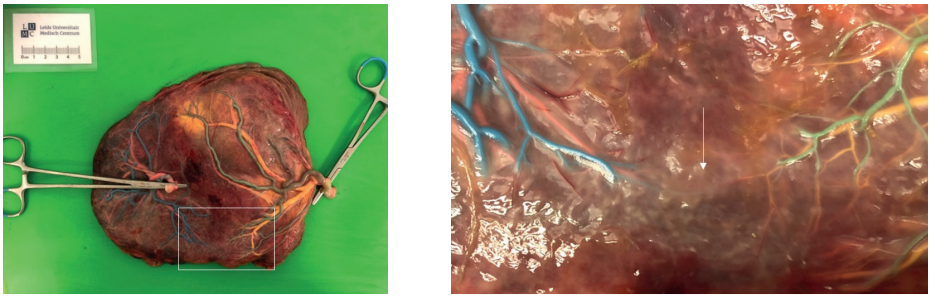

Figure 2. Monochorionic placenta of a post-laser TAPS case, showing the presence of a minuscule arterio-venous (blue-yellow) anastomosis responsible for TAPS

The role of anastomoses in TAPS

Through dedicated placental injection, it is now generally accepted that TAPS occurs as the result of chronic transfusion through one (or only few) small unidirectional arterio-venous (AV) anastomoses from the donor to the recipient, leading to a large inter-twin hemoglobin difference .[4] Arterio-arterial (AA) and veno-venous (VV) anastomoses are bidirectional, and the former is reported to play a protective and compensatory role against unbalanced inter-twin transfusion.[5] Accordingly, small single center studies have described the incidence of these anastomoses to be low in TAPS placentas.[4] In chapter 9 and 10, we have reported on the largest group of injected TAPS placentas in context of a large international study, and found AA anastomoses to be present in 8-18% of the population.[6, 7] Moreover, we observed several TAPS placentas which had only one AA or VV anastomosis and no other AV anastomoses, thereby raising questions about the specific role of bidirectional anastomoses in the development of TAPS. Future research should be aimed at investigating the influence of these anastomoses on the development and outcome of TAPS twins. Possibly, placentas with bidirectional anastomoses have a later time of onset of the disease, a smaller inter-twin hemoglobin difference and a more favorable outcome.

Another insufficiently investigated aspect in TAPS is placental sharing. A small study from our own research group has shown that the TAPS donor, while being the smaller infant, often has a paradoxically larger placental share than the recipient twin (this was also the case in the twins described in the 'Patient journey').4 This finding is only reported in TAPS and not in TTTS or sIUGR, in which growth restriction (in either donor or recipient) is associated with a smaller placental share. Future studies with larger samples should be

conducted to confirm this remarkable placental observation in TAPS, and to evaluate its potential consequences. Possibly, the larger placental share might be favorable feature, especially in the context of laser surgery. When laser surgery is performed, the chances of fetal demise due to a small remaining placental sharing might be lower. Moreover, TAPS donors might even show enhanced catch-up growth after the procedure.

Another interesting observation that we have made during the last three years of placental injection studies, is that a remarkable number of TAPS placentas were bilobar. Importantly, the other way around, 28% (9/29) of the bilobar monochorionic twin placentas in our placenta database had TAPS (to compare: only 5% of our total monochorionic placenta database has TAPS). A potential relationship between bilobarity in monochorionic twin and TAPS is not unlikely. Possibly, bilobar monochorionic placentas have smaller and fewer anastomoses due to the relatively early embryonic division, and might therefore be at increased risk for developing TAPS. Future research should be conducted to evaluate this hypothesis.

TTTS and TAPS: not so exclusive as previously thought?

Although TTTS and TAPS have been described as two mutually distinct conditions, we have shown in chapter 2 that 15% of TTTS twins treated with laser surgery presents with a large MCA-PSV discordance, indicative for anemia-polycythemia (AP), preoperatively.[8] This specific subgroup of 'TTTS+AP' cases was characterized by a later time of onset of TTTS and fewer placental anastomoses, similar to the pathogenesis of TAPS. These findings suggest that TAPS and TTTS might not be two exclusive entities but are part of a spectrum of findings. Unfortunately, we were only able to report on the number of anastomoses, and did not quantify the size. Knowledge on the diameter of anastomoses is crucial to further understand the pathogenesis of TTTS+AP cases, and the entire spectrum of unbalanced feto-fetal transfusion disorders. Potentially, placental anastomoses in TTTS+AP cases are generally smaller than in cases with TTTS-only, allowing more hemodynamic compensation to take place, leading to a delayed onset of the disease. To investigate this hypothesis, placentas from untreated TTTS+AP cases would be needed. However, as the majority of TTTS is now treated with laser surgery, conducting such research is not feasible. Therefore, the only way to gain quantified measurements on placental anastomoses, would be during fetoscopy. Ideally, future fetoscopes

would be equipped with integrated measuring software allowing to calculate the diameter and flow within a vessel.

Surprisingly, we also found that, despite comparable gestational age at birth, cases with TTTS+AP had a significantly more favorable short- and long-term outcome than cases with TTTS only. The cause for this difference is not entirely understood, but perhaps time of onset might play a role. Maybe the developing fetus is more prone to the detrimental effects of TTTS earlier in pregnancy. Future research comparing short- and long-term outcome between early-onset TTTS and late-onset TTTS could be performed to evaluate this hypothesis.

Antenatal diagnosis

Previous studies have shown that TAPS can occur spontaneously in 3-5% of monochorionic twins, or can develop iatrogenically due the presence of small residual anastomoses in 2-16% of TTTS twins treated with laser surgery.[9-12] It is likely that these numbers are an underestimation of the true incidence of TAPS, as many centers in the world do not use the adequate antenatal screening or postnatal diagnostics to identify the condition. At our department, we also facilitate placental injection, free of charge, for other Dutch hospitals that are in doubt about the diagnosis when a pale and plethoric twin pair is delivered. During the last three years a total of 19% (5/27) of the twins diagnosed with TAPS al the LUMC was born in peripheral hospital. This illustrates that, outside of the TAPS twins that are referred to fetal therapy centers, there is a subgroup of TAPS twins that will be left undiagnosed. Moreover, before the official introduction of the acronym TAPS, most monochorionic twins that presented with a pale and plethoric baby at birth were classified as acute peripartum TTTS cases. Since acute peripartum TTTS is even more rare than TAPS[13], we believe that the majority of these cases might actually have been (misdiagnosed) TAPS twins. As we now know that TAPS is associated with poor short- and long-term outcome,[6, 7, 14] it is crucial to improve antenatal detection to enable (intrauterine) intervention to cure or mitigate the condition.

Conflicting recommendations

TAPS can be identified antenatally using middle cerebral artery peak systolic velocity (MCA-PSV), showing an increased MCA-PSV in the donor, suggestive of anemia and a decreased MCA-PSV in the recipient, suggestive of polycythemia.[15] Although several studies have shown MCA-PSV to be an adequate predictor for

TAPS, there is still international controversy on the implementation of MCA-PSV screening in the bi-weekly ultrasound check-ups for monochorionic twins. The Society for Maternal and Fetal Medicine does not recommend MCA-PSV screening at all, due to the lack of evidence that routine screening improves outcome for TAPS twins.[16] The twin guideline from the International Society for Ultrasound in Obstetrics and Gynecology recommends bi-weekly MCA-PSV screening starting from 20 weeks of gestation, but primarily in twins that have been treated with laser surgery for TTTS.[17] The patient association for TAPS, the TAPS Support group, advocates for standardized routine MCA-PSV doppler screening for all patients expecting monochorionic twins.[18]

Time of onset of spontaneous TAPS – much earlier than expected

To develop adequate screening guidelines for TAPS, information on the time of onset of the condition is essential. We used the data collected in the TAPS Registry to investigate this in a large cohort of spontaneous TAPS twins. We found that spontaneous TAPS can develop within a very wide range in pregnancy, from the beginning of the second trimester until the end of the third trimester (15-35 weeks).[6] As 75% of the cases detected at 15 weeks were TAPS Stage 2 or higher, it is likely that condition manifested even earlier. Moreover, we showed that in half of the group TAPS was detected before 24 weeks, contradicting the current belief that spontaneous TAPS generally develops after viable gestation. Based on the mounting evidence of serious effects of TAPS (chapter 9 and 11) we recommend biweekly MCA-PSV Doppler screening starting from 14 weeks, to improve antenatal detection of TAPS.

Anticipate for post-laser TAPS - after every laser for TTTS, and at any time.

