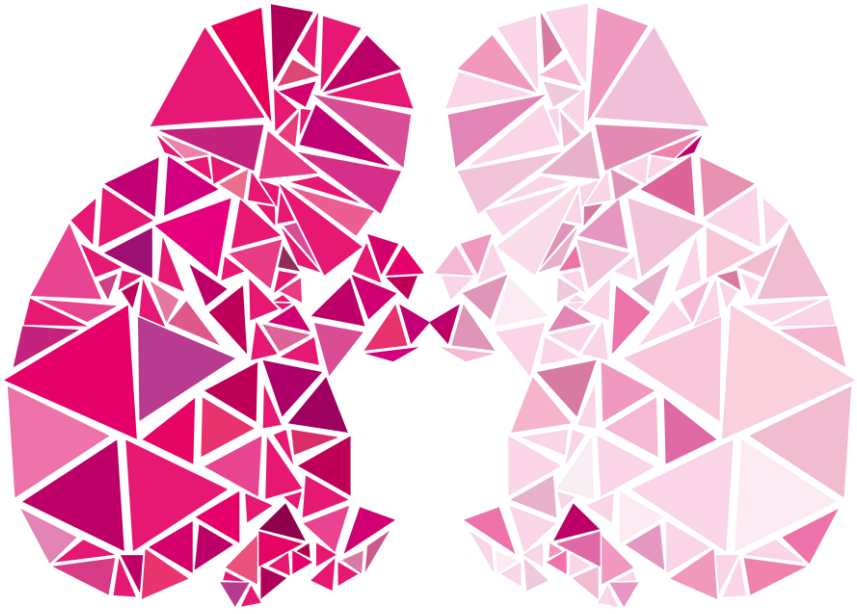
The TAPS Trial

Fetoscopic laser surgery for
Twin Anemia Polycythemia Sequence



a multicenter open-label randomized controlled trial

RESEARCH PROTOCOL

Fetoscopic laser surgery for Twin Anemia Polycythemia Sequence: a multicenter open-label randomized controlled trial

Protocol ID	NL64427.058.18
Short title	The TAPS Trial
EudraCT number	*Not applicable*
Version	3
Date	November, 2018
Coordinating investigator/project leader	Prof. Dr. D. Oepkes Leiden University Medical Center Building 1, Room K-06-35 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 28 96
Principal investigators	
Principal investigator	Prof. Dr. D. Oepkes Leiden University Medical Center Building 1, Room K-06-35 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 28 96
Coordinating investigators	L.S.A. Tollenaar Manager of The TAPS Trial Leiden University Medical Center Building 1, Room J-06-182 Albinusdreef 2, 2333 ZA Lieden Tel: +31(0)71 5262748 Dr. F. Slaghekke Leiden University Medical Center Building 1, Room K-06 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 2896 Prof. Dr. E. Lopriore Leiden University Medical Center Building 1, Room J-06-193 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 2909

Sponsor	*Leiden University Medical Center* Dick Oepkes Leiden University Medical Center Building 1, Room K-06-35 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 28 96
Subsidising party	*Not applicable*
Independent expert (s)	T. H. Van den Akker Leiden University Medical Center Building 1, Room K-06 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0) 71 5262896
Laboratory sites	*Not applicable*
Pharmacy	*Not applicable*

PROTOCOL SIGNATURE SHEET

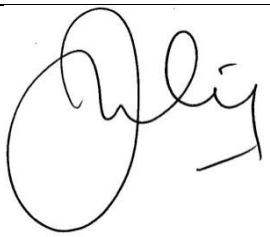
Name	Signature	Date
Sponsor or legal representative: Leiden University Medical Center **Head of Department:** *Prof. Dr. J.J.M. van Lith* Leiden University Medical Center Albinusdreef 2, 2333 ZA Leiden Tel: +317115262896		**13-11-2018**
Principal Investigator: Dick Oepkes Leiden University Medical Center Building 1, Room K-06-35 Albinusdreef 2, 2333 ZA Leiden Tel: +31 (0)71 526 28 96		**13-11-2018**

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
ASQ	**Ages and Stages Questionnaire**
BSID	**Bayley Scales of Infant and Toddler Development**
CA	**Competent Authority**
CBCL	**Child Behavior Checklist**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
eCRF	**Electronic Case Report Form**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
IUFD	**Intrauterine Fetal Death**
IUT	**Intrauterine Blood Transfusion**
MCA-PSV	**Middle Cerebral Artery – Peak Systolic Velocity**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MoM	**Multiples of the Mean**
PET	**Partial Exchange Transfusion**
PPROM	**Preterm Premature Rupture of the Membranes**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or**

	performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TAPS	**Twin Anemia-Polycythemia Sequence**
TOPS	**Twin Oligohydramnion-Polyhydramnion Sequence**
TTTS	**Twin-to-Twin Transfusion Syndrome**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Monochorionic twins share one placenta and are connected to each other via vascular anastomoses at the placental surface, allowing the blood to transfer bi-directionally between the two fetuses. Unbalanced inter-twin blood transfusion can result in twin anemia-polycythemia sequence (TAPS). Management options include: fetoscopic laser surgery, intrauterine blood transfusion (IUT) with or without partial exchange transfusion (PET), preterm delivery, selective feticide and expectant management. The optimal treatment for TAPS is not clear. Fetoscopic laser surgery is the only causative treatment option, but data on the feasibility of this procedure are mainly based on case reports and small cohort studies. A large randomized controlled trial is needed to evaluate the possible beneficial effect of fetoscopic laser surgery and to determine the optimal treatment option for TAPS.

Objective: The aim of this trial is to investigate whether fetoscopic laser surgery improves the outcome for TAPS twins as compared to the control group (standard care consisting of expectant management, IUT (with PET), preterm delivery). The hypothesis is that fetoscopic laser therapy will improve neonatal outcome by prolonging pregnancy.

Study design: International multi-centered open-label randomized controlled trial to assess whether fetoscopic laser surgery (experimental group) improves the outcome of TAPS twins compared to standard care (control group).

Study population: Monochorionic twin pregnancies with TAPS stage ≥ 2 (spontaneous or post-laser) diagnosed between 20 and 28 weeks of gestation.

Intervention: In the experimental group fetoscopic laser surgery is performed, whereas the control group is treated with standard care (expectant management, IUT (with PET), and/or preterm delivery, depending on the opinion of the fetal surgeon).

Main study endpoints: The primary outcome is gestational age at birth. Secondary outcomes include: perinatal mortality or severe neonatal morbidity, hematological complications, procedure related complications and long-term neurodevelopmental outcome at 2 years.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Fetoscopic laser surgery is performed for several decades now and is considered the golden standard for another feto-fetal transfusion syndrome, namely twin-twin

transfusion syndrome. Although fetoscopic laser surgery is associated with a higher risk on several complications (including single or double intrauterine fetal demise, iatrogenic monoamniocity, amnion dehiscention, intra-uterine infection and preterm premature rupture of the membranes), the natural course of TAPS on itself is characterized by high rates of morbidity and mortality as well. The additional risk of fetoscopic laser treatment on top of the risks that are already associated with the natural course of TAPS is therefore estimated as low. The benefit of participating is that TAPS twins allocated to the fetoscopic laser surgery group might be born at a higher gestational age and therefore have a better neonatal outcome.

1. INTRODUCTION AND RATIONALE

Introduction

Monochorionic twins share a single placenta and are connected to each other through inter-twin vascular anastomoses, which allow the blood to transfer bi-directionally 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 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 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. TAPS may occur spontaneously in monochorionic twin pregnancies or may develop in TTTS cases after incomplete laser surgery of the placental equator resulting in a few small residual anastomoses. The incidence of spontaneous TAPS and post-laser TAPS is 3-5% and 2-16%, respectively [1-5].

The pathogenesis of TAPS is based on the unique angioarchitecture of the placenta, characterized by the presence of only few minuscule anastomoses (diameter < 1 mm). These few small (residual) anastomoses between the two placental shares allow a chronic and slow transfusion of blood from the donor to the recipient twin. This process gradually leads to highly discordant Hb levels, causing the donor twin to become anemic and the recipient twin to become polycythemic (Figure 1.).

