Placental characteristics in twin-to-twin transfusion syndrome and twin anemia-polycythemia sequence

Villiers, S.F. de

Placental characteristics in twin-to-twin transfusion syndrome and twin anemia-polycythemia sequence

Suzanne F. de Villiers

ISBN 978-94-6233-405-2

Printed by: Gildeprint Drukkerijen, BV, Enschede

Cover illustration: M. Ruiter

Financial support for the publication of this thesis was provided by Afdeling Neonatologie LUMC, Afdeling Verloskunde LUMC, Piaget consulting, Dräger Nederland BV, Chiesi Pharmaceuticals BV, Isabelle von Notten

Placental characteristics in twin-to-twin transfusion syndrome and twin anemia-polycythemia sequence

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,

op gezag van
Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 13 oktober 2016
klokke 13:45 uur

door

Suzanne Francesca de Villiers
geboren te Johannesburg
in 1988

Promotores:	Prof. Dr. E. Lopriore
	Prof. Dr. D. Oepkes
Leden promotiecommissie:	Prof. Dr. J.M.M. van Lith
	Prof. Dr. L. Lewi (University Hospitals KU Leuven, België)
	Dr. D. Cohen
	Dr. A.J. Eggink (Erasmus Medical Center Rotterdam)

CONTENTS

Part I

Introduction

Chapter 1

Introduction to twin pregnancies with special attention to twin-to-twin transfusion syndrome and twin anemia-polycythemia sequence

S.F. de Villiers

Zygosity and chorionicity

Twin pregnancies occur in 1-2% of all pregnancies. Two-thirds of twin pregnancies are dizygotic and one-third are monozygotic. Dizygotic twins result from the fertilization of two different egg cells by two different sperm cells. Dizygotic twins or non-identical twins have the same genetic similarities as any other two siblings would have.[1] Dizygotic pregnancies are implicitly dichorionic (DC) and diamniotic (DA) which means there are two functionally separate placentas and two individual amniotic sacs.

Monozygotic twins or identical twins develop from a single egg fertilized by a single sperm cell. The zygote divides after fertilization. In monozygotic twins, the chorionicity is determined by the time interval between fertilization and division of the zygote and can be either DC or monochorionic (MC), with a shared placenta. The time interval of the division of the zygote in MC gestations also determines whether the embryos will have individual amniotic sacs (diamniotic) or share a single amniotic sac (monoamniotic (MA)).[2] DC-DA gestations occur in 25% of monozygotic gestations, MC-DA in 75% and MC-MA in less than 1%.[3]

The mortality and morbidity of twin pregnancies is 3-7 times higher than singleton pregnancies. This is mainly owing to prematurity, fetal growth restriction and both structural and chromosomal anomalies. However, the chorionicity also affects mortality and morbidity. Mortality in MC pregnancies is twice as high as DC pregnancies and four times higher than singleton pregnancies.[4] MC gestations have unique complications as a result of their shared placenta which will be discussed in more detail as well as other complications related to possible monoamnionicity, e.g. conjoined twins and umbilical cord entanglement.[4]

Placental angioarchitecture

Placental injection studies have shown that vascular anastomoses between the fetal circulations are ubiquitous in MC placentas, but occur extremely infrequently in DC placentas.[5]

There are three types of placental anastomoses: arterio-arterial (AA), veno-venous (VV) and arterio-venous (AV) anastomoses. AA and VV anastomoses are superficial, bidirectional and have a low resistance. They form direct connections between the two fetal circulations. The direction of flow can be in either direction and is dependent on the interfetal pressure gradient/difference.[6] After placental injection studies they can be seen as direct vascular connections running on the surface of the chorionic plate. In contrast, AV anastomoses are deep, unidirectional and have a high resistance. AV anastomoses consist of an artery from one twin and the vein of the other twin that are connected by a capillary network in a shared cotyledon below the chorionic plate. They can be visualized as a supplying artery and a draining vein that pierce the chorionic plate in close proximity

to each other.[6;7] As a result of the unidirectional flow in AV-anastomoses an imbalance in the net transfusion of blood can occur.

Vascular anastomoses and complications in monochorionic gestations

Placental vascular anastomoses are the basis for the development of various complications associated with MC gestations including chronic twin-to-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS). TTTS and TAPS will be discussed in more detail here below. Others complications in MC gestations include acute perinatal TTTS, acute perimortem TTTS, twin reversed arterial perfusion sequence (TRAP) and selective intrauterine growth restriction (sIUGR).[8;9]

Acute perinatal TTTS is described anecdotally as acute transfusion during birth through superficial AA or VV anastomoses, however there is very little data on it. This feto-fetal transfusion is possibly caused by uterine contractions or fetal position which influence fetal blood pressure.[7;9] This is not to be confused with placento-fetal transfusion which can occur at birth after clamping the umbilical cord of the first twin. This results in the second twin receiving blood not only from its own part of the placenta, but also from the placenta share of the co-twin. Hemoglobin levels are often higher in the second twin born of MC gestations due to placenta-fetal transfusion.[10]

Acute perimortem TTTS occurs after the intrauterine death of one twin. Transfusion or exsanguination takes place from the surviving twin through placental vascular anastomoses to the low pressure circulation of the dead twin and can lead to (double) fetal demise or hypoxic-ischemic damage of the surviving twin. It is believed to occur through large diameter, bidirectional AA or VV anastomoses.[9;11]