Chapter 10 showed that the development of post-laser TAPS is most often unexpected, as 81% of fetal surgeons assumed their laser for TTTS was complete.[7] The majority of cases (75%) was detected within 4 weeks after laser surgery for TTTS. However, a quarter of the population showed late onset of post-laser TAPS (from 5 up until 17 weeks after laser), indicating that ex-TTTS twins are not out of the woods after an uncomplicated first month. We therefore recommend to continue bi-weekly Doppler ultrasound examination in all TTTS cases after laser. The wide range in time of onset of post-laser TAPS is not entirely clear. Possibly, reversal of donor-recipient role and type of placental anastomoses are of influence. An in-depth analysis of the TAPS-Registry data is needed to investigate these hypotheses.

What diagnostic criterion to use?

In this thesis, we have shown that using a *delta* MCA-PSV > 0.5 multiples of the median (MoM) is more predictive of TAPS than the fixed cut-off values of 1.5 MoM for the TAPS donor and 1.0 MoM for the TAPS recipient.[15] In our study, we found a subgroup of TAPS twins that demonstrated a large *delta* MCA-PSV but had normal MCA-PSV values in either one of the twins. Interestingly, it were mainly the TAPS donors who had normal MCA-PSV values. This was also reported in a study that was published shortly after our work.[19] A possible explanation for this finding could be that MCA-PSV system we now use to identify anemia in monochorionic twins is originally based on reference values for anemia caused by erythrocyte alloimmunization in singletons.[20] Not only does this type of anemia arise from a completely different cause, the cardiovascular system of a singleton in not comparable to that of the shared circulation of monochorionic twins. Therefore, anemia might be better predicted when monochorionic twins have their own reference values. Klaritsch et al published such reference values previously.[21] An alternative explanation for the normal MCA-PSV values in TAPS donors might be found in the co-existing severe growth restriction, which we have reported to occur in 25-50% of TAPS donors. Potentially, the MCA-PSV is not solely dependent on the gestational age, but also on the size of the fetus. A growth-restricted anemic fetus might not be able to increase its cardiac output to achieve high MCA-PSV values in the same way as an anemic fetus with adequate growth can. Therefore, the measured MCA-PSV value in some TAPS donors might be regarded as normal for their current gestation, but is increased for the gestational age that would correspond with their fetal weight. Illustratively, the TAPS group with delta MCA-PSV and normal values in either donor or recipient showed a significantly higher birth-weight discordance than the group that met the MCA-PSV cut-off levels. This hypothesis also explains why in the 'Patient journey' the severely growth-restricted TAPS donor Max had a much lower hemoglobin value (3.0 g/dL) than would be expected based on his MCA-PSV value (1.7 MoM).

Importantly, we only evaluated the delta MCA-PSV > 0.5 MoM in a small population of TAPS twins and uncomplicated monochorionic twins. Perhaps, this new diagnostic tool might function less well in a more heterogeneous population, and might lead to more false positive cases. Of note, fetal MCA-PSV measures can fluctuate greatly due to physiological factors, such as sleep and fetal movements. A large *delta* MCA-PSV that is actually caused by TAPS

is therefore more probable if this difference is not a one-time observation, but persists during pregnancy. Looking at other ultrasound markers (including cardiomegaly in the TAPS donor, starry sky liver in the TAPS recipient, and placental dichotomy) could be helpful to support the diagnosis of TAPS, as we have shown that the majority of cases, including the ones with normal values in either donor or recipient, present with at least one of them. In order to evaluate the true potential of delta MCA-PSV > 0.5 MoM and (the combination of) additional ultrasound markers for the antenatal diagnosis of TAPS, a large prospective cohort of monochorionic twins is needed.

Antenatal management

Many options, many opinions

Antenatal management options for TAPS include expectant management, preterm delivery, intrauterine transfusion (IUT) with or without a partial exchange transfusion (PET), laser surgery and selective feticide. The best management option is unknown. Laser surgery is aimed at coagulating the responsible anastomoses at the vascular equator and is the only management option that directly tackles the cause of TAPS during pregnancy. Laser surgery has shown to drastically decrease mortality and morbidity in TTTS[22], however in TAPS, the procedure might be technically challenging due to the absence of TOPS and size of the anastomoses, resulting in reduced accessibility and visibility of the vascular equator. Treatment with IUT (with PET) is generally less invasive but is only a symptomatic procedure and therefore reintervention up to 1-6 times might be required. Selective feticide is aimed at increasing the chances of healthy survival in the co-twin, and can be considered in cases in of early severe TAPS when other options or technically not feasible, or in case of co-existent fetal abnormalities such as severe cerebral injury. With expectant management, no intrauterine intervention is performed, but twins are managed more intensively with ultrasound Doppler. Preterm delivery is only an option after viability is achieved and aims at treating the twins at the neonatal intensive care unit (NICU) instead of in the womb. Currently, there is a lot of debate with regard to the best management option for TAPS. Whereas some centers are strongly convinced about the benefits of laser surgery, other clinics refrain from any intrauterine intervention and manage their cases solely expectantly. The only way to escape from this impasse is by extensive international collaboration, allowing to combine data of this rare condition to

increase our sample size and provide more firm conclusions about diagnosis, management and outcome in TAPS.

Joining our forces

A revolutionary step toward increased understanding of this condition was the set-up our TAPS Registry, in which 17 fetal therapy centers enthusiastically participated and helped generate a sample size of 422 TAPS cases.[23] This number is extensively higher than the cohort of investigated TAPS twins (n = 62) reported in 2019 that was recorded as the largest thus far.[24] Moreover, the TAPS Registry is, aside from the International Fetal Cardiac Intervention Registry[25], a unique example of a successful registry within the field of fetal therapy, that hopefully might serve as a blueprint for other rare and insufficiently understood monochorionic twin conditions, such as selective fetal growth restriction.

What is the best management option?

Using the data of the TAPS registry, we found a vast diversity in management for TAPS, both within and between the 17 participating fetal therapy centers, reflecting the lack of international consensus on the best treatment choice.[23] When comparing expectant management, delivery, IUT (with PET), laser surgery and selective feticide, we found comparable perinatal mortality rates (ranging between 7-19%). The rate of severe neonatal morbidity was high in all groups, but especially in cases treated with IUT (with PET) or immediate delivery. Notably, our data showed that the occurrence of severe neonatal morbidities was strongly dependent on the gestational age at birth (chapter 9 and 10).[6, 7] It therefore is of paramount importance to prolong pregnancy to improve short- and long-term outcome rates for this condition. Comparing all treatments modalities, we discovered that prolongation of pregnancy was best achieved in cases managed expectantly, treated with laser surgery or selective feticide, and these three management strategies could therefore be regarded as equivalent management options if one mainly aims to reduce the risk of prematurity.[23]

Selective feticide - serious sacrifice

It must however be stressed that selective feticide comes with a high price, as parents lose at least one of their babies and do not have a guarantee of healthy survival for the co-twin.[23] Moreover, only little is known about the long-term consequences that selective feticide has on the quality of life of parents and families. Notably, parents are faced with an unprecedented tough and almost inhuman decision to sacrifice one of their own children in order to save its

brother or sister. It is not inconceivable that such an intervention will have impact on the psychological well-being of parents and thereby the quality of life of their children. More research is needed to investigate long-term (psychological) effects of not only selective feticide, but of all management options for TAPS. Only in this way, parents can be adequately counseled with regard to the expected lifelong consequences of their preferred treatment option.

Expectant management - extending exposure

Interestingly, our data showed that prolongation of pregnancy in expectant management was comparable with laser surgery and selective feticide.[23] The benefit of expectant management is that the pregnancy will not be exposed to iatrogenic risks of an intervention, while allowing the disease to resolve spontaneously. However, chances for spontaneous resolution are not high (only 16%)[23], and therefore the vast majority of TAPS twins will be continuously exposed to chronic anemia and polycythemia, allowing the condition to progress and potentially result in single or double fetal demise. Currently, there is no information available on the risk of fetal demise or severe cerebral damage in the co-twin after fetal demise in TAPS. Possibly, the effects of acute perimortem exsanguination through the placental anastomoses will be limited, due to the fact that TAPS placentas are characterized by the presence of one or only a few small unidirectional anastomoses.[4] As the demised fetus will most of the times be the TAPS donor[6, 7], the risk for the recipient might even be lower as the anastomoses are likely to be small AVs (from donor to recipient). Moreover, the polycythemic TAPS recipient might be protected by its erythrocyte surplus, mitigating the effects of acute anemia that occurs after perimortem exsanguination. An in-depth analysis of the cases with single fetal demise collected in the TAPS Registry could help exploring this hypothesis.

Laser surgery – definitive dichorionization (?)