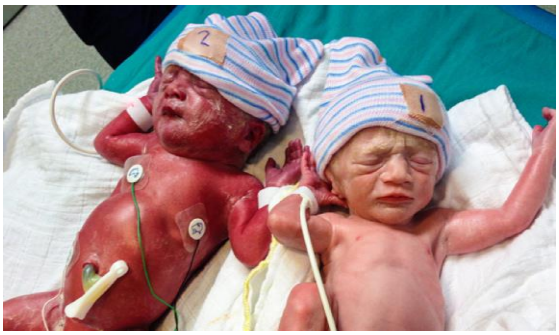


Figure 1 Spontaneous TAPS twins at birth. On the left the plethoric polycythemic recipient and on the right the pale anemic donor

TAPS can be diagnosed either antenatally or postnatally. Antenatal diagnosis is based on Doppler ultrasound measurements of the middle cerebral artery - the peak systolic velocity (MCA-PSV), a non-invasive test, which has become the standard test for the prediction of fetal anemia in singletons in a variety of fetal diseases. In TAPS, this test shows 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, with an inter-twin differences of MCA-PSV > 0.5 MoM. Postnatal diagnosis is based on a large inter-twin Hb 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.

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)[6, 7]

Antenatal Stage	Findings at Doppler ultrasound examination
Stage 1	Inter-twin MCA-PSV difference > 0.5 MoM, without other signs of fetal compromise
Stage 2	Inter-twin MCA-PSV difference > 0.7 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
Stage 4	Hydrops of donor
Stage 5	Intrauterine demise of one or both fetuses preceded by TAPS

Table 1. Antenatal 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

Table 2. Postnatal TAPS classification

The neonatal outcome in TAPS may vary from isolated large inter-twin Hb differences to severe neonatal morbidity including cerebral injury, and even neonatal death [8, 9]. Short term hematological complications are commonly seen in TAPS donors and recipients and may require IUT and PET, respectively. TAPS recipients are at risk of developing necrosis of the skin and multiple limb ischemia and thrombocytopenia [10-13], whereas TAPS donors often have short-term renal dysfunction and hypoalbuminemia[14] [15]. Both donors and recipients are at risk of cerebral injury and adverse long-term neurodevelopmental impairment[8] [16] [17].

When TAPS is detected during pregnancy, antenatal management options include expectant management, fetoscopic laser coagulation of the vascular equator of the placenta, (repeated) 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. Expectant management consists of close monitoring with MCA-PSV Doppler measurements, and can be considered in late onset (GA at diagnosis > 28w) or mild (stage 1) TAPS. In early onset (GA at diagnosis < 28w), and more severe TAPS twins ≥ stage 2 spontaneous recovery is considered unlikely and TAPS twins might benefit from fetal therapy.

However, the best antenatal treatment option for TAPS is not clear. Fetoscopic laser coagulation of the (residual) anastomoses at the vascular equator of the placenta is the only causative treatment. Although laser surgery is proven to be effective in decreasing neonatal mortality and morbidity in TTTS, 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 oligo-polyhydramnios sequence 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 re-intervention 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 more feasible than laser therapy.

There are only a few studies on the outcome of laser therapy for TAPS pregnancies [18-22]. In a 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 [23]. 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 [24].

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.

	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)
IUFD	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, IUFD = intrauterine fetal demise, NDI = neurodevelopmental impairment

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.

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

OBJECTIVES

The primary objective 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[25, 26].

In addition, we will study perinatal mortality, neonatal morbidity, hematological complications, procedure related complications and the long-term neurodevelopmental outcome in this population.

2. STUDY DESIGN

Study Design

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 the control group. We will randomly assign monochorionic twin pregnancies diagnosed with TAPS ≥ stage 2 between 20-28 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 (20-24 weeks vs. 25-28 weeks) and TAPS type (spontaneous vs. post-laser TAPS). Analysis will be by intention to treat. The outcome adjudication will be performed blinded to group allocation.

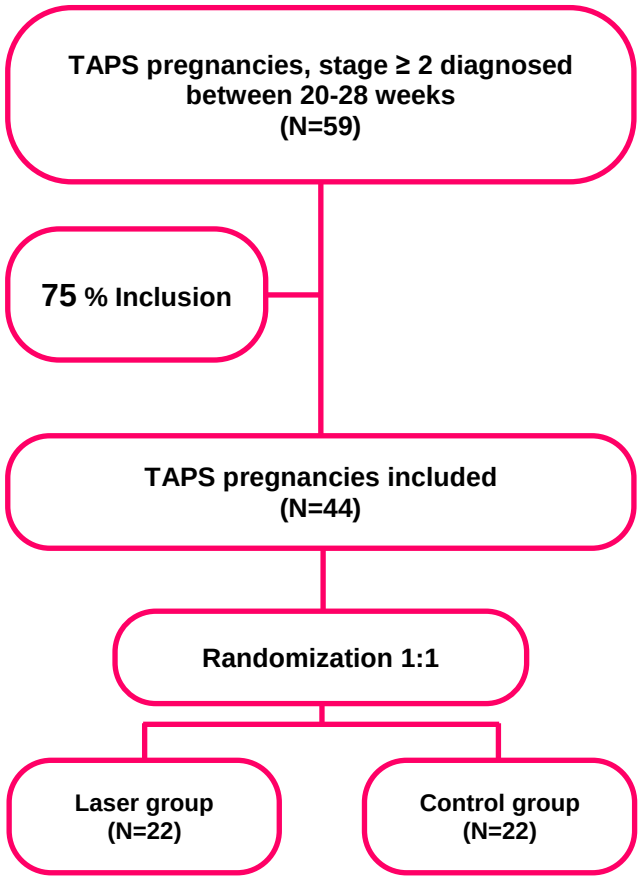


Figure 2. Flowchart of the study design of the TAPS TRIAL

Duration

The duration of the study will be approximately 2.5-3 years from time of randomization of the first TAPS pregnancy to assessing the primary outcome at 28 days after birth of the 44th TAPS twin. The duration of the participation for each TAPS twin will be approximately 2.5 years from time of randomization (20-28 weeks of gestation) to completion of the neurodevelopmental outcome assessment at a corrected age of 2 years.

3. STUDY POPULATION

3.1 Population (base)

Our study population will consist of patients diagnosed with a monochorionic twin pregnancy complicated with TAPS. The incidence of monochorionic twins is 0.3% of all pregnancies. TAPS may occur spontaneously in 3-5% of the monochorionic twin pregnancies and after incomplete laser surgery for TTTS in 2-16% of the TTTS cases. We expect to include 75% of the patients diagnosed with TAPS that are examined at our hospital.

3.2 Inclusion criteria

In order to be eligible to participate in this study, the patient must meet all of the following criteria:

- Monochorionic twin pregnancy complicated by either spontaneous or post-laser TAPS, stage ≥ 2, diagnosed between 20+0 and till 28+0 weeks of gestation
- Women aged 18 years or more, who are able to consent.
- Written informed consent to participate in this randomized controlled trial, forms being approved by the Ethical Committees.

3.3 Exclusion criteria

The following exclusion criteria will be applied:

- TAPS stage 1
- TAPS stage≥ 2, diagnosed within 1 week after laser surgery for TTTS*
- Triplet pregnancies, or higher order multiple pregnancies
- TAPS cases that already underwent an intrauterine treatment (with the exception of laser surgery for TTTS in post-laser TAPS cases)
- Congenital abnormalities (including severe cerebral injury) in one or both twins

** In some cases of TTTS treated with fetoscopic laser surgery, large differences in MCA-PSV values between the two fetuses are seen shortly after the intervention. Although these findings seem to be suggestive for the diagnosis of post-laser TAPS, the cause of this difference does not lie in the presence of small residual anastomoses, but in the feto-fetal transfusion during fetoscopic laser surgery. In time, these large differences in MCA-PSV values resolve spontaneously and therefore MCA-PSV differences within one week after fetoscopic laser surgery will be excluded.*

3.4 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. For more details regarding baseline characteristics and outcome parameters, see chapter 7.2.1 and Table 4, respectively. The collected data for the non-randomized patients will include the same parameters as collected for the randomized patients, with the exception of long-term neurodevelopmental outcome. Data will be stored in the CASTOR database.

In analogy with the data collection for randomized patients, data management for non-randomized patient will be implemented according to Good clinical practice (GCP) guidelines and will comply with the General Data Protection Regulation (GDPR) and the 'Algemene Vordering Gegevensbescherming' (AVG).

3.5 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.