TRAP sequence, also known as ‘acardiac twinning’, is a rare complication of MC gestations. One twin is acardiac and as the name suggests has no functional heart and may have other severe malformations. The co-twin is known as the pump twin and has no malformations. The pump twin perfuses the acardiac twin by pumping blood trough both fetal circulations. The pump twin is at risk for congestive heart failure. This extreme form of feto-fetal transfusion only occurs in 1 of 35 000 gestations and in <1% of MC gestations.[12;13]

sIUGR and/or birth weight discordance (BWD) are important complications of MC gestations. sIUGR is defined as an estimated fetal weight of one fetus below the 10th centile. BWD is defined as a discrepancy in the birth weights of more than 25%. sIUGR and BWD are strongly associated with each other and result from unequal placental sharing. The growth restricted fetus often has a small placental share and a velamentous cord insertion, while the larger fetus has a larger placental share and a para-central cord insertion.[6;14]

TWIN-TO-TWIN TRANSFUSION SYNDROME (TTTS)

Usually blood flow across placental anastomoses is balanced. However, in TTTS there is chronic and unbalanced feto-fetal transfusion from the donor-twin to the recipient-twin. Although placental anastomoses are ubiquitous in MC placentas, TTTS develops only in 9-15%.[9;15] Clinical symptoms usually occur during the second or third trimester because of severe polyhydramnios of the recipient and can include maternal discomfort, preterm prelabor rupture of membranes (PPROM), or premature labor.[3;9;16]

Placental angioarchitecture and placental characteristics

The pathophysiology of TTTS is described as resulting from a net imbalance of blood flow between the fetuses through communicating placental anastomoses. TTTS only develops in the presence of unidirectional AV-anastomoses when blood from one twin (the donor) is pumped through the artery to the shared cotyledon and then drains through a vein to the other twin (the recipient).[8;9] TTTS develops therefore in the presence of at least one AV-anastomosis, unless blood is pumped back to the donor through another AV-anastomosis in the opposite direction or through a bidirectional AA or VV anastomosis. The unbalanced blood flow causes the donor to become progressively hypovolemic, often growth restricted and oliguric, which causes oligohydramnios in the amniotic sac. In contrast the recipient becomes progressively hypervolemic and polyuric, congestive heart failure can develop as a result of the volume overload. Polyhydramnios develops in the recipient's amniotic sac.

AA anastomoses are thought to be protective against the development of TTTS. Bidirectional anastomoses can therefore theoretically compensate for unbalanced blood flow through an AV anastomosis.[3;9] A mathematical computer model showed that AA anastomoses compensate better for the hemodynamic imbalance in TTTS than contra directional AV anastomoses because of the lower resistance over AA anastomoses.[17] Previously, not much attention has been paid to the possible role VV anastomoses may have in the development of transfusion syndromes in MC gestations.

Unequal placental sharing and the incidence of velamentous cord insertions in TTTS placentas corresponds with those found in normal monochorionic placentas. In TTTS placentas, in a significant majority of cases, the donor twin has a velamentous cord insertion and a smaller placental territory.[18] Whether this has any bearing on the development of TTTS still needs to be elucidated.

Other pathophysiological mechanisms

Other pathophysiological theories have been proposed because intertwin hemoglobin differences are not always large which might disprove pathophysiological theories based solely on placental angioarchitecture, although placental anastomoses are undeniably

a prerequisite for the development of TTTS.[9] These theories include utero-placental insufficiency and activation of vasoactive and hormonal factors, including insulin-like growth factor (IGF)-II, leptin, endothelin-1 and the renin-angiotensin system.[6;9;16] When related to the expected blood pressure for birth weight, donors had a lower than expected blood pressure and recipients a higher.[19]

Diagnosis

Chorionicity is determined by antenatal ultrasound in the first trimester. Other signs of monochorionicity include a single placental mass and fetuses of the same sex. TTTS is diagnosed by twin oligo-polyhydramnios (TOPS) seen on prenatal ultrasound. It can occur at any time during the pregnancy, but is most common in the second trimester. Severe oligohydramnios or anhydramnios is defined as the deepest vertical amniotic fluid pool < 2 cm and polyhydramnios is described as the deepest amniotic fluid pool > 8cm.[3] Furthermore, signs of oligo- or anuria can be seen in the donor as a small or empty bladder and the donor may be `stuck` to the uterine wall with the amniotic membrane is tightly wrapped around it. This can be incorrectly interpreted as a MA gestation if careful attention is not paid to the reduction of movement of the fetus as is the case in a DA pregnancy with a stuck twin. Conversely, the signs of polyuria can be seen in the recipient as an extended bladder. In severe cases, Doppler investigations can show signs of congestive heart failure in the recipient as a result of hypervolemia. Sonographically, growth discrepancy may be noted with the recipient being the larger twin.