Laser surgery in TAPS was associated with a large diagnosis-to-birth interval and a high rate of TAPS resolution after the procedure.[23] As we know now that the severity of TAPS is a strong predictor for perinatal mortality and severe neonatal morbidity (chapter 9 and 10)[6, 7], treatment with laser surgery (thereby blocking ongoing transfusion and preventing further deterioration), might be the most optimal intervention to improve perinatal outcome for this condition. It should however be noted that laser surgery is not always successful, since

recurrence of TAPS is seen in 15% of cases.[23] Moreover, we demonstrated that, if anastomoses are missed during the procedure, there is a 100% chance that TAPS will recur. Additionally, laser surgery can lead to complications such as preterm premature rupture of the membranes (PPROM), intrauterine infection, iatrogenic monoamnionicity and pseudo amniotic band syndrome. We report the prevalence of PPROM to be 37%, which is comparable to the prevalence of PPROM after laser for TTTS.[26] Other procedure-related complications are insufficiently investigated and will be evaluated in future research (in the TAPS trial).[27]

Apples and oranges

Although it's tempting to draw conclusions based on the results of the TAPS registry, we should be very cautious in doing so. Importantly, management groups differed considerably in gestational age at diagnosis, antenatal TAPS stage and type of TAPS.[23] As a higher TAPS stage and post-laser TAPS are associated with higher mortality and morbidity rates, these factors might have played an important role in perinatal outcome. Illustratively, despite a comparable diagnosis-to-birth interval, expectant management was performed in milder TAPS cases than laser surgery or selective feticide. Therefore, expectant management might be associate with even higher mortality and morbidity rates if it would be performed in equally severe TAPS cases. Furthermore, the high neonatal morbidity rate in the IUT (with PET) group might partly be attributed to the fact that 64% of the group were post-laser TAPS cases, which have higher rates of perinatal mortality and morbidity than spontaneous TAPS cases.[23] Although delivery (within a week after diagnosis) was performed in milder TAPS cases and at a later gestational age, we found high rates of perinatal morbidity in this group. Possibly, other factors besides the severity of TAPS might have played a role in decision-making, such as decreased fetal movements or fetal distress. An alternative explanation for the high morbidity rate could be that part of the TAPS twins were not prepared with steroids and magnesium sulfate, making them more prone for prematurity-related problems. The case described in the 'Patient journey' is an illustrative example that these factors can play a role when a preterm delivery is decided.

Reliability through randomization

The only way to adequately compare treatment groups is to perform randomization and stratify for potential risk factors. In chapter 6, we presented

the protocol of the TAPS Trial, an international open-label randomized controlled trial that we are currently conducting to evaluate the potential beneficial role of laser surgery on the outcome in TAPS twins.[27] In this study, women pregnant with monochorionic twins diagnosed with TAPS stage 2 or higher between 20^{+0} and 27^{+6} will be randomized to laser treatment or standard treatment (expectant management, IUT (with PET), preterm delivery). We will stratify for gestational age at diagnosis (20^{+0} – 23^{+6} vs. 24^{+0} – 27^{+6}) and type of TAPS (post-laser vs. spontaneous). A total of 5 other centers plan to participate, 3 are currently recruiting and 5 patients have been included so far.

Postnatal diagnosis

The power of the placenta

TAPS is not the only feto-fetal transfusion problem that is characterized by a large inter-twin hemoglobin difference at birth. Acute peripartum TTTS, the rarer form of the well-known chronic TTTS that is believed to develop during labor, presents with a pale and plethoric twin pair as well.[13] As neonatal management for these two conditions calls for a different approach, quick distinction at birth is vital. In chapter 7 and 8 we have shown that despite their similar presentation at birth, the maternal side of the TAPS placenta shows a remarkable color difference, whereas acute peripartum TTTS placentas have a uniformly colored surface.[28, 29] Importantly, visual examination of the placenta can be performed on-site, shortly after delivery of the placenta, and before reticulocyte counts are available or the placenta is injected. We therefore strongly encourage clinicians in the obstetric and neonatal field to examine the maternal side of the placenta when a pale and plethoric twin pair is born and obstetrical data is lacking or inconclusive. Due to the rarity of the conditions, our findings are based on small numbers. Future research should be focused at evaluating the diagnostic benefit of color difference in a larger population of twins with TAPS and acute peripartum TTTS. Since our publication in 2017 we have recorded five more acute peripartum TTTS cases and none showed a color difference on the maternal side of the placenta.

Short- and long-term outcome

Short term outcome – fatal fetal anemia

Through extensive international collaboration on the TAPS Registry, we were able to report on the outcome of large group of TAPS twins .[6, 7] We found that

outcome in both spontaneous and post-laser TAPS was poor. In spontaneous TAPS, mortality occurred in 1 in 10 fetuses; in post-laser TAPS in 1 in 4. Donors had a three- to four-fold increased risk for mortality, highlighting the profound impact of anemia on fetal survival. Severe neonatal morbidity was seen in approximately 30% of spontaneous TAPS twins and in 40% of post-laser TAPS twins, and was strongly predicted by gestational age at birth. Despite the increased risk for demise in the donor antenatally, donors and recipients had comparable rates of severe neonatal morbidity. This could be a reflection of the big impact of prematurity (which is comparable for donors and recipients), but might also be due to the fact that the most severely ill donors already demised in utero. If all donors would have survived, the neonatal morbidity rate might have been higher in donors. Notably, post-laser TAPS twins showed a far worse outcome than spontaneous TAPS twins, which could be explained by preceding TTTS, a different placental angioarchitecture (less compensating blood flow in post-laser TAPS), and the type of management (post-laser TAPS was frequently managed expectantly or with IUT (with PET), which might have allowed the condition to progress).[6, 7] Although it is clear that adverse outcome rates in TAPS twins are high, we did not compare the perinatal mortality and severe neonatal morbidity rates to those of uncomplicated monochorionic twins. Future research should address this subject in order to quantify the added risk of TAPS on an uncomplicated monochorionic twin pregnancy.

Long term outcome - deafness and developmental delay in donors

We conducted the first study into long-term neurodevelopmental and behavioral outcome in spontaneous TAPS twins. Overall neurodevelopmental impairment (mild and severe) was detected in 30% of our cohort of TAPS twins.[14] Moreover, we found that TAPS donors do not only have increased risk antenatally (chapter 9 and 10)[6, 7], but also show poorer outcome later in life. TAPS donors had significantly higher rates of overall neurodevelopmental impairment than recipients (44% vs 18%) and demonstrated a high prevalence of bilateral deafness (15% vs. 0%). Although the small numbers prevented statistical significance in the latter, we found the high rate of deafness clinically striking and warranting more attention. Notably, this high rate of deafness is not reported in TTTS survivors nor in children that suffered from anemia based on erythrocyte alloimmunization.[30] Moreover, the prevalence of hearing problems in TAPS donors is substantially higher than in NICU infants (1-3%). In the TAPS donors, hearing loss was in all cases based on auditory neuropathy

spectrum disorder (ANSD), a form of sensorineural hearing loss, in which the cochlea is unaffected but the inner hair cells, connecting synapses and/or auditory nerve is damaged.[31] The cause of ANSD is not entirely clear but perinatal hypoxia might play an important role. We hypothesize that the chronic anemic state of the donor might have led to a hypoxic environment, gradually damaging the developing brain and auditory nerve system. To investigate this hypothesis, future research should be aimed at comparing umbilical cord pH- and lactate values between donors and recipients. According to our theory, TAPS donors might present with lower pH values and higher lactate values on day 1 after birth. Additionally, a thorough evaluation of cerebral magnetic resonance images of the five donors with hearing problems might shine more light on the exact cause of ANSD in these children.

The value of long-term follow-up

This long-term outcome study reinforces crucial importance of long-term follow-up. Without this study, we would not have been aware of the long-term consequences in TAPS twins, thereby withholding this high-risk population the adequate follow-up care. With these new insights, we now have implemented specialized hearing screening for every twin diagnosed with TAPS born at our hospital (Figure 3). When hearing screening is performed in TAPS neonates, it is important to use the correct test. The standard neonatal hearing screening that uses otoacoustic emission (OAE) is not sufficient in detecting sensorineural hearing loss and therefore a specialized test, the 'automated auditory brainstem response' (AABR), should be used in these infants.[32] Notably, early detection of hearing loss by newborn hearing screening (and subsequent early intervention) is of utmost importance as it has shown to drastically improve speech- and language development in children with impaired hearing.[33, 34]

This study has also led to the implementation of routine long-term follow-up at 2-, 5- and 8-years into the standard care for all TAPS twins managed, born or treated at our center. We subsequently advise all fetal medicine centers around the world caring for twins diagnosed with TAPS to follow our example and do routine pediatric long-term follow-up in this high-risk population, not only to provide parents and children with the aftercare that they deserve, but even so important: to be able to evaluate the outcome and do quality control of their performed intervention.