TREATMENT OF SUBJECTS

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 judgment 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. This is part of standard clinical practice for monochorionic twin pregnancies managed in our hospital. 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).

3.6 Investigational treatment

In the experimental group, laser therapy must be performed within 72 hours after diagnosis of TAPS stage ≥ 2. 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. In case of a wandering inter-twin membrane or not enough distention of the uterus, the amniotic sac of the recipient can be infused with saline and amniotic fluid can be drained from the donor. 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.

3.7 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 the disease. In the control group treatment with laser surgery will not be performed, this procedure will solely be performed in the experimental group. In ongoing TAPS twin pregnancies, labor will be induced or cesarean section will be performed at 36 weeks of gestational age.

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 decrease 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 or without 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. The amount of blood transfused is based on the estimated fetal weight and will be calculated using the formula proposed by Rodeck et al [27]. The calculator can be found at: perinatology.com/protocols/rhc.htm. The necessary volume of donor blood is calculated using the following formula: $V_{donor\ blood} = V_{feto\text{-}placental} \bullet (Ht_{final} - Ht_{inititial})/Ht_{donor\ blood}$
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. The total blood volume to be exchanged is determined as follows: $V_{exchange} = [V_{blood} \bullet (Ht_{initial} - Ht_{final})]/(Ht_{initial})$, where blood volume = the patients weight in kilograms multiplied by 90 mL/kg.

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.

Generally, IUTs (with or without PET) are performed up to a maximum of 3 to 4 times in donors of TAPS pregnancies, but an absolute maximum is not reported before. The number of IUTs that the fetal surgeon is able to perform depends on several factors, including gestational age at intervention, position of the donor and clinical condition of both fetuses. When the fetal surgeon decides to stop performing IUTs (with or without PET) and go over to (preterm) delivery, the reason for stopping should be recorded. Based on the recent analysis of our data, the mean number of IUT in TAPS ≥ stage 2 is 2 (range 1-4).

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 TAPS cases (see below for criteria for preterm delivery). During neonatal period, no additional follow-up examinations other than the regular care for premature neonates will be performed.

Criteria for (preterm) delivery (for both the experimental group and control group)

Criteria for proceeding to an induced preterm delivery are defined as follows;

- 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), intra-uterine infection)
- Iatrogenic monoamnionicity: an elective cesarean will be planned at 32 weeks of gestation

3.8 Use of co-intervention (not applicable)

3.9 Escape medication (not applicable)

4. INVESTIGATIONAL PRODUCT (not applicable)

4.1 Name and description of investigational product(s)

N/A

4.2 Summary of findings from non-clinical studies

N/A

4.3 Summary of findings from clinical studies

N/A

4.4 Summary of known and potential risks and benefits

N/A

4.5 Description and justification of route of administration and dosage

N/A

4.6 Dosages, dosage modifications and method of administration

N/A

4.7 Preparation and labelling of Investigational Medicinal Product

N/A

4.8 Drug accountability

N/A

5. NON-INVESTIGATIONAL PRODUCT (not applicable)

5.1 Name and description of non-investigational product(s)

N/A

5.2 Summary of findings from non-clinical studies

N/A

5.3 Summary of findings from clinical studies

N/A

5.4 Summary of known and potential risks and benefits

N/A

5.5 Description and justification of route of administration and dosage

N/A

5.6 Dosages, dosage modifications and method of administration

N/A

5.7 Preparation and labelling of Non Investigational Medicinal Product

N/A

5.8 Drug accountability

N/A

6. METHODS

6.1 Primary study outcome

- Gestational age at birth (completed weeks and additional days)

6.2 Secondary study outcomes

- Composite outcome of perinatal mortality and severe neonatal morbidity
    - Perinatal mortality: intrauterine fetal demise or neonatal death until 28 days after birth
    - Severe neonatal morbidity
        - Respiratory distress syndrome
        - Proven early onset neonatal sepsis
        - Retinopathy of prematurity
        - Necrotizing enterocolitis
        - Patent ductus arteriosus
        - Severe cerebral injury, includes at least one of the following
            - intraventricular hemorrhage
            - cystic periventricular leukomalacia
            - ventricular dilatation
            - porencephalic or parenchymal cysts
            - severe cerebral lesions associated with adverse neurological outcome
- Hematological complications
    - Anemia in donor requiring a blood transfusion within 24 hours after birth
    - Polycythemia in recipient requiring a partial exchange transfusion within 24 hours after birth
    - Other neonatal complications associated with TAPS
        - necrotic skin injury
        - limb ischemia
        - thrombocytopenia
        - severe hypoalbuminemia
        - severe hypoproteinemia
- Procedure related complications
    - 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:

- Instruments: Formal neurologic examination, Bayley Scales of Infant and Toddler Development version 3 (BSID-III) and/or Ages and Stages Questionnaire (ASQ-3), Child Behavior Checklist (CBCL 1.5-5y)
- Neurodevelopmental impairment (NDI)
  - Cerebral palsy
  - Impaired cognitive or motor development
  - Impaired functioning in communication, fine and gross motor, problem solving and/or personal and social functioning
  - Severe visual loss
  - Severe hearing loss
- Severe NDI
  - Cerebral palsy
  - Impaired cognitive or motor development
  - Impaired functioning in communication, fine and gross motor, problem solving and/or personal and social functioning
  - Bilateral blindness
  - Bilateral deafness
- Behavioral Problems

In Table 4, a list with definitions for each outcome parameter is provided. Figure 3 presents a timeline including timeframes for assessment for each outcome parameter.

Outcome parameter	Definition	Timeframe for assessment
Gestational age at birth		
Gestational age at birth	Gestational age: completed weeks + additional days since the first day of the last menstruational period of the mother.	Birth
Perinatal mortality		
Perinatal mortality	Intrauterine fetal demise (IUFD): absence of cardiac activity on ultrasound Neonatal death: absent brain stem reflexes combined with no cardiac activity	Randomization → 28 days after birth
Neonatal morbidity		
Respiratory distress syndrome	Requiring mechanical ventilation and/or surfactant	Birth → 28 days after birth
Proven early-onset neonatal sepsis	Positive blood cultures within 72 hours postpartum	Birth → 28 days after birth
Retinopathy of prematurity	ICROP stage 3 or higher **[28]**	Birth → 28 days after birth
Necrotizing enterocolitis	Stage 2 or higher **[29]**	Birth → 28 days after birth
Patent ductus arteriosus	Requiring medical therapy or surgical closure	Birth → 28 days after birth
Severe cerebral injury		

Intraventricular Hemorrhage	Grade 3 or higher **[30]**	Birth → 28 days after birth
Cystic periventricular leukomalacia	Grade 2 or higher **[31]**	Birth → 28 days after birth
Ventricular dilatation	> 97th percentile **[32]**	Birth → 28 days after birth
Porencephalic or parenchymal cysts	Presence of porencephalic or parenchymal cysts Porencephalic cyst: focal cystic area of encephalomalacia that communicates with the ventricular system and/or the subarachnoid space. Parenchymal cyst: smooth, rounded borders and minimal-to-no surrounding signal intensity abnormality.	Birth → 28 days after birth
Hematological complications		
Anemia	Requiring blood transfusion	Day of birth (day 1)
Polycythemia	Requiring partial exchange transfusion	Day of birth (day 1)
Complications due to anemia or polycythemia		
Necrotic skin injury	Clinical evaluation of either eschar necrotic tissue (dry, thick, leathery tissue that is often tan, brown or black) or slough (yellow, tan, green or brown in color and may be moist, loose and stringy in appearance).	Day of birth (day 1)
Limb ischemia	a condition characterized by chronic ischemic at-rest pain, ulcers, or gangrene in one or both arms/legs attributable to objectively proven arterial occlusive disease **[33] [34]**	Day of birth (day 1)
Thrombocytopenia	Platelet count < 150,000/microL**[35]**	Day of birth (day 1)
Severe hypoalbuminemia	Albumin levels < 20 g/L **[36]**	Day of birth (day 1)
Severe hypoproteinemia	Protein levels < 40 g/L **[37]**	Day of birth (day 1)
Procedure related complications		
Amniotic band syndrome	Amniotic band causing strangulation of body parts of the fetus or umbilical cord **[38]**	Day of birth (day 1)
Iatrogenic monoamniocity	Rupture of the inter-twin membrane resulting in mono-amnionicity. Diagnosis: ultrasound visualization of a free-floating inter-twin membrane and/or the presence of both twins in one amniotic cavity and/or the suspicion of umbilical cord entanglement**[39]**.	Randomization → birth
Preterm premature rupture of the membranes	Premature rupture of the membranes occurring before 37 weeks of gestation. For the diagnosis of PPROM there must be evidence of diminished amniotic fluid volume on ultrasound in combination with objectification of amniotic fluid leakage **[40]**.	Randomization → birth