Staging: Quintero

Quintero et al. devised a staging system with which to categorize TTTS based on antenatal ultrasound criteria. Stage I is the mildest form of TTTS with oligohydramnios of the donor (deepest pocket < 2cm) with the bladder still visible and polyhydramnios of the recipient (deepest pocket > 8cm). Stage II describes a `stuck` twin and the bladder of the donor is not visible. In stage III there are severely abnormal Doppler flow patterns with absent or reverse end-diastolic flow in the umbilical artery of the donor and/or venous abnormalities in the recipient with reverse flow in the ductus venosus or pulsatile umbilical venous flow or tricuspid regurgitation. Stage IV shows fetal hydrops and stage V is described as death of one or both twins.[20]

Treatment:

The two main therapeutic options for TTTS are amniodrainage and fetoscopic laser surgery.

Serial amniodrainage is a relatively simple, symptomatic treatment and was the standard care before laser coagulation was developed.[3] Depending on the gestational age it can be used to prolong the pregnancy and postpone premature birth. It reduces

the polyhydramnios and the amniotic fluid pressure on the uterus and also improves the placental perfusion which in turn improves the condition of the fetus. The risk of complications is small, however it would seem that serial amniodrainage is only effective for MC twin gestations with mild signs of TTTS. Usually the procedure needs to be repeated a number of times as amniotic fluid reaccumulates.[6;9]

In contrast to amniodrainage, fetoscopic laser coagulation addresses the cause of TTTS by coagulating the placental anastomoses, stopping intertwin transfusion and creating two functionally separate placentas. The most frequently occurring complication is PPROM in approximately 10% of procedures, other complications occur infrequently. Fetoscopic laser coagulation is the preferred treatment before 26 weeks gestation.[9] The aim of the treatment is to completely separate the two fetal circulations, however, in our center, about a quarter to a third of lasered placentas had residual anastomoses. Most residual anastomoses were located near the edge of the placenta and were very small. However, residual anastomoses can lead to a number of complications including reversed TTTS or TAPS.[21;22]

Studies differ on whether laser coagulation improves overall survival rates, but it does reduce the risk of neurological morbidity. For this reason, laser coagulation should be considered in all TTTS cases.[6;23] The Leiden University Medical Centre (LUMC) is the national referral center in The Netherlands for complicated monochorionic gestations whe fetoscopic laser coagulation is the preferred treatment option for TTTS.

Table 1. Diagnostic criteria of TTTS and TAPS

TTTS	TAPS
Antenatal criteria Confirmed MC gestation *and* Oligohydramnios or anhydramnios in the donor with as the deepest vertical amniotic fluid pool < 2 cm *and* Polyhydramnios in the recipient with the deepest amniotic fluid pool > 8cm *and* Discordant fetal bladders with a small or empty bladder in the donor and a large bladder in the recipient	**Antenatal criteria** Confirmed MC gestation *and* Middle cerebral artery peak systolic velocity (MCA-PSV) > 1.5 multiples of the mean (MoM) in the donor *and* MCA-PSV < 1.0 MoM in the recipient **Postnatal criteria** Intertwin Hb difference > 8.0 g/dl *and* at least one of the following: • Reticulocyte count ratio > 1.7 • Placenta with only small vascular anastomoses (diameter < 1 mm)

TWIN ANEMIA-POLYCYTHEMIA SEQUENCE (TAPS)

TAPS is a form of chronic feto-fetal transfusion and is defined as a discordant hemoglobin level at birth as a result of chronic intertwin transfusion without signs of twin oligo-polyhydramnios sequence (TOPS) as seen in TTTS. It has been recently described in 2007 and can occur spontaneously or iatrogenically after laser coagulation of TTTS.

[9;24;25] Interestingly, in iatrogenic TAPS, it is usually the former TTTS recipient who becomes anemic and vice versa, the former TTTS donor who becomes polycythemic. The incidence of spontaneous TAPS has been estimated to be about 3-5%[15;26] and iatrogenic TAPS occurs in up to 2-13% of cases after laser coagulation.[27;28]

A new technique called the Solomon technique has been proposed to decrease the number of residual anastomoses. When the Solomon technique is practiced, anastomoses are identified and lasered, hereafter a line is lasered along the vascular equator. This technique shows a significant reduction in the number of post-laser TAPS cases, as well as a reduction in the recurrence of TTTS [29].

Pathophysiology

The maternal side of the placenta can be distinguished by the pale donor side and the plethoric recipient side of the placenta, as is seen in the anemic and polycythemic neonate. TAPS is caused by miniscule AV anastomoses. It is postulated that less volume and a much slower blood flow occurs in TAPS than in TTTS. This would potentially allow more time for hemodynamic compensation to take place.[9] A few additional reasons may possibly explain why inter-twin fetal transfusion in TAPS does not lead to amniotic fluid imbalance. The renin angiotensin system may play a role in achieving euvolemia and therefore the absence of oligo- and polyhydramnios. Another explanation is that TOPS had not yet developed in the progression of TTTS.[30]

Diagnosis

TAPS can be diagnosed antenatally or postnatally. Antenatally, Doppler ultrasound criteria include an increased middle cerebral artery peak systolic velocity (MCA-PSV) > 1.5 multiples of the mean (MoM) in one fetus with a simultaneous decrease in the MCA-PSV < 1 MoM in the other fetus.[27] Postnatal diagnosis of TAPS includes anemia in the donor and polycythemia in the recipient as well as reticulocytosis in the donor as a sign of chronic anemia. In 2014 diagnostic cutoff criteria were proposed with an intertwin hemoglobin difference > 8.0 g/dl and an intertwin reticulocyte count ratio donor/recipient > 1.7. The detection of minuscule AV anastomoses in placental injection is supportive of the diagnosis.[9;26]

Treatment

As with TTTS there are a number of treatment options which need to be considered, including induced labor, intrauterine transfusion (IUT) in the donor with or without partial exchange transfusion (PET) in the recipient and (repeat) laser coagulation of the vascular anastomoses.