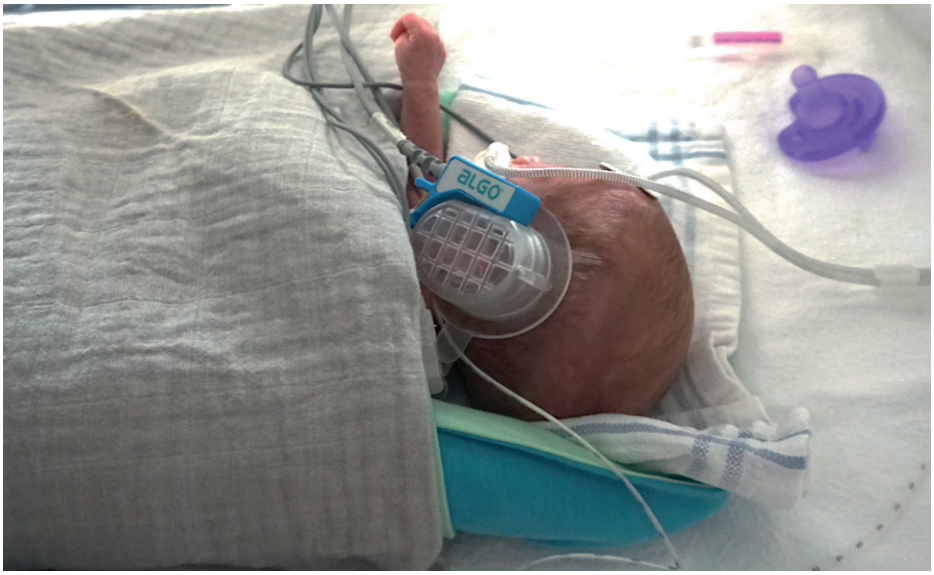

Figure 3. TAPS donor born at our hospital in 2019 receiving the neonatal hearing test using automated auditory brainstem response.

Last but not least

A topic that has been underexposed in this thesis, but is equally as relevant, is the role of a proper social support group for TAPS. As TAPS is a very rare condition in the general population, the chances of meeting a fellow TAPS family are not likely. Notably, being diagnosed with TAPS -on top of having an already risky monochorionic twin pregnancy – places parents in a very uncertain and stressful situation. Knowing that there are families out there that gone through the same, might help in coping. A few years ago, the TAPS Support group was founded (www.tapssupport.com), an online place where parents can meet each other and share their stories. Notably, almost 200 families have already joined this group, even leading to (inter)national TAPS family meet-ups (Figure 4).

Figure 4. TAPS twins Emilie and Mathilde (5 years old; picture of them as babies can be seen in chapter 1), holding another twin that was diagnosed with TAPS. Both donors are seen on the left-hand side of the picture, and both recipients are seen on the right-hand side of the picture.

Final conclusion

With this thesis we have further unraveled diagnosis, management and outcome in TAPS. We have optimized antenatal and postnatal diagnostic criteria for TAPS, unveiled time of onset for the condition and presented new insights into short- and long-term outcome rates of this high-risk population. To further improve our care for TAPS, the future holds some major challenges. One of the biggest challenges will be the implementation and uniformization of routine MCA-PSV measurements into the biweekly ultrasound exams for monochorionic twins to timely reach the diagnosis. Moreover, every center caring for TAPS pregnancies should perform a complete postnatal diagnostic work-up including hemoglobin, reticulocytes and placental injection, to be able to adequately diagnose TAPS and distinguish the condition from other monochorionic twin problems such as acute peripartum TTTS or hemoglobin differences based on placenta-fetal transfusion, and check whether laser therapy was successful. Moreover, all centers managing TAPS twins should register short- and long-term outcomes

in order to evaluate the effects of their treatment choice. As we have shown that the consequences of TAPS are not limited to the neonatal phase but also manifest later in life, routine long-term follow-up in this population is of paramount importance. Lastly, investigation of the best treatment option for TAPS pregnancies is vital to prevent severe adverse outcome. Results of the TAPS Trial, an international randomized controlled trial that compares outcome of laser treatment with standard treatment, are eagerly awaited.

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Patient journey, in light of our new insights

In the following paragraphs we will discuss the questions that have arisen based on the story of Daan an Max and we will evaluate whether we are now able to answer them.

Antenatal diagnosis

Are the currently cut-off levels of < 1.0 MoM and > 1.5 MoM accurate enough to diagnose TAPS during pregnancy, our should we use an inter-twin MCA-PSV difference?

Chapter 3 has shown that the cut-off levels of < 1.0 MoM and > 1.5 MoM are indeed not accurate enough to diagnose TAPS. We have investigated delta (difference) MCA-PSV > 0.5 MoM for the diagnosis of TAPS and found that this criterion was associated with a higher sensitivity and specificity than the cut-off values. We have subsequently updated the antenatal classification system for TAPS.

What is known about the prevalence of cardiomegaly and a difference in placental echogenicity in TAPS? Are these findings unique for this case, or are they more ubiquitous in the TAPS population?

In chapter 4, we have investigated the prevalence of additional ultrasound markers and found that placental dichotomy (based on a difference in echogenicity), cardiomegaly in the donor and a starry-sky liver in the recipient (not seen in Daan) were detected in 41%, 65% and 61% of TAPS cases, respectively. Therefore, Daan and Max do not represent a unique case.

Antenatal therapy

If TAPS would have been detected early on, what management strategy would have been best for Daan and Max?

The best treatment option for TAPS is unclear. In chapter 9 and 10 we have presented the results of a large international TAPS registry and found that expectant management, laser surgery and selective feticide were all associated with a long (8-10 weeks) diagnosis-to-birth interval. However, we were not able to draw firm conclusions with regard to the best management option, as our treatment groups were not comparable at baseline, and differed greatly in severity of TAPS, time of onset of TAPS, and type of TAPS. We therefore need to await the results of the international randomized controlled trial to be able

to determine what would potentially have been the best treatment option for Daan and Max, if we would have detected TAPS early on.

Growth restriction

What is the prevalence of severe growth restriction in TAPS? Is Max an exceptional case, or is severe growth restriction more frequent in TAPS?

We have shown in chapter 9 and chapter 11 that severe growth restriction (birthweight < third centile) is common in spontaneous TAPS and is seen in almost half (49%) of donor twins. Max is therefore not an exceptional case. In contrast to isolated selective growth restriction in monochorionic twins, growth restriction in TAPS is not a result of unequal placental sharing. On the contrary, TAPS donors often show a larger placental share. This was also seen in Max, who's placental share was 60% of the total placental mass. The exact cause of growth restriction in TAPS is unclear, but is likely to be attributed to the chronic erythrocyte and albumin loss.

Postnatal diagnosis

In line with the sonographic observation antenatally, the maternal side of the TAPS placenta showed a striking color difference. Is this feature related to the hemoglobin difference in TAPS twins? Could looking at the maternal side of the placenta help in achieving the diagnosis of TAPS shortly after birth (even before reticulocytes are available and placental injection is performed) ?

In chapter 7 and 8 we have shown that a color difference on the maternal side of the placenta is a unique diagnostic feature for TAPS twins, and is not seen in twins that demonstrate a large hemoglobin difference (>8 g/dL) based on acute peripartum twin-twin transfusion syndrome. Moreover, we found that the color difference ratio was positively correlated with the inter-twin hemoglobin difference, meaning that the bigger the inter-twin hemoglobin difference the more profound color difference between the two placental shares. We therefore advise caregivers in the perinatal field to inspect the maternal side of the placenta when a pale and plethoric twin pair is born in order to achieve a quick diagnosis of TAPS.

Long-term outcome

What is the long-term neurodevelopmental and behavioral outcome in spontaneous TAPS survivors? Are there differences in long-term outcome between TAPS donors and recipients? Does Max represent a unique case of deafness in TAPS, or are hearing problems more prevalent in this population?

In chapter 11 we have shown that long-term neurodevelopmental impairment (mild and severe) occurs in 31% of spontaneous TAPS twins. We found remarkable differences between donors and recipients, with donors having a significantly higher risk for neurodevelopmental impairment, based on a high prevalence of cognitive delay, and bilateral deafness. To our surprise, we discovered that Max is not the only TAPS survivor with bilateral deafness. Remarkably, there were four other donor twins with bilateral hearing loss, all based auditory neuropathy spectrum disorder (same as in Max). We do not know what the exact pathophysiological mechanism is behind the hearing loss in anemic donors. Possibly, chronic anemia results in a relatively hypoxic state of the fetus, gradually damaging both the developing brain (reflected by high rates of cognitive delay) and auditory nerve (reflected by high rates of hearing problems). More research is needed to investigate this hypothesis.