Placental abruption	Premature separation of the placenta before the delivery of the fetus, diagnosed by vaginal bleeding during uterine contractions, with non-reassuring fetal cardiotocography, confirmed both by the operator and pathologist**[41]**.	Randomization → Birth
Clinical chorioamnionitis	An acute inflammation of the membranes and chorion of the placenta. The key clinical findings include fever, uterine fundal tenderness, m aternal tachycardia (>100/min), fetal tachycardia (>160/min) and purulent or foul amniotic fluid.	Randomization → birth
Histological Chorioamnionitis	Diagnosis is based on the presence of neutrophilic granulocytes in the chorionic plate or the extraplacental membranes. Chorioamnionitis is defined as one or more of the following categories: 1) acute subchorionitis 2) acute chorionitis 3) acute chorioamnionitis 4) necrotizing chorioamnionitis.	Birth
Histological Funisitis	Diagnosis is based on the presence of neutrophilic granulocytes in the wall of the umbilical vessel(s) and Wharton's jelly Funisitis is defined as one or more of the following categories: 1) chronic vasculitis 2) umbilical phlebitis 3) umbilical vasculitis (inflammation in one or two umbilical arteries ± umbilical vein) 4) umbilical panvasculitis (inflammation in 3 vessels)	Birth
Long-term outcome		
NDI		
Cerebral palsy	Spastic bilateral, spastic unilateral, or mixed**[42]**. Classification by European CP Network.	At the corrected age of 2 years
Impaired cognitive or motor development	Score < 85 (1 SD below the mean) as assessed by Bayley Scales of Infant and Toddler Development version 3 (BSID-III)	At the corrected age of 2 years
Impaired functioning in communication, fine and gross motor, problem solving, personal and social functioning	Score > 1 SD below the mean as assessed by Ages and Stages Questionnaire version 3 (ASQ-III)	At the corrected age of 2 years
Severe visual loss	Blind or partially sighted	At the corrected age of 2 years
Severe hearing loss	Needing hearing aids	At the corrected age of 2 years

Severe NDI		
Cerebral palsy	Gross motor function classification system (CMFCS) grade > 1	At the corrected age of 2 years
Impaired cognitive or motor development	Score < 70 (2 SD below the mean) as assessed by the Bayley Scales of Infant and Toddler Development version 3 (BSID-III)	At the corrected age of 2 years
Impaired functioning in communication, fine and gross motor, problem solving, personal and social functioning	Score > 2 SD below the mean as assessed by Ages and Stages Questionnaire version 3 (ASQ-III)	At the corrected age of 2 years
Bilateral blindness	Visual acuity of less than 3/60 in the better eye **[43]**	At the corrected age of 2 years
Bilateral deafness	Severe or profound hearing loss in both ears (severe hearing loss: a person can only hear sounds > 70-89 dB, profound hearing loss: a person can only hear sounds > 90dB)**[44]**	At the corrected age of 2 years
Behavioural problems		
Behavioural problems	Presence of emotional and behavioral problems is 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) Instrument: Child Behaviour Checklist 1.5-5 years	At the corrected age of 2 years

Table 4. List of definitions and timeframe for assessment specified for each outcome parameter

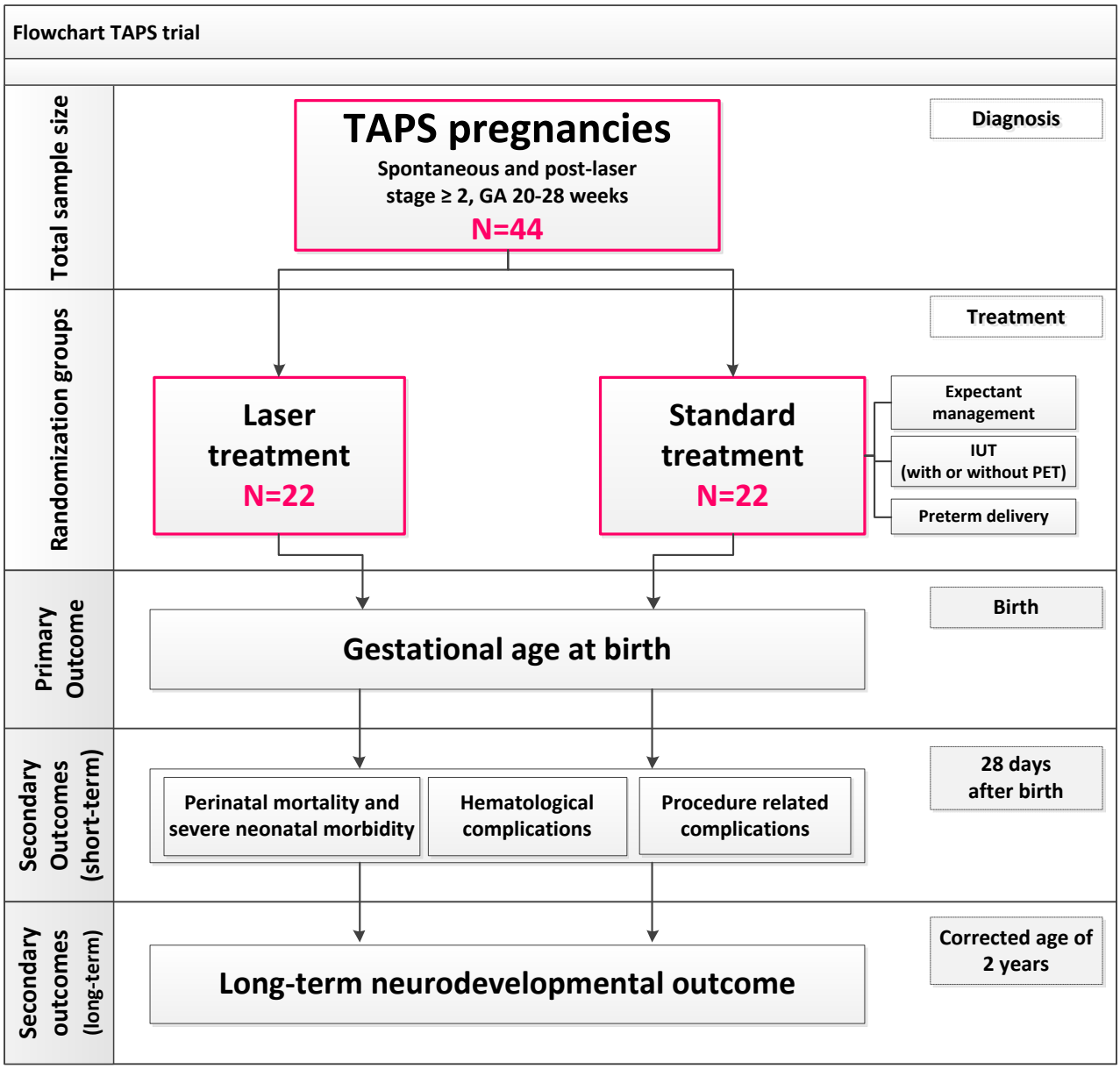


Figure 3. Design for the TAPS trial, including timeframe for assessment for each outcome parameter

6.2.1 Other study parameters (if applicable)

Maternal and fetal baseline characteristics

- Gestational age at presentation
- TAPS stage at randomisation
- Localisation of the placenta (predominantly anterior or posterior)
- Mode of delivery (caesarean or vaginal birth)
- Weight
- Height
- Gravidity

- Parity
- The presence of the following findings on ultrasound:
    - Pericardial effusion in the TAPS donor
    - Cardiomegaly in the TAPS donor
    - Pleural effusion in the TAPS donor
    - Skin edema in the TAPS donor
    - Ascites in the TAPS donor
    - Starry sky liver in the TAPS recipient