The option of induced labor depends on the duration of gestation. The advantages of stopping the feto-fetal transfusion need to be weighed up against the disadvantages of preterm delivery.

Intrauterine transfusion is a symptomatic treatment for the anemic donor, but possibly deleterious to the recipient. However, depending on the placental angioarchitecture, one runs the risk of iatrogenically increasing the recipient's polycythemia and all risks associated with it. A computer model has shown that potentially combining this with PET in the recipient may minimize these risks. However further study is needed to verify this theory. [33]

As in the treatment of TTTS, laser coagulation remains the only causal therapy. Repeat laser coagulation in iatrogenic TAPS is technically more difficult than it is for TTTS as there is less amniotic fluid (no polyhydramnios) and the uterine wall is less tense making the visualization of anastomoses more difficult. Until recently the preference has been to avoid invasive procedures (IUT and laser coagulation) as the complications associated with preterm delivery outweigh the benefits of the procedures.[9] However, new studies indicate that laser coagulation may prolong the pregnancy, thereby improving survival and neonatal outcome.[32]

Outcome

The outcome of TAPS have not been well studied. There is only limited long-term data available. The short-term data that is available shows a low mortality and low neurological morbidity.[33;34]

PLACENTAL STUDIES IN TTTS AND TAPS

In the past two decades, major improvements in diagnosis and treatment of complicated MC pregnancies have led to improved perinatal outcome in MC twins. Placental studies with colored dye injection have played a crucial role in the understanding of the pathophysiology of the various complications and the detection of new disorders in MC twins. In addition, placental injection studies play a crucial role in the evaluation of fetoscopic laser coagulation in TTTS and TAPS. Injection studies with color dye allow accurate detection of residual anastomoses and give invaluable feedback information to the fetal surgeons in regard to the success or completeness of the laser coagulation intervention.

Placental injection studies should therefore be regarded as standard postnatal evaluation in all complicated MC twin pregnancies.

Nevertheless, various severe complications in MC twin pregnancies still pose major diagnostic and therapeutic challenges for fetal surgeons and the outcome in these complicated MC pregnancies is far from optimal. To continue improving our understanding of the

development of various complications in MC twins, routine injection of all MC placentas is of paramount importance.

The exact role of the size, number and type of anastomoses (particularly AA and VV anastomoses) in the development of TTTS and TAPS is still not fully understood. In addition, other etiological placental factors such as placental sharing and type of umbilical cord insertion also require further investigation.

The objective of our studies is to investigate several aspects of the placental angio-architecture in TTTS and TAPS cases to enhance our understanding in the development of these disorders.

OUTLINE OF THIS THESIS

PART I – Introduction

Chapter 1 – General introduction

Chapter 2 – Review on the literature on injection technique and analysis of MC twin placentas

PART II – TTTS

Chapter 3 – Study on the velamentous cord insertion in MC twins with TTTS compared to a control group of MC placentas without TTTS.

Chapter 4 – Study on arterio-arterial vascular anastomoses in MC placentas with TTTS compared to a control group of MC placentas without TTTS.

Chapter 5 – Study on veno-venous anastomoses in in MC twins with and without TTTS with special attention to the perinatal mortality.

PART III – TAPS

Chapter 6 – Study on arterio-arterial vascular anastomoses in MC placentas with spontaneous TAPS compared to a control group of MC placentas without TAPS.

Chapter 7 – Study on the placental characteristics in MC twins with spontaneous versus post-laser TAPS.

PART IV – Discussion and Summary

Chapter 8 – General discussion of the most important findings of this thesis and future perspectives.

REFERENCE LIST

[1] Hollenbach KA, Hickok DE. Epidemiology and diagnosis of twin gestation. Clin Obstet Gynecol 1990; 33(1):3-9.

[2] Lewi L, Gucciardo L, Van Mieghem T, de Koninck P, Beck V, Medek H et al. Monochorionic Diamniotic Twin Pregnancies: Natural History and Risk Stratification. Fetal Diagnosis and Therapy 2010; 27(3):121-133.

[3] Huber A, Hecher K. How can we diagnose and manage twinΓÇôtwin transfusion syndrome? Best Practice & Research Clinical Obstetrics & Gynaecology 2004; 18(4):543-556.

[4] Sherer DM. Adverse perinatal outcome of twin pregnancies according to chorionicity: review of the literature. Am J Perinatol 2001; 18(1):23-37.

[5] Robertson EG, Neer KJ. Placental injection studies in twin gestation. Am J Obstet Gynecol 1983; 147(2):170-174.

[6] Lewi L, Schoubroeck DV, Gratac+¦s E, Witters I, Timmerman D, Deprest J. Monochorionic diamniotic twins: complications and management options. Current Opinion in Obstetrics and Gynecology 2003; 15(2).