The major questions that arises now is: would the care for Daan and Max have been different, with the knowledge that we have now?

Firstly, it should be stated that this will always remain a tough question when it is retrospectively asked. If we look at their MCA-PSV curve, Max and Daan showed a delta MCA-PSV > 0.5 MoM at the same day that Max was already showing signs of distress, leading to an emergency cesarean. Before that moment, Daan and Max demonstrated a delta MCA-PSV of exactly 0.5 MoM. Therefore, they would not have officially classified as a TAPS case even when the new criteria would have been applied. We would however have been more aware now that this persisting MCA-PSV difference of 0.5 MoM could also point towards TAPS, especially because we are now aware of the fact that TAPS is often accompanied by severe growth restriction in the TAPS donor. Moreover, the additional finding of placental dichotomy would have further supported the diagnosis of TAPS. It remains a complicated question whether we now would have intervened with fetal therapy, if we would indeed have strongly suspected TAPS before 30 weeks. Notably, the hemoglobin value of Max was extremely low at birth (3.0 g/dL) and he demonstrated a strikingly high (363) reticulocyte value, indicating that he was anemic for a considerably long time. Treatment

with laser surgery blocking the ongoing chronic transfusion from Max to Daan could have prevented Max his condition to deteriorate, ideally leading to a prolonged pregnancy and eventually a potentially better outcome for both boys. Moreover, laser treatment could potentially have improved growth in Max. As growth restriction in TAPS is not problem related to a small placental share but is potentially related to chronic anemia and hypoalbuminemia, Max might have showed catch-up growth after a successful laser. Although this all sounds promising, we have no scientific proof that laser indeed improves outcome compared to other treatment strategies, and we therefore need to await the results of the TAPS Trial before we can draw firm conclusions on this subject.

One thing that would have been different now, is the perinatal counseling for TAPS. If Max and Daan would have been born in 2019, we would have discussed risks of hearing problems with the parents and have instructed them to be alert towards signs of hearing loss and speech delay in their children. Moreover, we would strongly stress the importance of neonatal hearing screening also for the hospitals to which the care was transferred. This would have hopefully led to a timelier detection of Max his hearing loss, leading to earlier intervention with hearing aids, allowing his speech and language skills to develop earlier than the age of four.

Nederlandse samenvatting

In Nederland is ongeveer 2% van alle zwangerschappen per jaar een tweelingzwangerschap. Twee derde van deze tweelingen is een twee-eiige tweeling, en een derde is een eeneiige tweeling. Twee-eiige tweelingen hebben altijd elk hun eigen placenta (chorion) en vruchtzak (amnion) en worden dichoriaal diamniotisch genoemd. Bij eeneiige tweelingen hangt het aantal placenta's en vruchtzakken sterk af van het moment van het splitsen van de bevruchte eicel (zie ook Figure 1, General Introduction). Wanneer deze splitsing in de eerste drie dagen na bevruchting plaatsvindt zullen beide foetussen, net als bij twee-eiige tweelingen, ook hun eigen placenta en vruchtzak hebben (dichoriaal diamniotisch). Wanneer de deling plaatsvindt na 3 dagen, zal de tweeling één placenta delen, maar wel nog elk hun eigen vruchtzak hebben. Dit wordt een monochoriaal diamniotische zwangerschap genoemd. Als de bevruchte eicel na 8 dagen splitst, is er sprake van een monochoriale monoamniotische tweeling en delen de foetussen zowel de placenta als de vruchtzak. Alle monochoriale tweelingen hebben, ongeachte het aantal vruchtzakken, placentaire anastomosen (vaatverbindingen) tussen de twee foetale circulaties. Door deze anastomosen stroomt er bloed van de ene naar de andere foetus, en vice versa. De anastomosen kunnen unidirectioneel (arterio-veneus of veno-arterieel) of bidirectioneel zijn (arterio-arterieel of veno-veneus). Bij de meerderheid van de monochoriale tweelingen is de transfusie tussen de foetussen in balans. Echter, bij 15% gaat er te veel bloed van de ene foetus naar de andere foetus en ontstaat er een onevenwichtige verdeling in bloed. Afhankelijk van de grootte van de anastomosen kan deze onevenwichtige bloedstroom zich uiten in twee verschillende soorten aandoeningen: tweelingtransfusiesyndroom (TTS) of tweeling anemie polycytemie sequentie (TAPS) (Figure 2, General Introduction).

Tweelingtransfusiesyndroom

Bij TTS stroomt er door grote anastomosen te veel bloed van de ene foetus (donor) naar de andere foetus (recipiënt). Bij de donor ontstaat een tekort aan bloed waardoor deze foetus eerst minder en later helemaal niet meer gaat plassen. Dit leidt tot een oligohydramnion: er is weinig of helemaal geen vruchtwater meer in de vruchtzak van de donor, waardoor deze foetus krap in zijn vruchtzak komt te liggen. De recipiënt krijgt juist te veel bloed, waardoor deze veel gaat plassen en er een teveel aan vruchtwater ontstaat.

Hierdoor groeit de buik van de zwangere heel hard, en kunnen de vliezen breken of kunnen er weeën ontstaan. De beste behandeling voor TTS is een laserbehandeling, een kijkoperatie waarbij de anastomosen met een laserstraal dicht gebrand worden.

Tweeling anemie polycytemie sequentie

TAPS is een relatief nieuw ziektebeeld en werd in 2006 pas voor het eerst beschreven. Bij TAPS stroomt er door minuscule anastomosen (diameter < 1 mm) langzaam te veel bloed van de ene foetus (donor) naar de andere foetus (recipiënt). Hierdoor wordt de donor chronisch anemisch (dun bloed) en de recipiënt chronisch polycytemisch (dik bloed). Bij TAPS is er geen sprake van vruchtwaterverschille zoals deze bij TTS gezien worden. Bij de geboorte is de TAPS-donor bleek en de TAPS-recipiënt juist heel rood. TAPS kan tijdens de zwangerschap worden vastgesteld door met echo Doppler de bloedstroomsnelheid in de arteria cerebri media (MCA-PSV) te meten. Het dunne bloed van de anemische donor stroomt snel en het dikke bloed van de recipiënt stroomt juist heel langzaam. Wanneer de bloedstroomsnelheid van de donor groter is dan 1,5 'multiples of the median' en bloedstroomsnelheid bij de recipiënt < 1,0 MoM is er sprake van TAPS. Zowel anemie als polycytemie is niet goed voor de ontwikkeling van de foetus en kan leiden tot handicaps of sterfte. Het is nog niet bekend wat de beste behandeling voor TAPS is.

In de studies verzameld in dit proefschrift worden verschillende aspecten van TAPS onderzocht. Een overzicht van de literatuur staat beschreven in hoofdstuk 1. In hoofdstuk 2 onderzoeken we een bijzondere groep tweelingen die gediagnosticeerd zijn met een mengbeeld van TTS en TAPS en gaan we in op de eigenschappen van de placenta en de korte- en langetermijnuitkomsten van deze kinderen. In hoofdstuk 3 testen we de diagnostische accuraatheid van een nieuw criterium voor de diagnose van TAPS tijdens de zwangerschap, namelijk delta (verschil) MCA-PSV > 0.5 MoM. In hoofdstuk 4 gaan we in op additionele echobevindingen bij tweelingen met TAPS, zoals een verschil in echogeniciteit van de placenta, cardiomegalie bij de donor, en een starry-sky liver bij de recipiënt. In hoofdstuk 5 en 6 worden twee studies gepresenteerd waarin met betrekking tot de antenatale behandeling voor TAPS: de TAPS Registry en de TAPS Trial. In hoofdstuk 7 berekenden we het kleurverschil op de maternale zijde van de TAPS-placenta. In hoofdstuk 8 onderzochten we of een kleurverschil op de maternale zijde van de placenta ook bij tweelingen met acute peripartum

TTS te vinden is. Deze aandoening presenteert zich ook met een bleke en rode baby, maar vereist een ander neonataal beleid. Hoofdstuk 9 en 10 beschrijven studies naar de diagnose en uitkomsten bij tweelingen met spontane TAPS en post-laser TAPS. Hoofdstuk 11 richt zich op de langetermijnuitkomsten bij tweelingen met spontane TAPS.

Review

Hoofdstuk 1 geeft een overzicht van de literatuur en somt bevindingen en inzichten op van ruwweg 100 studies, die tien jaar na onze eerste publicatie over TAPS gepubliceerd zijn. Daarnaast bevat deze review een flowchart voor behandeling van TAPS, afhankelijk van de zwangerschapsduur waarbij de ziekte vastgesteld wordt en de ernst van de ziekte.