<u>Intervention characteristics</u>

- General:
    - Cervical length before procedure
    - TAPS stage at intervention
    - Gestational age at procedure
- Laser therapy
    - Fetoscopy time
    - Maximal power used (Watt)
    - Total amount of energy used (Joule)
    - Diameter work shaft
    - Number of intrauterine insertions
    - Visibility score (0-10)
    - Visibility scale (defined as clear, blurred or blood stained)
    - Number and type of vascular anastomoses at fetoscopic laser coagulation
    - Completeness of procedure (surgeon's opinion)
    - Maternal complications (related to the procedure)
        - bleeding at the induction site
        - intra-uterine infection
        - membrane separation
        - abdominal bleeding
- IUT and PET
    - Site of transfusion (umbilical cord, intrahepatic vein, intraperitoneal)
    - Volume of blood/saline transfused/exchanged
    - Hemoglobin levels (g/dL) before and after transfusion
    - Amount of IUTs and/or PETs
    - Reason for discontinuing IUTs/PET
        - Unfavorable position of the fetus
        - Fetal compromise in donor
        - Fetal compromise in recipient
        - PPROM
        - Patients preference
        - Technically challenging or not favourable
        - Other

- Selective feticide
  - Technique used
    - RFA
    - Interstitial laser
    - Bipolar cord occlusion

Placental evaluation

- Amount of (residual) anastomoses
- Diameter of anastomoses (in mm)
- Type of anastomoses (aterio-venous, veno-arterial, arterio-arterial, veno-venous)
- Type of umbilical cord insertion for each twin (central, marginal, velamentous)
- Placental share (%)
- Presence of single umbilical artery
- Presence of bipartite placenta
- Digital picture of the injected fetal side of the placenta (including measuring tape)
- Digital picture of the maternal side of the placenta (including measuring tape)

Neonatal evaluation (for each twin)

- Birth weight
- Gender
- Apgar score at 1, 5 and 10 minutes
- Birth order of twins
- Hemoglobin value at birth (g/dL)
- Reticulocyte count at birth (‰)

6.3 Randomisation, blinding and treatment allocation

Women eligible for participation in the study will be invited for counselling, to ensure that they are fully informed on the nature of the study. They will also be provided written information (Participant Information Form). Women who agree to participate will be asked to sign a written informed consent of which they will receive a copy.

Consenting eligible women will then be randomly allocated to either the experimental group (laser therapy) or the control group (standard care) with an 1:1 allocation using a web-based data system called CASTOR, using a permuted block design. The block size will be 2 and 4. Stratification will be by gestational age (20-24 weeks vs. 25-28 weeks) and type of TAPS (post-laser vs. spontaneous TAPS). CASTOR will not release the randomization code until the participant has been recruited into the trial,

which takes place after baseline measurements have been entered in the system. In this way, allocation concealment will be ensured. The unique number generated cannot be deleted afterwards. The randomization sequence will not be accessible by the recruiters. Researchers who collect and analyse the data will not be masked. Women who decline randomization will be asked permission for using data including reasons for decline and participant characteristics.

This study is open-label since the nature of this intervention makes it impossible to disguise the type of intervention. However, neonatologist who assess the nature and degree of severe cerebral injury in TAPS survivors will be blinded for treatment allocation. In addition, treatment allocation will also be masked for pediatric psychologists who examine the long-term neurodevelopmental follow-up.

6.4 Study procedures and required clinical evaluation

Data on gestational age, TAPS stage, localisation of the placenta, height and weight will be retrieved from medical history.

Laser treatment

Participating patients will be randomly allocated to the experimental group or the control group. Patients allocated to the experimental group will receive laser therapy within 72 hours after randomization. The following data will be noted: time of fetoscopy, the maximal power that was used (Watt), diameter of the work shaft, number of intrauterine insertions, visibility score and scale, number and type of vascular anastomoses that were coagulated, whether the procedure was complete or not (opinion of the fetal surgeon directly after laser) and if there occurred any complications related to the laser surgery. A follow-up ultrasound scan will be made the following day.

Standard treatment

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 judgment of the fetal surgeon and the preference of the patient. The following data will be collected: site of transfusion (intravascular, intraperitoneal or both), volume of blood/saline transfused and the haemoglobin values before and after

transfusion. 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.

In both treatment groups, 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.

Birth

Generally all monochorionic twins receive induction of labor around 36 weeks of gestational age. Data on mode of delivery and gestational age at delivery will be collected.

Neonatal evaluation

After birth of the TAPS twins, a full blood count including hemoglobin and reticulocyte count will be performed on blood of the umbilical cord to determine the rate of anemia in the donor and polycythemia in the recipient twin. Data regarding hematological complications including blood transfusion or partial exchange transfusion on day 1, necrotic skin injury, limb ischemia, thrombocytopenia, severe hypoalbuminemia, severe hypoproteinemia will be gathered. In addition, the following data will be collected at birth: birth order (first or second born child), birth weight, gender and Apgar score at 1, 5 and 10 minutes. During a timeframe of 0-28 days after birth, neonatal mortality and neonatal morbidities including respiratory distress syndrome, retinopathy of prematurity, patent ductus arteriosus requiring treatment, necrotizing enterocolitis, severe cerebral injury will be assessed. Serial cranial ultrasound examinations will be performed by a neonatologist (who is blinded for treatment allocation) to detect the presence and type of cerebral injury.

Placental evaluation

1. Placental injection

All participants will be asked to use their placenta for color dye injection. This is part of standard clinical practice for monochorionic twin pregnancies managed in our hospital. After birth, the placenta is collected and stored in the fridge for color dye injection. A detailed protocol and accompanying video showing each step of placental injection created by our institution can be found at http://www.jove.com/video/3208/accurate-simple-evaluation-vascular-anastomoses-monochorionic.

During this procedure, at least two digital pictures are made:
1. A picture of the maternal side of the placenta (before injection)
2. A picture of the injected fetal side of the placenta (after injection)
On all pictures a measuring tape must be present.

After placental injection, the following data will be collected: the amount and type of anastomoses, type of umbilical cord insertion, placental share, presence of single umbilical artery, presence of bipartite placenta. In addition, a digital picture of both the maternal side and the fetal side injected with color dye will be made.

2. Histologic evaluation of the placenta

After injection, the placental will be sent to the department of Pathology for histological examination. The placenta will be examined according to a fixed protocol. Placental tissues will be collected using a minor adaptation of the method described by Burton et al.[45] In brief, tissue samples will be collected from three areas:

(1) the umbilical cord
   a. one tissue sample close to the fetus
   b. one tissue sample at the middle of the cord
   c. one tissue sample near the placental insertion
(2) the placenta
   a. macroscopically normal placental parenchyma from the placental share of twin 1
   b. macroscopically normal placental parenchyma from the placental share of twin 2
(3) the membrane area of each twin
   a. membrane roll of the ruptured site to the placental margin of twin 1
   b. membrane roll of the ruptured site to the placental margin of twin 2

Long-term follow up

At the corrected age of 2 years, 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).

6.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

6.5.1 Specific criteria for withdrawal (if applicable)

N/A

6.6 Replacement of individual subjects after withdrawal

When the participant withdraws from the study, replacement will not take place.

6.7 Follow-up of subjects withdrawn from treatment

Since the statistical analysis is planned according to the intention-to-treat principle, participants who discontinue the study will be analysed in the group that they were allocated to.

6.8 Premature termination of the study

The DSMB can decide to terminate the study prematurely in case of a substantial overrepresentation of SAE's in the experimental treatment or standard treatment group. Due to the low number of patients in this study and the large number of potential SAE types (tables 5 and 6), the decision what constitutes a substantial overrepresentation will be based on clinical judgement. For the expected SAEs presented in table 5, this judgement is further supported by the following 'warning flag'.

If in either of the two treatment groups the lower limit of an exact two sided 80% confidence interval for the proportion of twin pregnancies in which at least one of the fetus experiences one of the following SAEs: intrauterine death, extreme prematurity (<26 weeks), severe cerebral damage or neonatal death exceeds 40%, a warning flag will be raised that triggers a further in-depth clinical investigation of the safety data. The degree of imbalance in both expected and unexpected SAEs between the two groups will be taken into account in the consideration whether or not to prematurely terminate the trial.