[7] Nikkels PG, Hack KE, van Gemert MJ. Pathology of twin placentas with special attention to monochorionic twin placentas. J Clin Pathol 2008; 61(12):1247-1253.

[8] Benirschke K. The placenta in twin gestation. Clin Obstet Gynecol 1990; 33(1):18-31.

[9] Lopriore E, Oepkes D. Fetal and neonatal haematological complications in monochorionic twins. Seminars in Fetal and Neonatal Medicine 2008; 13(4):231-238.

[10] Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Vandenbussche FP, Walther FJ. Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses. J Pediatr 2005; 147(5):597-602.

[11] Fusi L, McParland P, Fisk N, Nicolini U, Wigglesworth J. Acute twin-twin transfusion: a possible mechanism for brain-damaged survivors after intrauterine death of a monochorionic twin. Obstet Gynecol 1991; 78(3 Pt 2):517-520.

[12] Chalouhi GE, Stirnemann JJ, Salomon LJ, Essaoui M, Quibel T, Ville Y. Specific complications of monochorionic twin pregnancies: twinΓÇôtwin transfusion syndrome and twin reversed arterial perfusion sequence. Seminars in Fetal and Neonatal Medicine 2010; 15(6):349-356.

[13] Bornstein E, Monteagudo A, Dong R, Schwartz N, Timor-Tritsch IE. Detection of Twin Reversed Arterial Perfusion Sequence at the Time of First-Trimester Screening: The Added Value of 3-Dimensional Volume and Color Doppler Sonography. Journal of Ultrasound in Medicine 2008; 27(7):1105-1109.

[14] Lewi L, Cannie M, Blickstein I, Jani J, Huber A, Hecher K et al. Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. American Journal of Obstetrics and Gynecology 2007; 197(6):587.

[15] Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T et al. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. American Journal of Obstetrics and Gynecology 2008; 199(5):514.

[16] Lopriore E, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Twin-to twin transfusion syndrome: from placental anastomoses to long-term neurodevelopmental outcome. Curr Pediatr Review 2005; 15:177-194.

[17] Umur A, van Gemert MJC, Nikkels PGJ, Ross MG. Monochorionic Twins and TwinΓÇôTwin Transfusion Syndrome: The Protective Role of Arterio-arterial Anastomoses. Placenta 2002; 23(2ΓÇô3):201-209.

[18] Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Walther FJ, Vandenbussche FPHA. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. American Journal of Obstetrics and Gynecology 2007; 196(2):159.

[19] Mercanti I, Boivin A, Wo B, Vlieghe V, Le RC, Audibert F et al. Blood pressures in newborns with twin-twin transfusion syndrome. J Perinatol 2011; 31(6):417-424.

[20] Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. J Perinatol 1999; 19(8 Pt 1):550-555.

[21] Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ, Vandenbussche FPHA. Residual Anastomoses After Fetoscopic Laser Surgery in Twin-to-Twin Transfusion Syndrome: Frequency, Associated Risks and Outcome. Placenta 2007; 28(2ΓÇô3):204-208.
[22] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. American Journal of Obstetrics and Gynecology 2009; 201(1):66.
[23] Roberts D, Neilson JP, Kilby MD, Gates S. Interventions for the treatment of twin-twin transfusion syndrome. Cochrane Database Syst Rev 2014; 1:CD002073.
[24] Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FPHA. Twin AnemiaΓÇôPolycythemia Sequence in Two Monochorionic Twin Pairs Without Oligo-Polyhydramnios Sequence. Placenta 2007; 28(1):47-51.
[25] Lopriore E, van den Wijngaard JP, Middeldorp JM, Oepkes D, Walther FJ, van Gemert MJ et al. Assessment of feto-fetal transfusion flow through placental arterio-venous anastomoses in a unique case of twin-to-twin transfusion syndrome. Placenta 2007; 28(2-3):209-211.
[26] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ et al. Twin Anemia-Polycythemia Sequence: Diagnostic Criteria, Classification, Perinatal Management and Outcome. Fetal Diagnosis and Therapy 2010; 27(4):181-190.
[27] Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J et al. Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. American Journal of Obstetrics and Gynecology 2006; 194(3):796-803.
[28] Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R et al. Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009; 201(4):417.
[29] Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS 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):2144-2151.
[30] van den Wijngaard JP, Lewi L, Lopriore E, Robyr R, Middeldorp JM, Vandenbussche FP et al. Modeling severely discordant hematocrits and normal amniotic fluids after incomplete laser therapy in twin-to-twin transfusion syndrome. Placenta 2007; 28(7):611-615.
[31] Slaghekke F, van den Wijngaard JP, Akkermans J, van Gemert MJ, Middeldorp JM, Klumper FJ et al. Intrauterine transfusion combined with partial exchange transfusion for twin anemia polycythemia sequence: Modeling a novel technique. Placenta 2015.
[32] Slaghekke F, Favre R, Peeters SH, Middeldorp JM, Weingertner AS, van Zwet EW et al. Laser surgery as a management option for twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2014; 44(3):304-310.
[33] Slaghekke F, van Klink JM, Koopman HM, Middeldorp JM, Oepkes D, Lopriore E. Neurodevelopmental outcome in twin anemia-polycythemia sequence after laser surgery for twin-twin transfusion syndrome. Ultrasound Obstet Gynecol 2014; 44(3):316-321.
[34] Lopriore E, Slaghekke F, Kersbergen KJ, de Vries LS, Drogtrop AP, Middeldorp JM et al. Severe cerebral injury in a recipient with twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2013; 41(6):702-706.