Pathogenese

In **hoofdstuk 2** onderzochten we de prevalentie van anemie-polycytemie (AP; gedefinieerd als MCA-PSV > 0.5 MoM) bij TTS-tweelingen voordat zij een laserbehandeling kregen voor TTS. Onze resultaten laten zien dat er bij 15% van de tweelingen met TTS gelijktijdig sprake is van AP. Tweelingen met TTS+AP werden bij een latere zwangerschapsduur behandeld met laser, wat erop wijst dat het ziektebeeld bij deze groep waarschijnlijk later in de zwangerschap ontwikkelt. Daarnaast hadden tweelingen met TTS+AP minder placentaire anastomosen dan tweelingen die geïsoleerde TTS hadden. Ondanks gelijke zwangerschapsduur bij geboorte hadden tweelingen met TTS+AP een significant betere uitkomst dan tweelingen met geïsoleerde TTS. Het percentage ernstige neonatale morbiditeit (samengestelde uitkomstmaat van respiratoir distress syndroom (RDS), necrotiserende enterocolitis (NEC), patente ductus arteriosus (PDA) en ernstige hersenschade) was significant lager bij tweelingen met TTS+AP. Ook op de lange termijn functioneerde tweelingen met TTS+AP beter dan tweelingen met geïsoleerde TTS: ziektevrije overleving (overleving zonder handicaps) was significant hoger in de groep met TTS+AP.

Antenatale diagnose

In **hoofdstuk 3** bepaalden we hoe goed een MCA-PSV-verschil > 0.5 MoM (delta MCA-PSV > 0.5 MoM) TAPS kon voorspellen ten opzichte van de MCA-PSV-afkapwaarden van >1.5 MoM voor de donor en <1.0 MoM voor de recipiënt. Onze resultaten lieten zien dat een MCA-PSV-verschil > 0.5 MoM gekenmerkt

werd door hogere percentages sensitiviteit en specificiteit dan MCA-PSV-afkapwaarden van 1.5 MoM en 1.0 MoM. Bovendien toonden we aan dat er een significante positieve correlatie was tussen een MCA-PSV-verschil en het hemoglobineverschil tussen de kinderen na geboorte: hoe groter het MCA-PSV-verschil, hoe groter het verschil in hemoglobine. In **hoofdstuk 4** onderzochten we de prevalentie van drie verschillende additionele echobevindingen bij TAPS: (1) een verschil in echogeniciteit van de placenta (hyperdens placentadeel voor de donor en een hypodens placentadeel voor de recipient) (2) cardiomegalie bij de donor (3) starry-sky liver bij de recipiënt. Bij tweelingen met TAPS werd bij 44% een verschil in echogeniciteit van de placenta gezien. Van de donoren had 70% cardiomegalie en bij de recipiënten liet 66% het beeld van een starry-sky liver zien. In totaal had 86% van de TAPS-tweelingen tenminste één van deze drie echokenmerken en 14% dus helemaal geen extra echokenmerken. De prevalentie van alle drie de echomarkers steeg naarmate de ernst van TAPS toenam.

Antenatale behandeling

In **hoofdstuk 5** onderzochten we hoe TAPS behandeld wordt in 17 verschillende foetale therapiecentra en wat de perinatale uitkomst is na expectatief beleid, vroegtijdig bevallen, intra-uteriene transfusie (IUT) bij de donor met of zonder een partiële wisseltransfusie (PWT) bij de recipiënt, foetoscopische laserbehandeling en selectieve reductie. We ontdekten dat er een zeer grote variëteit is in behandeling voor TAPS, niet allen binnen een centrum maar ook tussen centra onderling. De perinatale mortaliteit was vergelijkbaar voor de vijf verschillende behandelgroepen. Het percentage ernstige neonatale morbiditeit was significant hoger in de groep TAPS-tweelingen die behandeld was met IUT (± PWT) of een vroegtijdige bevalling kreeg, dan bij TAPS-tweelingen die behandeld werden met laser, selectieve reductie of expectatief beleid. De zwangerschapsduur werd significant meer verlengd wanneer er gekozen was voor expectatief beleid, laserbehandeling of selectieve reductie. Verschillen tussen de groepen moeten met grote terughoudendheid worden geïnterpreteerd, omdat behandelgroepen geen vergelijkbare uitgangssituatie hadden en aanzienlijk verschilden met betrekking tot de ernst van TAPS, de zwangerschapsduur waarbij TAPS gediagnosticeerd was en het type TAPS (spontaan of post-laser).

In **hoofdstuk 6** presenteren we het protocol van de TAPS Trial, een internationale multicenter open-label gerandomiseerd gecontroleerde studie, waarbij patiënten die in verwachting zijn van een monochoriale tweeling gediagnosticeerd met TAPS ≥ stadium 2 bij een zwangerschapsduur tussen 20^{+0} weken en 27^{+6} weken geloot worden tussen laserbehandeling en standaardbehandeling. Bij de standaardbehandeling vindt geen laserbehandeling plaatst, maar kan gekozen worden tussen afwachtend beleid, IUT (± PWT) en/of vroegtijdige bevalling. De primaire uitkomstmaat is zwangerschapsduur bij geboorte. Secundaire uitkomstmaten zijn perinatale mortaliteit, ernstige neonatale morbiditeit, procedure-gerelateerde complicaties, hematologische complicaties en neurologische ontwikkeling op lange termijn.

Postnatale diagnose

De postnatale diagnose van TAPS is gebaseerd op drie criteria. De eerste is een hemoglobineverschil > 8.0 g/dL (of > 5 mmol/L). Echter presenteert acute peripartum TTTS zich ook met een groot verschil in hemoglobine en een bleke en een rode baby. Bij acute peripartum TTS gaat er in heel korte tijd, waarschijnlijk tijdens de bevalling, acuut te veel bloed van de donor naar de recipiënt, waardoor de donor acuut anemisch en hypovolemisch wordt en de ontvanger acuut polycytemisch en hypervolemisch. Gezien het neonatale beleid voor TAPS en acute peripartum TTS anders is, is het cruciaal om bij geboorte tussen de twee aandoeningen een onderscheid te maken. Daarvoor zijn twee criteria opgesteld. De diagnose TAPS kan gesteld worden wanneer er sprake is van tenminste een van de volgende aspecten: een reticulocytenratio > 1.7 en de aanwezigheid van louter minuscule (diameter < 1 mm) anastomosen op de placenta. Bij de TAPS-donor is het reticulocytenpromillage hoog, als uiting van de reactie van de foetus op chronische anemie. Bij de TAPS-recipiënt is het aantal reticuloycten juist laag. Wanneer het reticulocytenpromillage van de donor gedeeld wordt door dat van de recipiënt, zal hier een hoge reticulocytenratio uitkomen. Bij acute peripartum TTS is de reticuloyctenratio laag (< 1.7), als uiting van het onvermogen van de TTS-donor om zich in zo'n korte tijd aan te passen aan de anemie. Daar waar TAPS gekenmerkt wordt door de aanwezigheid van louter minuscule vaatverbindingen, is er bij acute peripartum TTTS juist sprake van grote vaatverbindingen (waarvan tenminste één bidirectionele vaatverbinding). Helaas worden reticuloycten vaak niet bepaald en is het opspuiten van de placenta een uitdagend en tijdrovend

onderzoek, dat vaak alleen in gespecialiseerde centra uitgevoerd wordt. Daarom zijn we op zoek gegaan naar een simpelere diagnostische tool, die gemakkelijk te gebruiken is in ieder ziekenhuis. In een aantal case reports is beschreven dat TAPS-placenta's, in lijn met het verschil in huidskleur van de kinderen bij geboorte, een groot kleurverschil op de maternale zijde van de placenta tonen (bleke placentahelft voor bleke donor, donkerrode placentahelft voor polycytemische recipiënt). In hoofdstuk 7 en 8 hebben we de dit aanwezigheid van het kleurverschil zowel bij TAPS-tweelingen als bij acute TTS-tweelingen onderzocht.

In **hoofdstuk 7** berekenen we bij een groep TAPS-placenta's en placenta's van ongecompliceerde monochoriale tweelingen het kleurintensiteitsverschil tussen de twee placentahelften. We toonden aan dat TAPS-placenta's vrijwel altijd een kleurverschil laten zien op de maternale zijde en een significant hogere kleurintensiteitsratio hebben dan placenta's van ongecompliceerde monochoriale tweelingen, die een egale kleur hebben. Ook lieten we zien dat er een positieve correlatie bestaat tussen het verschil in kleurintensiteit en het verschil in hemoglobine tussen de kinderen: hoe groter het kleurverschil op de placenta, hoe groter het hemoglobineverschil. In **hoofdstuk 8** onderzoeken we het kleurverschil op de maternale zijde van placenta's van tweelingen met acute peripartum TTTS en vergelijken we dit met het kleurverschil op TAPS-placenta's en een controlegroep van placenta's van ongecompliceerde monochoriale tweelingen. Het onderzoek laat zien dat, hoewel TAPS en acute TTS zich beide presenteren met een bleke en een rode baby bij geboorte, alleen TAPS-placenta's een kleurverschil op de maternale zijde laten zien. Placenta's van tweelingen met acute peripartum TTS zijn egaal van kleur. Wanneer er een bleek-rode tweelingen geboren en wordt en er getwijfeld wordt tussen TAPS of acute peripartum TTS, kan een vlugge blik op de maternale zijde van de placenta dus snel een richting geven aan de diagnose.