According to the abovementioned rule, a warning flag will be raised in the following cases:

- When assessing 5 pregnancies per group at the DSMB meeting: if at least 1 of the expected SAEs occurs in one of the groups in ≥4/ 5 pregnancies
- When assessing 10 pregnancies per group at the DSMB meeting: if at least 1 of the expected SAEs occurs in one of the groups in ≥7/10 of the pregnancies

- When assessing 15 pregnancies per group at the DSMB meeting: if at least 1 of the expected SAEs occurs in one of the groups in ≥ 9/15 pregnancies

7. SAFETY REPORTING

7.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO and section 22 of the Embryowet (Embryo's Act), the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

7.2 AEs and SAEs

7.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

7.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Based on the definitions described above, we expect to report many adverse events. However, a large part of these adverse events are inherent to the common and well-known complications of (untreated) TAPS, and are not always attributed to the effect of the treatment. Therefore a list of expected and unexpected adverse events is presented below.

Expected SAEs

Expected SAEs in The TAPS Trial will include the following fetal and neonatal complications:

SAE	Time Frame
Intra-uterine fetal death	Randomization → birth of twins
Neonatal mortality	Birth → 28 days after birth of twins
Extreme prematurity (Gestational age at birth < 26 w + 0 d)	Birth
Severe cerebral injury (for definition, see table 4.)	Birth → 28 days after birth of twins

Table 5. Expected SAEs for the TAPS Trial

Unexpected SAEs

Expected SAEs in the TAPS trial will include the following maternal complications:

SAE	Time Frame
Need for transfusion or operation for postoperative hemorrhage	Intervention → 24 hours after intervention
Placental abruption	Randomization → birth of twins
Sepsis (positive blood cultures)	Randomization → 24 hours after birth of twins
Bowel perforation	Intervention → 7 days after intervention
Admission of the patient to the intensive care unit	Intervention → 24 hours after birth of the twins
Death of the patient	Randomization → 28 days

Table 6. Unexpected SAEs for The TAPS Trial

All adverse events occurring in women, fetuses, and neonates must be recorded and are an integral part of the study outcome.

All SAE's that are life-threatening or result in death of the mother must be reported to the initiator of The TAPS Trial (LUMC) within 24 hours. SAE's occurring in participating centers will be reported via the 'SAE Report Form' designed for this study. Participating centers will send this completed form to the sponsor of the study (LUMC). He will perform an initial check of the report, request any additional information, and he will notify it to the steering committee and the DSMB. Occurrence of a life-threatening SAE or SAE that results in death of the mother should lead to a

meeting of the DSMB who can decide to discontinue the study. All SAE information will be recorded.

The sponsor (LUMC) will report all the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

7.2.3 Suspected unexpected serious adverse reactions (SUSARs)

N/A

7.3 Annual safety report

N/A

7.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

7.5 Data Safety Monitoring Board (DSMB)

There will be an independent DSMB composed of:

- 1 Statistician
- 2 Independent experts from departments that are committed to the study

A DSMB is established to perform ongoing safety surveillance which means that (S)AE will be monitored. The DSMB will have insight into the total number of (S)AEs and will, as needed, review individual records to be able to analyse potential associations between complications and the study protocol. Accumulating data on (S)AEs will be send to the DSMB for safety review by researchers. All members have independent positions from the trial study group. The composition and responsibilities of the DSMB are described in detail in the DSMB charter.

The advice(s) of the DSMB will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

8. STATISTICAL ANALYSIS

All data will be analysed in accordance with the intention-to-treat principle, meaning that, for the purpose of the analysis, all TAPS pregnancies will be analysed in the group to which they have been randomized. If TAPS twins are lost to follow up, they will be included in their randomized group for all outcomes for which data is collected. Descriptive statistics will be calculated to check for major dissimilarities between study groups with respect to baseline information. The null hypothesis for this trial assumes that both study arms will result in a comparable gestational age at birth. All comparisons on outcomes measured on the per fetus or per neonate level, we will use generalized estimating equations (GEE) procedure for statistical inference to account for the fact that observations between co-twins are not independent. A p-value < 0.05 is considered to indicate statistical significance. All data will be analysed using SPSS (IBM, Armonk, NY, USA).

8.1 Primary study parameter(s)

In the primary analysis, gestational age at birth will be compared between the fetoscopic laser surgery group and the control group using a Mann-Whitney-U test.

8.2 Secondary study parameter(s)

In the secondary analysis, data on the presence of procedure related complications will be analyzed using a Chi-square test. Inter-twin hemoglobin difference will be compared between the groups using a Mann-Whitney-U test. Data on other hematological complications, perinatal mortality, neonatal morbidity and long-term (neuro)developmental impairment will be expressed as odds ratios, with a confidence interval of 95% calculated by the GEE procedure.

In a secondary analysis we will adjust this comparison for covariates ‘type of TAPS’ and ‘gestational age at diagnosis’ that were used as stratification factors during randomisation and possibly for other baseline factors if large dissimilarities at baseline are observed.

8.3 Other study parameters

No subgroup analyses will be performed.

8.4 Interim analysis (if applicable)

The DSMB will perform an interim analysis on safety parameters after delivery of the 22th TAPS twin (halfway through the study).

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and the Embryo's Act .

9.2 Recruitment and consent

All eligible women will be invited to participate in the study, when they present at one of the participating centers. Women eligible for participation in the study will be invited for additional counselling by a fetal surgeon or a member of the research team of the center, to ensure that they are fully informed on the nature of the study by means of both oral and written information (Participant Information Form). Women who agree to participate will be asked to sign a written informed consent of which they will receive a copy

9.3 Objection by minors or incapacitated subjects (if applicable)

N/A

9.4 Benefits and risks assessment, group relatedness

The intervention strategies in the standard treatment arm are already applied in current practice. Fetoscopic laser surgery, performed in the experimental treatment arm, is applied for several decades now in the treatment for TTTS and is considered the golden standard for this syndrome. Although fetoscopic laser surgery is associated with higher risk on several complications (including single or double intrauterine fetal demise, iatrogenic monoamniocity, amnion dehiscention, intra-uterine infection and preterm premature rupture of the membranes), the natural course of TAPS on itself is characterized by high rates of morbidity and mortality, as well (Table 3). The additional risk of fetoscopic laser treatment on top of the risks that are already associated with the natural course of TAPS is therefore estimated as low. The benefit of participating is that TAPS pregnancies allocated to the fetoscopic laser surgery group might be delivered at a higher gestational age, and will thus have improved neonatal outcome.

More information regarding risk assessment is presented in Chapter 11 'Structured Risk Analysis'.

9.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9.6 Incentives (if applicable)

N/A

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

Data management will be implemented according to Good clinical practice (GCP) guidelines and will comply with the General Data Protection Regulation (GDPR) and the 'Algemene Vordering Gegevensbescherming' (AVG). Data will be coded and handled confidentially.The key to the code will be safeguarded by one of the investigators. The coded patient data will be entered in CASTOR, a GCP conform internet based electronic data capture tool secured with login codes. Participating centers will be supplied with a username and password to be able to entry their data into the online database (Castor). Data will only be transferred encrypted. Data will be stored for 15 years according to the WGBO.

10.2 Monitoring and Quality Assurance

Monitoring will be performed in compliance with Good Clinical Practice (GCP) and other rules and regulations in order to achieve high quality research and secure patients safety. An independent monitor will take care for the monitoring in the TAPS trial in the LUMC and will have access to the data and source documents of the trial.

The monitoring plan will consist of the following visits:

1. Before the start of the trial
2. After every 10 included TAPS pregnancies
3. After closing of the study

Each participating center will be sponsor of the TAPS trial in their own center, and is therefore responsible for monitoring in their own hospital.

10.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

10.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed

the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the patient's last visit. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

10.6 Public disclosure and publication policy

After completion of the study, we aim to publish our results as soon as possible (within 6-12 months). According to the collaboration agreement (paragraph 4.1 and 4.2) the participating centers and their principal investigator and members of research staff involved in The TAPS Trial will be permitted to independently present at symposia, national or regional professional meetings, and to publish in journals, theses or dissertations or otherwise of their own choosing, only those methods and results generated by them under the The TAPS Trial.

The responsibility for the preparation and maintenance of the single database and the first publication or presentation of the results of the The TAPS Trial shall reside with LUMC and LUMC Investigator. Any independent publication based on the results obtained in The TAPS Trial will not be made before the first multi-centre publication or presentation of the multi-centre results of the Studies by LUMC and LUMC Investigator unless otherwise agreed in writing.

