Next steps towards improved care for twin anemia polycythemia sequence

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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.

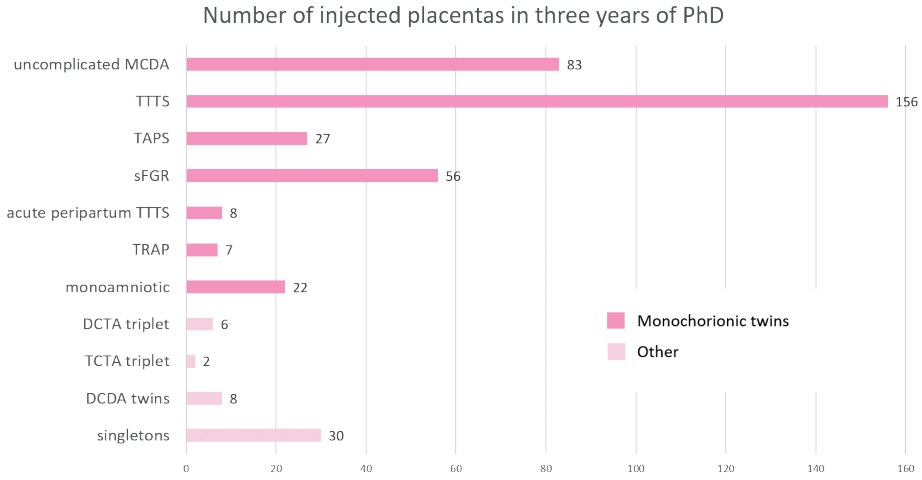

Figure 1. Number of injected monochorionic placentas in three years of PhD divided into type of complications.

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.

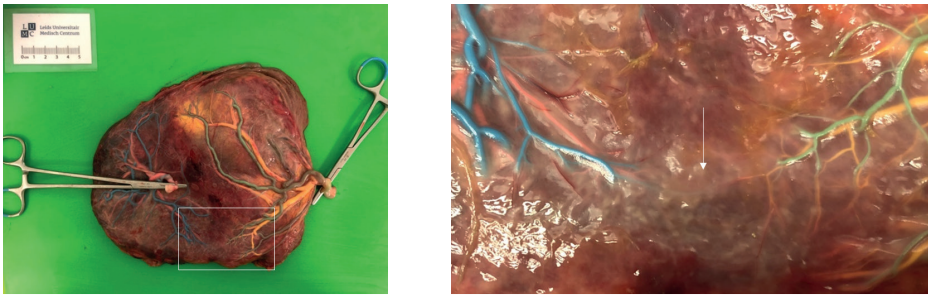

Figure 2. Monochorionic placenta of a post-laser TAPS case, showing the presence of a minuscule arterio-venous (blue-yellow) anastomosis responsible for TAPS

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.

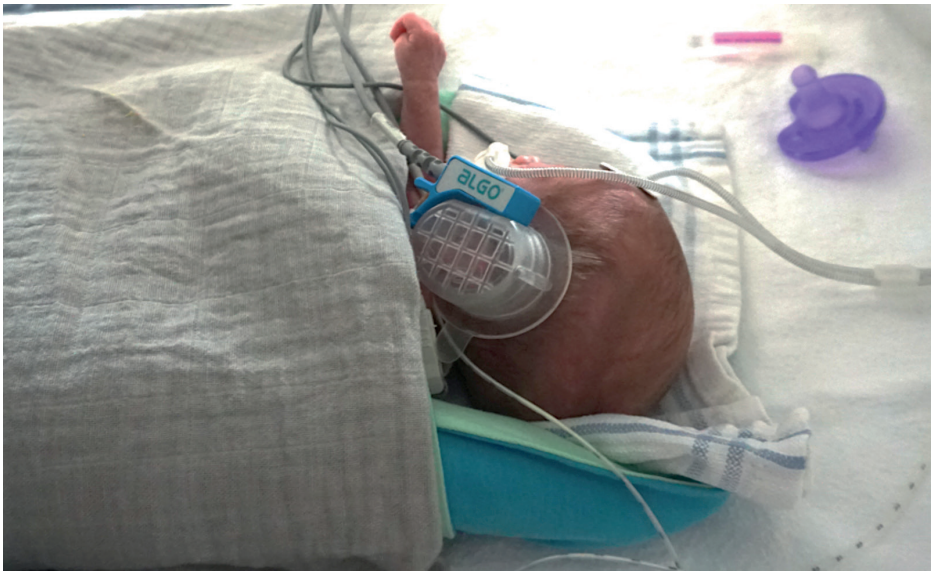

Figure 3. TAPS donor born at our hospital in 2019 receiving the neonatal hearing test using automated auditory brainstem response.

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).