Uitkomsten op korte termijn

In deel 6 van het proefschrift worden twee andere studies gepresenteerd die net als hoofdstuk 5 gebaseerd zijn op de data verzameld in de TAPS Registry. In **hoofdstuk 9** onderzoeken we in een grote groep spontane TAPS-tweelingen (N = 249) wanneer de ziekte zich openbaart, wat het beleid was bij deze tweelingen, en wat de neonatale uitkomsten zijn. De resultaten laten zien dat spontane TAPS zich gedurende een heel lange periode in de zwangerschap

kan ontwikkelen, vanaf 15 weken tot en met 35 weken zwangerschapsduur. Er is veel variatie in het beleid voor spontane TAPS, echter wordt de meerderheid behandeld met lasertherapie. Perinatale mortaliteit treedt op in 15% van de tweelingen, waarbij TAPS-donoren een vier keer zo hoog risico op overlijden hebben als TAPS-recipiënten. Behalve donor-status zijn andere onafhankelijke risicofactoren voor perinatale mortaliteit de ernst van TAPS tijdens de zwangerschap en een lage zwangerschapsduur bij geboorte. Er is sprake van ernstige neonatale morbiditeit bij 33% van de tweelingen met spontane TAPS, waarbij er geen verschil is tussen donoren en recipiënten. Het voorkomen van ernstige neonatale morbiditeit was onafhankelijk geassocieerd met een ernstige vorm van TAPS (stadium 4) en een lage zwangerschapsduur bij geboorte. In **hoofdstuk 10** onderzoeken we een grote groep (N= 164) tweelingen die post-laser TAPS hebben ontwikkeld, en beschrijven we wanneer de ziekte ontstaat, hoe post-laser TAPS behandeld wordt en wat de uitkomsten zijn in deze groep. Post-laser TAPS werd in het merendeel van de groep gedetecteerd binnen 4 weken na de laserbehandeling voor TTS, echter kan de ziekte zich nog tot 17 weken na de initiële laserbehandeling openbaren. Behandeling voor post-laser TAPS is divers, maar bestaat voornamelijk uit afwachtend beleid. Perinatale mortaliteit treedt op bij 25% van tweelingen met post-laser TAPS, en was net als bij spontane TAPS sterk afhankelijk van donor-status, de ernst van TAPS tijdens de zwangerschap en een lage zwangerschapsduur bij geboorte. Bij 40% van de post-laser TAPS-tweelingen worden ernstige neonatale morbiditeiten gezien, waarbij geen verschil wordt gevonden tussen donor en recipiënt. Een lage zwangerschapsduur bij geboorte was een sterke risicofactor voor het optreden van ernstige neonatale morbiditeiten.

Uitkomsten op lange termijn

De eerste studie naar de langetermijnontwikkeling van tweelingen met spontane TAPS wordt beschreven in **hoofdstuk 11**. Voor deze langetermijnstudie hebben we ouders van kinderen die tussen 2005 en 2017 in het LUMC gediagnosticeerd waren met spontane TAPS, benaderd voor deelname aan follow-up onderzoek. Van de 81 kinderen die geschikt waren, deden 74 (91%) kinderen mee aan het onderzoek. Bij de deelnemende kinderen werd er een ontwikkelingstest afgenomen. Een ernstig ontwikkelingsprobleem werd vastgesteld bij 9% van de kinderen, en vaker bij TAPS-donoren (18%) dan bij TAPS-ontvangers (3%). In vergelijking met TAPS-recipiënten hebben TAPS-donoren een lager IQ en een

grotere kans op een milde vertraging in de verstandelijke ontwikkeling. Ook was er bij 15% van de TAPS-donoren sprake van doofheid. Bij de TAPS-recipiënten was geen enkel kind doof. In totaal had 10% van de kinderen gedragsproblemen, waarbij er geen verschillen werden gevonden tussen donoren en recipiënten. Dit percentage is vergelijkbaar met percentage gedragsproblemen bij kinderen in de algehele Nederlandse populatie. In de gedragsvragenlijsten gaven ouders aan meer zorgen te hebben over de ontwikkeling van de TAPS-donor, dan over de ontwikkeling van de TAPS-recipiënt. De studie laat zien dat TAPS gekenmerkt wordt door een hoog percentage aan ernstige ontwikkelingsproblemen, met name bij de donor. Hoewel TAPS over het algemeen gezien wordt als een relatief milde vorm van onevenwichtige transfusie bij monochoriale tweelingen, toont deze studie aan dat de consequenties op lange termijn niet onderschat moeten worden en dat routinematig vervolgonderzoek geïndiceerd is.

Conclusie

Dit proefschrift levert een substantiële bijdrage aan de kennis over de pathofysiologie, diagnose, therapie en korte- en langetermijnuitkomsten bij TAPS. De beste behandeling voor TAPS blijft tot op heden onduidelijk, maar wordt momenteel grondig onderzocht in de TAPS Trial.

Next steps towards improved care for TAPS

A step-by-step manual for management for TAPS, based on the insights gained through our studies and experience. A visual summary can be found at the end of this thesis.

Step 1 – Antenatal diagnosis

- Perform bi-weekly middle cerebral artery peak systolic velocity (MCA-PSV) measurements using a delta of > 0.5 MoM to detect TAPS starting at 14 weeks of gestation.
- To detect TAPS after laser for TTTS, MCA-PSV measurements should be performed bi-weekly in every case of TTTS.
- Pay attention to signs of cardiomegaly in the TAPS donor, a starry-sky liver in the TAPS recipient and placental dichotomy (based on a hyperechogenic part of the donor and a hypoechogenic part of the recipient) to support the diagnosis of TAPS
- As MCA-PSV dopplers can fluctuate during pregnancy, a repeated MCA-PSV assessment (within a week) might be needed in twins with solely mild inter-twin MCA-PSV differences (and no signs of cardiac decompensation, other additional ultrasound markers) to confirm or rule out the diagnosis.

Step 2 – Antenatal management

- Expectant management
  - Can be considered in mild or stable TAPS cases
  - Progression: depending on the judgement of the caretaker regarding the condition of the fetuses, ultrasound evaluation can be performed more frequent and admission to the hospital for fetal monitoring with cardiotocography can take place.
  - Regression or resolution: Repeated weekly MCA-PSV measurements are needed to confirm spontaneous resolution in TAPS. Notably, MCA-PSV values can fluctuate greatly during pregnancy; an episode of spontaneous regression of TAPS followed by sudden progression of the disease is

not uncommon. Therefore, remain aware of recurrence of TAPS after spontaneous regression. In case of persisting normalized MCA-PSV values and subsequent referral to the referring center, postnatal evaluation of hemoglobin, reticulocytes and placental injection (step 4) should still be performed to evaluate the presence postnatal TAPS.

- Immediate delivery
  - Is not preferable before 32 weeks when other intrauterine treatment options are still possible and preterm birth is associated with high risks for prematurity-related problems.
  - If the condition of the fetuses allows for it, prepare the twins with steroids and magnesium sulfate
- IUT (with PET)
  - The site of transfusion is depending on position of the fetus and the placenta
  - Intravascular IUT can be combined with intraperitoneal IUT for delayed uptake of erythrocytes and prolonged effect of the transfusion.
  - In case of (severe) polycythemia in the recipient, an IUT can be combined with a PET.
  - Median days between interventions with IUT (with PET) is approximately 2 weeks, but might be shorter/longer depending on the severity of the disease.
  - Record the occurrence of procedure-related complications such as iatrogenic monoamnionicity, PPROM, intrauterine infection, placental abruption, pseudo amniotic band syndrome, bleeding from the puncture site, fetal distress leading to an emergency cesarean section, or fetal death.
- Laser surgery
  - Is the only causal treatment option
  - Consider before 30 weeks in severe (> stage 2) or progressive TAPS

  - Record the occurrence of procedure-related complications such as iatrogenic monoamnionicity, PPROM, intrauterine infection, placental abruption, pseudo amniotic band syndrome, fetal distress leading to an emergency cesarean section, or fetal death.
  - Continue at least biweekly MCA-PSV Doppler measurements
- Selective feticide
  - Can be considered in early, severe cases of TAPS, when other treatment options are not feasible, in case of co-existing congenital abnormalities (including severe cerebral injury) or on request of the parents
  - Record the occurrence of procedure-related complications such as iatrogenic monoamnionicity, PPROM, intrauterine infection, placental abruption, pseudo amniotic band syndrome, fetal distress leading to an emergency cesarean section, or fetal death.