11. STRUCTURED RISK ANALYSIS

11.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The rationale of this procedure is to separate both fetal circulations by occluding all vascular anastomoses on the placental surface. This is achieved by introducing a small fetoscope. With this fetoscope the vascular equator is identified and all anastomoses are selectively coagulated with a high powered medical laser. Thereafter, a line is drawn from one placenta margin to the other along the vascular equator connecting all coagulated anastomoses making sure even the smallest anastomoses are coagulated.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

Fetoscopic laser surgery for pregnancies diagnosed with TTTS has been performed since 1990[46]. Since then, the effects of laser therapy are extensively studied in TTTS pregnancies and currently, laser surgery is considered the golden standard for the management of TTTS. Moreover, the laser technique has been significantly improved, resulting in a decreased perinatal mortality and neonatal morbidity[47]. TAPS is only discovered a decade ago[48], and knowledge regarding the effects of fetoscopic laser coagulation for this disorder is not yet elucidated. Just like TTTS, TAPS is a result of a distorted balance in inter-twin blood transfusion through placental anastomoses, and therefore the only causative treatment is considered the same. There are small studies and case reports endorsing the potential benefit of laser therapy[18-20, 22-24].

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

N/A

d. Selectivity of the mechanism to target tissue in animals and/or human beings

N/A

e. Analysis of potential effect

Fetoscopic laser therapy is associated with the following complications: PPROM, iatrogenic monoamnionicity, amnion dehiscence, intrauterine infection, and IUFD of one or both children. In Table 7, the risk of each complication is presented based on the multicenter, international Solomon trial in which TTTS cases are treated with laser (Solomon technique)[47]. Table 8 shows the risk for procedure-related complications in TAPS twins ≥stage 2 treated with laser surgery, IUT (+PET) and expectantly.

Complication	Laser in TTS (N=137)
PPROM	42% (57/137)
Iatrogenic monoamnionicity	12% (17/137)
Amnion dehiscention	5% (7/137)
Intrauterine infection	1% (1/137)
IUFD	16% (45/274)

Table 7. The risk for complications associated with fetoscopic laser surgery based on the results of the Solomon trial[49].

Complication	Laser (N=15)	IUT (+PET) (N=16)	Expectant (N=16)
PPROM	40% (6/15)	19% (3/16)	31% (5/16)
Iatrogenic monoamnionicity	17% (5/30)	6% (1/16)	-
Intrauterine infection	7% (1/15)	19% (3/16)	19% (3/16)
IUFD	17% (5/30)	19% (6/32)	9% (3/32)

Table 8. The risk for complications associated with fetoscopic laser surgery, IUT (+PET) and expectant management in TAPS pregnancies ≥ stage 2, GA at diagnosis < 28w, based on the October 2018- analysis of our own data (2003-2018).

f. Pharmacokinetic considerations

N/A

g. Study population

This study will be conducted in pregnant women diagnosed with TAPS. It needs to be clarified that the natural course of TAPS is progressive of nature and characterized by high rates of perinatal mortality and neonatal morbidity. In TAPS cases treated expectantly, the perinatal mortality and neonatal morbidity rate is 25% and 43%, respectively (based on the analysis in oct. 2018 of our own data) (Table 9.). Donors with severe anemia are at risk for developing heart failure, hydrops and intra-uterine fetal demise. In the recipient twin, severe polycythemia may lead to poor blood flow and thrombosis in skin, extremities, the brain and other organs, resulting in necrosis, cerebral injury and death of the fetus. Since the best treatment option for TAPS is still unclear, we propose to conduct a RCT in which TAPS pregnancies are allocated to either the laser surgery group, or the group treated with standard care.

	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)
IUFD	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, IUFD = intrauterine fetal demise, NDI = neurodevelopmental impairment

Table 9. 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.

h. Interaction with other products

N/A

i. Predictability of effect

- *IUFD*: The cause of IUFD shortly after laser therapy is not always clear. Therefore, it is difficult to predict which twin pregnancies managed with laser therapy are at risk for single or double fetal demise. In TTTS, growth restriction and the severity of the disease pre-operatively might play a role. We expect the chance of IUFD after laser to be lower in TAPS pregnancies. TAPS, in contrast to TTTS, is characterized by only a small and slow transfusion through a few miniscule anastomoses, resulting in a smaller difference in circulating capacity before and after laser. Additionally, TAPS fetuses may be less ill than in TTTS
- *Iatrogenic monoamnionicity*: unintentional perforation of the inter-twin dividing membranes can result in an iatrogenic monoamniotic twin pregnancy. Iatrogenic monoamniotic twin pregnancies are at risk for umbilical cord entanglement and are therefore managed as spontaneous monoamniotic twins (admission for fetal CTG monitoring and delivery by caesarean section at gestational age 32weeks and 4 days.
- *Amnion dehiscention:* in case of amnion dehiscence, the amnion detaches from the chorion, resulting in the amnion lying free in the amniotic cavity. Twin pregnancies diagnosed with amnion dehiscence are at risk for amniotic band syndrome and iatrogenic monoamnionicity (and therefore umbilical cord entanglement).
- *Intrauterine infection:* Fetoscopic laser therapy is performed in a strict sterile environment. There is, however, always a small chance that pathogens will be introduced in the uterus, resulting in an intrauterine infection.
- *PPROM:* When the endoscope is inserted in the uterus, a small insertion hole is made in the amniotic and chorionic membrane. In most cases, the two membranes slide over each other after laser surgery, so that the insertion hole is sealed. However, several studies show that there is an increased chance of PPROM in pregnancies in which laser surgery has been performed[23, 50]. PPROM is associated with preterm birth [51]

<u>j. Can effects be managed?</u>

- *IUFD*: After diagnosis of IUFD, the pregnancy is managed as a singleton pregnancy or the care is left up to the local caretaker.
- *Iatrogenic monoamniocity*: In pregnancies diagnosed with iatrogenic monoamnionicity, monitoring will be intensified. At a gestational age of 26-28

weeks, the patient may be admitted to the hospital for fetal surveillance. At 32 +4 weeks of gestation a primary caesarean section will be planned.

- *Amnion dehiscention:* depending on the severity of the dehiscention, the intensity of the monitoring will be increased. When amnion dehiscence is accompanied by a ruptured inter-twin membrane, the pregnancy is managed as if it were an iatrogenic monoamniotic pregnancy.
- *Intrauterine infection*: in case of an intrauterine infection, a delivery will be pursued to prevent severe neonatal and maternal sepsis.
- *PPROM*: When PPROM is diagnosed, steroids can be given to the mother to speed up fetal lung development. In addition, tocolytics can be considered.

11.2 Synthesis

We propose to conduct a randomized controlled trial in which patients diagnosed with TAPS ≥ stage 2 will be randomized to two different groups: fetoscopic laser surgery and the standard care group. Although fetoscopic laser surgery is associated with higher risk on several complications (including single or double IUFD, iatrogenic monoamniocity, amnion dehiscention, intra-uterine infection and PPROM), the natural course of TAPS on itself is characterized by high rates of morbidity and mortality as well. The additional risk of laser treatment on top of the risks that are already associated with the natural course of TAPS is therefore estimated as low.

12. REFERENCES

1. Lopriore, E., et al., *Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences.* Am J Obstet Gynecol, 2009. **201**(1): p. 66 e1-4.
2. Yokouchi, T., et al., *Incidence of spontaneous twin anemia-polycythemia sequence in monochorionic-diamniotic twin pregnancies: Single-center prospective study.* J Obstet Gynaecol Res, 2015. **41**(6): p. 857-60.
3. Casanova, J., et al., *Twin anemia polycythemia sequence: a report of three cases.* J Reprod Med, 2014. **59**(11-12): p. 596-8.
4. Lewi, L., et al., *The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study.* Am J Obstet Gynecol, 2008. **199**(5): p. 514 e1-8.