Part II

TTTS

Chapter 2

Monochorionic twin placentas: Injection technique and analysis

S.F. de Villiers

D.P. Zhao

D. Oepkes

E. Lopriore

Diagnostico Prenatal 2014, vol. 25(2) pp. 35–42

ABSTRACT

Careful placenta examination and injection studies are crucial to understand the differences between the various complications in monochorionic (MC) pregnancies. In this review, we will first describe an accurate and simple method of placental injection and then discuss the placental characteristics of normal MC, twin–twin transfusion syndrome (TTTS), twin anemia-polycythemia sequence (TAPS), selective intrauterine growth restriction (sIUGR), monoamniotic (MA) and other special cases.

INTRODUCTION

Twin pregnancies can be classified into two different groups: monochorionic (MC) and dichorionic. MC twins have a 3 to 6-fold increased risk of adverse perinatal outcome.[1, 2] Adverse outcome in MC twinning is due to complications associated with the presence of placental vascular anastomoses. Vascular anastomoses connecting the circulation of the twins are ubiquitous in MC placentas but are extremely rare in dichorionic placentas. These placental vascular anastomoses may lead to several complications including twin-twin transfusion syndrome (TTTS), spontaneous twin anemia-polycythemia sequence (TAPS), selective intrauterine growth restriction (sIUGR) and twin reversed arterial perfusion (TRAP).[2-6] Imbalance of volume of blood flow through the vascular anastomoses may cause hypovolemia and/or anemia in one twin (donor) and hypervolemia and/or polycythemia in the co-twin (recipient). In addition, MC twins may also be monoamniotic (MA) which may lead to complications such as cord entanglement and double fetal demise.[7]

Careful placenta examination and injection studies are crucial to understand the differences between the various complications in MC pregnancies. In this review, we will first describe an accurate and simple method of placental injection and then discuss the placental characteristics of normal MC, TTTS, TAPS, sIUGR, MA and other special cases.

A. Dye-colored injection of MC placentas

All MC placentas should be routinely examined and injected after birth in order to understand the pathogenesis of the various complications. In addition, in TTTS placentas treated with fetoscopic laser coagulation, injection studies are of paramount importance to evaluate the accuracy and completeness of laser surgery, an important tool for laser therapy specialists. A detailed protocol for placental injection used at our center is reported here below and can be viewed using the following links: http://www.youtube.com/watch?v=Qm4bdLkl9BE).[8]

1. Preparation of the placenta after delivery

Use clamps to label the umbilical cords of the twins with one for the first-born or two for the second-born. Then, inspect the maternal and fetal surface of the placenta for completeness or disruption and record the following data: type of cord insertion (central, eccentric, marginal or velamentous), number of blood vessels in the umbilical cord (usually one vein and two arteries, sometimes only one artery) and color difference between both placental shares. A section of the dividing membranes can be sent to Pathology to confirm the type of chorionicity. The placenta can then be placed in a plastic bowl and refrigerated until the final examination (best within one week) and color dye injection. The placenta must not be frozen or fixed (do not use formalin).

2. Catheterization of the umbilical vessels

Wash the placenta with warm water or saline, trim the peripheral membrane, remove the inter-twin dividing membrane and peel off the amnions (for better visualization of the vascular anastomoses and better quality of the placental pictures). Transect each umbilical cord at approximately 5 cm distance from the cord insertion and gently squeeze out blood clots from the umbilical vessels and placental vessels. Then, cannulate the umbilical cord vessels. Cannulate the umbilical vein with an appropriately sized catheter, avoiding false passages. Cannulate one of the two umbilical arteries with a smaller catheter using tweezers to widen the lumen of the artery. Only one of the 2 umbilical arteries needs to be catheterized since an anastomosis (of Hyrtl) connects the 2 arteries near the cord insertion. Cannulation of the vessels of the other cord is same. Placement of the catheter can be facilitated by gentle back and forth massaging of the umbilical vessels. Any type of catheter can be used for this procedure. We choose to use (and recycle) the catheters used at our neonatology ward for umbilical catheterization in neonates. Tie a piece of tape around both cords to avoid back flow of the colored dye during dye injection.

3. Injection with colored dye

Connect a 20 ml syringe filled with colored dye to each catheter. Any viscous colored dye can be used to visualize the placental angio-architecture. Use contrasting colors to allow good visualization of the anastomoses (dark colors for the arteries, bright colors for the veins). Gently inject (with low pressure) the colored dye in the vein while an assistant gently pushes the dye to allow the colored dye to fill all placental vessels, also the smallest ones. Pay particular attention to the small vessels near the vascular equator (the vascular equator is the place where the anastomoses from either twin connect with each other). Repeat the previous steps to inject colored dye into the artery. Of note: arteries may be more difficult to inject and require more patience. Repeat above steps for the other umbilical cord.

4. Evaluation and documentation of the placenta after colored dye injection

Carefully examine the vascular equator and record the number and types of anastomoses. Place a measuring tape on the placenta to measure the diameters and placental shares on the digital picture. Use a high-resolution digital camera and take pictures of the injected placenta. Make sure that the pictures are taken perpendicular to the placenta.