Step 3 – Delivery and birth

- Location of delivery: In case of ongoing TAPS, delivery should be planned in a hospital experienced in performing neonatal blood transfusions and partial exchange transfusions
- Shortly after birth: if possible, take a picture of both babies together to record the striking difference in skin color. This picture might be a valuable memory for parents and can serve as a visual tool to help educate the medical team on TAPS.

STEP 4, 5 and 6 need to be carried out for all infants that have been diagnosed with TAPS (antenatally and/or postnatally), and after every management option.

Step 4 – Postnatal diagnosis

- Perform a full blood count including the following: hemoglobin and reticulocyte values
  - Determine the reticulocyte ratio by dividing the highest reticulocyte value (‰) by the lowest reticulocyte value (‰). If your lab only provides absolute

reticulocyte values, reticulocyte (‰) can be calculated by dividing the absolute reticulocyte value by the erythrocyte value (absolute).

- Perform placental examination:
  - NB. In case of fetal demise (spontaneous or intended), consider placental injection if delivery occurs within 2 weeks after fetal demise.
  - Inspect the maternal side of the placenta to identify a potential color difference between the placental shares. Take a picture of the maternal side. Try to avoid light reflection. Store the picture in a dedicated database.
  - Inspect the fetal side of the placenta.
    - Assess the type of umbilical cord insertion
    - Pay attention to the appearance of the placental vessels. In the TAPS recipients the vessels are usually dark and congested. In the anemic TAPS donors, the vessels are thin and might appear empty.
  - Inject the placenta with color dye
    - An elaborate tutorial can be found at:
      - https://www.jove.com/video/3208/accurate-simple-evaluation-vascular-anastomoses-monochorionic
    - NB. Extensive placental massing might be needed in TAPS cases to help guide the color dye into the most distant minuscule vessels.
    - Assess the number, size and type of anastomoses
    - When finished, take a picture of the injected placenta and store it in a dedicated database.
  - Digital pictures are can be used to measure the following:
    - Placental sharing. The surface area can be easily measured using the freely available image-processing program ImageJ. A tutorial can be found at: https://www.youtube.com/watch?v=Qsxvnby7aCM
    - The color difference ratio can be quantified using freely available image processing program ImageJ. A step-by-step tutorial to calculate

the difference can be found at: https://www.youtube.com/watch?v=_OSd6utv2Bw

Step 5 – Neonatal care

- Be alert to the following potential complications related to TAPS
  - Donor: anemia requiring (multiple) erythrocyte transfusions, hypoalbuminemia, hypoproteinemia, thrombocytopenia, leukopenia, short-term renal dysfunction, severe cerebral injury
  - Recipient: polycythemia-hyperviscosity syndrome requiring a partial exchange transfusion, thrombocytopenia, necrotic skin injury, severe cerebral injury
- Perform a cerebral ultrasound in both babies
- Record severe neonatal morbidities including respiratory distress syndrome, patent ductus arteriosus, necrotizing enterocolitis, retinopathy of prematurity, early-onset neonatal sepsis and severe cerebral injury.
- Perform a neonatal hearing test, preferably using automated auditory brainstem response in both infants to detect (sensorineural) hearing loss timely.
- Instruct parents to be aware of signs of hearing issues in their children, such as a speech and language delay

Step 6 – Long-term follow-up

- Perform long-term follow-up at 2, 5 and 8 years, including the following appointments
  - Pediatrician (for general physical check-up)
    - With special attention to hearing abilities and speech and language development, mainly in the donor
  - Physical therapy to thoroughly assess motor development
  - Child psychologist, to assess cognitive development, using

- Bayley Scales for Infant and Toddler development (2 years)
- Wechsler Preschool and Primary Scale of Intelligence (5 years)
- Wechsler Intelligence Scale for Children (8 years)
- Child Behavior Checklist age 1.5-5 (for 2 and 5 years) and Child Behavior Checklist 6-16 (for 8 years).

PART 11

appendices

Publications

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Curriculum Vitae

Lisanne Tollenaar werd in 1993 thuis geboren in Leiden. Als oudste van drie kinderen bracht zij haar jeugd door in Den Haag. Na haar basisschooltijd op de Willem de Zwijgerschool, behaalde ze haar eindexamen aan het Gymnasium Haganum in 2011. Na uitgeloot te zijn voor geneeskunde, begon ze aan de studie pedagogische wetenschappen aan de Universiteit Leiden en verhuisde weer terug naar haar geboortestad. In datzelfde jaar deed zij onder begeleiding van Wilma Mesker onderzoek naar de tumor-stroma ratio in tumor-positieve lymfeklieren bij borstkankerpatiënten. In 2012 lootte zij voor een tweede keer uit voor geneeskunde. Ze besloot haar studie pedagogische wetenschappen te continueren, maar tevens terug te keren naar de middelbare school om opnieuw eindexamen te doen. In 2013 slaagde zij alsnog cum laude aan het Luzac College te Den Haag, waarna zij startte met de studie geneeskunde aan de Universiteit Leiden. In het tweede jaar van geneeskunde werd zij toegelaten tot het MD/PhD-traject van het Honours College. In het kader daarvan deed zij haar eerste onderzoek naar tweeling anemie polycytemie sequentie onder begeleiding van prof. Oepkes, prof. Lopriore, en dr. Slaghekke. In 2016 ontving ze de MD/PhD-beurs van het Leids Universitair Medisch Centrum (LUMC) en begon ze, na het afronden van de bachelor, officieel aan haar promotietraject op de afdeling Verloskunde en afdeling Kindergeneeskunde van het LUMC. Tijdens haar onderzoekstijd verdiepte Lisanne zich in grafisch ontwerpen en illustreerde met de verkregen kennis en vaardigheden haar wetenschappelijke presentaties en dit proefschrift. Oorspronkelijk stond de verdediging van haar proefschrift gepland op 23 april 2020, maar deze moest verplaatst worden naar 10 september 2020 in verband met de uitbraak van de COVID-19-pandemie. Vanaf juli 2020 werkte Lisanne bij Sanquin om daar het immunohematologie-onderwijs te herstructureren en illustreren. Na de verdediging van haar proefschrift pakt ze haar studie geneeskunde weer op en begint ze met de coschappen.

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Vanaf september 2016 mocht ik fulltime onderzoek doen aan de afdeling Verloskunde en de afdeling Neonatologie. Velen hebben bijgedragen aan de mooie tijd die ik gehad heb en een aantal van hen wil ik in het bijzonder bedanken.

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Stafleden, arts-assistenten en verpleegkundigen van de afdeling verloskunde, neonatologie en kindergeneeskunde. Dank voor de fijne leeromgeving en welkome sfeer. Ik heb mij enorm thuis gevoeld!

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List of Abbreviations

AA anastomosis	Arterio-arterial anastomosis
ACM Vmax	Arteria cerebri media Vmax (maximale bloedstroomsnelheid)
ANSD	Auditory neuropathy spectrum disorder
AV anastomosis	Arterio-venous anastomosis
BSID-III	Bayley Scales of Infant and Toddler Development – third edition
CDR	Color difference ratio
CTG	Cardiotocogram
DVP	Deepest vertical pocket
GA	Gestational Age
GEE	Generalized estimating equations
Hb	Hemoglobin
IUT	Intrauterine transfusion
IUFD	Intrauterine fetal demise
MC	Monochorionic
MCA-PSV	Middle cerebral artery – peak systolic velocity
MoM	Multiples of the median
PET	Partial exchange transfusion
PPROM	Preterm premature rupture of membranes
PWT	Partiële wisseltransfusie
RA	Residual anastomosis
RCT	Randomized controlled trial
sFGR	Selective fetal growth restriction
SGA	Small for gestational age
sIUGR	Selective intra uterine growth restriction
TAPS	Twin Anemia Polycythemia Sequence
TOP	Termination of pregnancy
TOPS	Twin oligohydramnios-polyhydramnios sequence
TTTS	Twin-Twin Transfusion Syndrome
TTS	Tweeling-Transfusie syndroom
VA anastomosis	Veno-arterial anastomosis
VV anastomosis	Veno-venous anastomosis
WISC-III	Wechsler Intelligence Schale for Children – third edition
WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence – third edition