5. Gucciardo, L., et al., *Twin anemia polycythemia sequence from a prenatal perspective.* Prenat Diagn, 2010. **30**(5): p. 438-42.
6. Slaghekke, F., et al., *Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome.* Fetal Diagn Ther, 2010. **27**(4): p. 181-90.
7. Tollenaar, L.S., Lopriore, E., Middeldorp, J.M., Haak, M.C., Klumper F.J., Oepkes, D., Slaghekke, F., *Improved Antenatal Prediction of Twin Anemia Polycythemia Sequence by Delta Middle Cerebral Artery Peak Systolic Velocity – A New Antenatal Classification System.* Ultrasound Obstet Gynecology, 2018.
8. Lopriore, E., et al., *Severe cerebral injury in a recipient with twin anemia-polycythemia sequence.* Ultrasound Obstet Gynecol, 2013. **41**(6): p. 702-6.
9. Luminoso, D., et al., *Fetal brain lesion associated with spontaneous twin anemia-polycythemia sequence.* Ultrasound Obstet Gynecol, 2013. **42**(6): p. 721-2.
10. Stranak, Z., et al., *Prenatally Acquired Multiple Limb Ischemia in a Very Low Birth Weight Monochorionic Twin.* Fetal Diagn Ther, 2015.
11. Robyr, R., et al., *Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome.* Am J Obstet Gynecol, 2006. **194**(3): p. 796-803.
12. Sarkar, S. and T.S. Rosenkrantz, *Neonatal polycythemia and hyperviscosity.* Semin Fetal Neonatal Med, 2008. **13**(4): p. 248-55.
13. Lopriore, E., et al., *Hematological characteristics in neonates with twin anemia-polycythemia sequence (TAPS).* Prenat Diagn, 2010. **30**(3): p. 251-5.
14. Verbeek, L., et al., *Hypoalbuminemia in donors with twin anemia-polycythemia sequence: a matched case-control study.* Fetal Diagn Ther, 2013. **33**(4): p. 241-5.
15. Verbeek, L., et al., *Short-Term Postnatal Renal Function in Twin Anemia-Polycythemia Sequence.* Fetal Diagn Ther, 2016. **39**(3): p. 192-7.
16. Taniguchi, K., et al., *Twin anemia-polycythemia sequence after laser surgery for twin-twin transfusion syndrome and maternal morbidity.* Fetal Diagn Ther, 2015. **37**(2): p. 148-53.
17. Slaghekke, F., et al., *Neurodevelopmental outcome in twin anemia-polycythemia sequence after laser surgery for twin-twin transfusion syndrome.* Ultrasound Obstet Gynecol, 2014. **44**(3): p. 316-21.
18. Abdel-Sattar, M., et al., *Treatment of Complicated Spontaneous Twin Anemia-Polycythemia Sequence via Fetoscopic Laser Ablation of the Vascular Communications.* Fetal Diagn Ther, 2015. **38**(3): p. 233-7.
19. Ishii, K., et al., *Therapy by laser equatorial placental dichorionization for early-onset spontaneous twin anemia-polycythemia sequence.* Fetal Diagn Ther, 2014. **35**(1): p. 65-8.
20. Diehl, W., et al., *Twin anemia-polycythemia sequence in a case of monoamniotic twins.* Ultrasound Obstet Gynecol, 2013. **42**(1): p. 108-11.
21. Groussolles, M., et al., *Evolution of middle cerebral artery peak systolic velocity after a successful laser procedure for iatrogenic twin anemia-polycythemia sequence.* Ultrasound Obstet Gynecol, 2012. **39**(3): p. 354-6.
22. Assaf, S.A., K. Benirschke, and R.H. Chmait, *Spontaneous twin anemia-polycythemia sequence complicated by recipient placental vascular thrombosis and hydrops fetalis.* J Matern Fetal Neonatal Med, 2011. **24**(3): p. 549-52.
23. Slaghekke, F., et al., *Laser surgery as a management option for twin anemia-polycythemia sequence.* Ultrasound Obstet Gynecol, 2014. **44**(3): p. 304-10.
24. Sananes, N., et al., *Evaluation of the Utility of in utero Treatment of Twin Anemia-Polycythemia Sequence.* Fetal Diagn Ther, 2015. **38**(3): p. 170-8.
25. Xiong, T., F. Gonzalez, and D.Z. Mu, *An overview of risk factors for poor neurodevelopmental outcome associated with prematurity.* World J Pediatr, 2012. **8**(4): p. 293-300.
26. Chen, P.C., P.W. Wang, and L.J. Fang, *Prognostic predictors of neurodevelopmental outcome or mortality in very-low-birth-weight infants.* Acta Paediatr Taiwan, 2005. **46**(4): p. 196-200.
27. Rodeck, C.H., et al., *The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions.* Am J Obstet Gynecol, 1984. **150**(6): p. 769-74.

28. *An international classification of retinopathy of prematurity. The Committee for the Classification of Retinopathy of Prematurity.* Arch Ophthalmol, 1984. **102**(8): p. 1130-4.
29. Bell, M.J., et al., *Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging.* Ann Surg, 1978. **187**(1): p. 1-7.
30. Volpe, J.J., *Intraventricular hemorrhage and brain injury in the premature infant. Diagnosis, prognosis, and prevention.* Clin Perinatol, 1989. **16**(2): p. 387-411.
31. de Vries, L.S., P. Eken, and L.M. Dubowitz, *The spectrum of leukomalacia using cranial ultrasound.* Behav Brain Res, 1992. **49**(1): p. 1-6.
32. Levene, M.I., *Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound.* Arch Dis Child, 1981. **56**(12): p. 900-4.
33. Broadbent, R.S., *Recipient twin limb ischemia with postnatal onset.* J Pediatr, 2007. **150**(2): p. 207-9.
34. Rutherford, R.B., et al., *Recommended standards for reports dealing with lower extremity ischemia: revised version.* J Vasc Surg, 1997. **26**(3): p. 517-38.
35. Wiedmeier, S.E., et al., *Platelet reference ranges for neonates, defined using data from over 47,000 patients in a multihospital healthcare system.* J Perinatol, 2009. **29**(2): p. 130-6.
36. Cartlidge, P.H. and N. Rutter, *Serum albumin concentrations and oedema in the newborn.* Arch Dis Child, 1986. **61**(7): p. 657-60.
37. Reading, R.F., R. Ellis, and A. Fleetwood, *Plasma albumin and total protein in preterm babies from birth to eight weeks.* Early Hum Dev, 1990. **22**(2): p. 81-7.
38. Karunaratne, S., S. Mukherjee, and R. Ramanan, *Laser therapy for twin-to-twin transfusion syndrome causing amniotic band syndrome.* Arch Dis Child Fetal Neonatal Ed, 2011. **96**(1): p. F35.
39. Peeters, S.H., et al., *Iatrogenic perforation of intertwin membrane after laser surgery for twin-to-twin transfusion syndrome.* Ultrasound Obstet Gynecol, 2014. **44**(5): p. 550-6.
40. Caughey, A.B., J.N. Robinson, and E.R. Norwitz, *Contemporary diagnosis and management of preterm premature rupture of membranes.* Rev Obstet Gynecol, 2008. **1**(1): p. 11-22.
41. Lanna, M.M., et al., *Increased risk of placental abruption after solomon laser treatment of twin-twin transfusion syndrome.* Placenta, 2017. **53**: p. 54-56.
42. Palisano, R., et al., *Development and reliability of a system to classify gross motor function in children with cerebral palsy.* Dev Med Child Neurol, 1997. **39**(4): p. 214-23.
43. Gogate, P., C. Gilbert, and A. Zin, *Severe visual impairment and blindness in infants: causes and opportunities for control.* Middle East Afr J Ophthalmol, 2011. **18**(2): p. 109-14.
44. Kushalnagar, P., et al., *Infants and children with hearing loss need early language access.* J Clin Ethics, 2010. **21**(2): p. 143-54.
45. Burton, G.J., et al., *Optimising sample collection for placental research.* Placenta, 2014. **35**(1): p. 9-22.
46. De Lia, J.E., D.P. Cruikshank, and W.R. Keye, Jr., *Fetoscopic neodymium:YAG laser occlusion of placental vessels in severe twin-twin transfusion syndrome.* Obstet Gynecol, 1990. **75**(6): p. 1046-53.
47. Slaghekke, F., et al., *Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial.* American Journal of Obstetrics and Gynecology, 2014. **211**(3).
48. Lopriore, E., et al., *Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence.* Placenta, 2007. **28**(1): p. 47-51.
49. Slaghekke, F., et al., *Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial.* Lancet, 2014. **383**(9935): p. 2144-2151.
50. Slaghekke, F., et al., *Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial.* Am J Obstet Gynecol, 2014. **211**(3): p. 285 e1-7.
51. Mackeen, A.D., et al., *Tocolytics for preterm premature rupture of membranes.* Cochrane Database Syst Rev, 2014(2): p. CD007062.