Vascular anastomoses include 3 types: arterioarterial (AA), venovenous (VV) and arteriovenous (AV) anastomoses. The first two types are superficial with bidirectional blood flow and directly linking the arteries and veins of two umbilical cords, while AV anastomoses form at a deep capillary level within shared cotyledons and allow only unidirectional blood flow. Of note, color dye injected in AA and VV anastomoses mixes and crosses the vascular equator, whereas color dye in AV or VA anastomoses does not mix and does not cross over the vascular equator.

B. Differences between the various types of MC placentas

Between June 2002 and January 2013 a total of 654 MC placentas were examined at our center. We were not able to inject 46 placentas due to damage caused by maceration or destruction (n=41) or formaline (n=5). The results of the 608 injected placentas are summarized in Table 1 showing the differences in angio-architecture between the various subtypes. A detailed description of the differences between each subtype of MC placentas is reported here below.

Table 1 Placental characteristics of MC placentas of various types

	Normal MC (n=178)	TTTS without laser (n=47)	TAPS (n=22)	sIUGR (n=73)	MA (n=18)	P_1 Value	P_2 Value	P_3 Value	P_4 Value
Overall no. of anastomoses	8.3±5.2	9.2±7.8	3.8±2.2	9.4±5.6	8.5±5.6	0.787	0.000	0.129	0.859
Placentas with AA anastomoses - n (%)	160(90)	22(47)	3(14)	73(100)	41(98)	0.000	0.000	0.002	0.410
Placentas with VV anastomoses - n (%)	45(22)	15(32)	0	17(23)	18(43)	0.360	—	0.739	0.031
Placental sharing discordance -%	28.3±17.6	30.9±18.1	31.7±19.8	54.8±19.5	25.8±23.8	0.431	0.427	0.000	0.711
Velamentous cord insertion* -n (%)	75(21)	28(30)	7(16)	48(33)	3(4)	0.073	0.421	0.005	0.000

Data are shown as mean ± SD.
P_1: normal MC vs TTTS without laser; P_2: normal MC vs TAPS; P_3: normal MC vs sIUGR; P_4: normal MC vs MA.
*refers to the type of cord insertion per fetus

B.1. Normal MC placenta

Although vascular anastomoses are always present in MC placentas, most MC pregnancies proceed well without complications, suggesting a balance in inter-twin blood exchange.

The mean number of vascular anastomoses in normal MC placentas varies in different studies from 2 to 7.[9-12] This may be attributed to different techniques of placenta examination: from detection with the naked eye to colored dye injection with milk, water or color dye. We routinely inject MC placentas with color dye and injected to date a total of 178 normal MC placentas (**Figure 1**). The mean number of anastomoses per placenta was 8.3±5.2. The prevalence of AV (and VA) anastomoses, AA anastomoses and VV anastomoses was 99% (176/178), 90% (160/178) and 25% (45/178), respectively. The high rate of AA anastomoses is typical of normal MC placentas. AA anastomoses are thought to prevent the development of various complications due to compensation through bidirectional blood flow. [13]The role of VV anastomoses is not clear and remains to be elucidated. [9]

In normal MC twins, placental sharing discordance is usually small.[14] The rate of velamentous cord insertion is approximately 21%, which is higher than in singleton placentas (2%) or dichorionic placentas (7%).[15] The results of sharing discordance and type of cord insertion in normal MC placentas in our cohort are shown in Table 1.

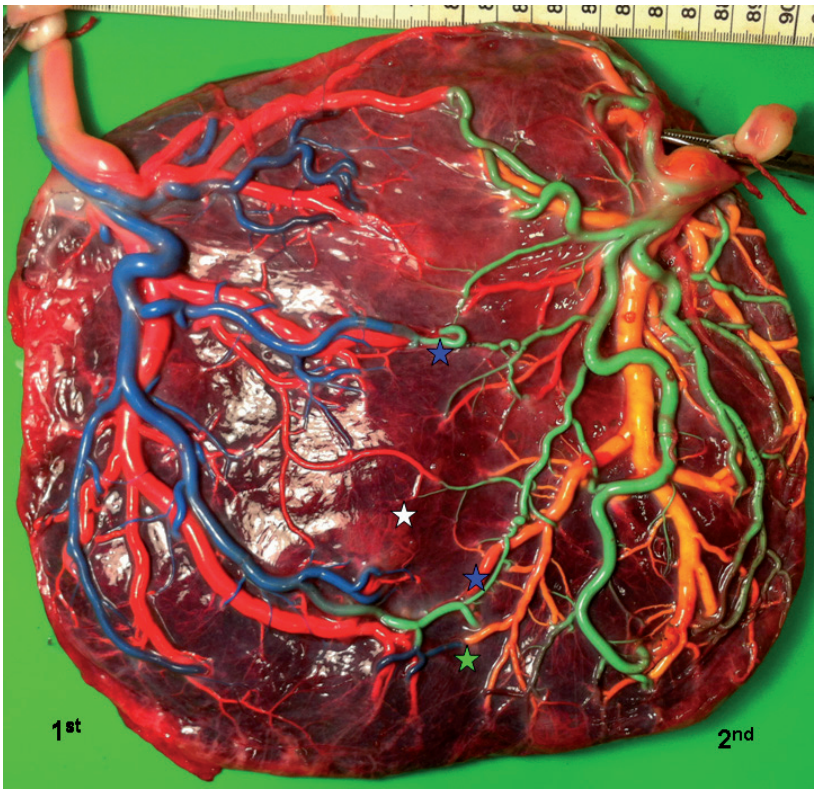

Figure 1: Normal MC placenta (gestational age at delivery: 28 weeks) showing several AV and VA anastomoses (green and white stars, respectively) and 2 AA anastomoses (blue stars).

B.2. TTTS placentas (with and without laser treatment)

TTTS is the most severe complication in MC twin pregnancies and develops in about 10% of MC pregnancies.[4] The diagnosis of TTTS is based on ultrasound signs: oligohydramnios (deepest vertical pocket ≤ 2cm) present in the sac of one twin with a collapsed bladder (the donor) and polyhydramnios (deepest vertical pocket ≥ 8cm) present in the sac of the other twin with a distended bladder (the recipient).[16]

The development of TTTS is mainly attributed to imbalanced blood flow between donor and recipient and hormonal imbalance leading to twin oligo-polyhydramnios sequence (TOPS).[17-19] TTTS may be treated with serial amnioreduction or fetoscopic laser coagulation of the vascular anastomoses. Randomized controlled trials and systematic reviews of the literature have shown that laser surgery is the optimal treatment for TTTS. [20, 21] Laser surgery was introduced as the treatment of choice at our center in August 2000.

B.2.1.TTTS placentas treated without laser: We injected a total of 41 TTTS placentas not treated with laser (**Figure 2**). The mean number of anastomoses per placentas was 9.2±7.8. The prevalence of AV (and VA) anastomoses, AA anastomoses and VV anastomoses was 96% (45/47), 47% (22/47) and 32% (15/47). The mean number of

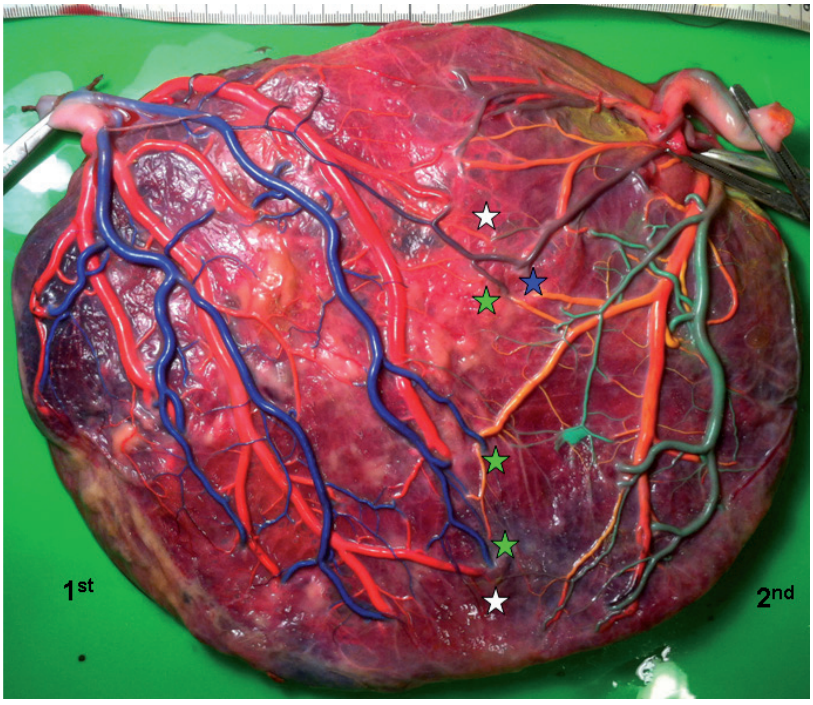

Figure 2: TTTS placenta treated with amnioreduction (gestational age at delivery: 33 weeks) showing several AV anastomoses (green stars) and VA anastomoses (white stars) and 1 AA anastomosis (blue star).

anastomoses in TTTS is similar to that of normal MC placentas. In TTTS placentas, however, the net blood flow and/or number of AV anastomoses are not balanced, which causes the transfusion from donor to recipient. The low rate of AA anastomoses is typical of TTTS placentas.[9] The absence of AA anastomoses is thought to lead to insufficient compensation of blood loss of the donor twin and promote the chronic inter-twin polyhydramnios-oligohydramnios sequence.

The placental sharing discordance and incidence of velamentous cord insertion is similar between TTTS and normal MC placentas.[22]The results of sharing discordance and type of cord insertion in TTTS placentas in our cohort are shown in Table 1.

The velamentous insertion in TTTS usually belongs to the donor twin.[22] The exact role of placental sharing discordance and velamentous cord insertion in the development of TTTS is controversial and requires further study.

B.2.3. TTTS treated with laser: We injected a total of 270 TTTS placentas treated with laser (**Figure 3**). In TTTS placentas treated with fetoscopic laser surgery, injection studies are useful in determining the presence of residual anastomoses, which can be associated with recurrent TTTS and post-laser TAPS.[23, 24] Residual anastomoses may thus be difficult to visualize with the naked eye and can only be evaluated using careful injec-

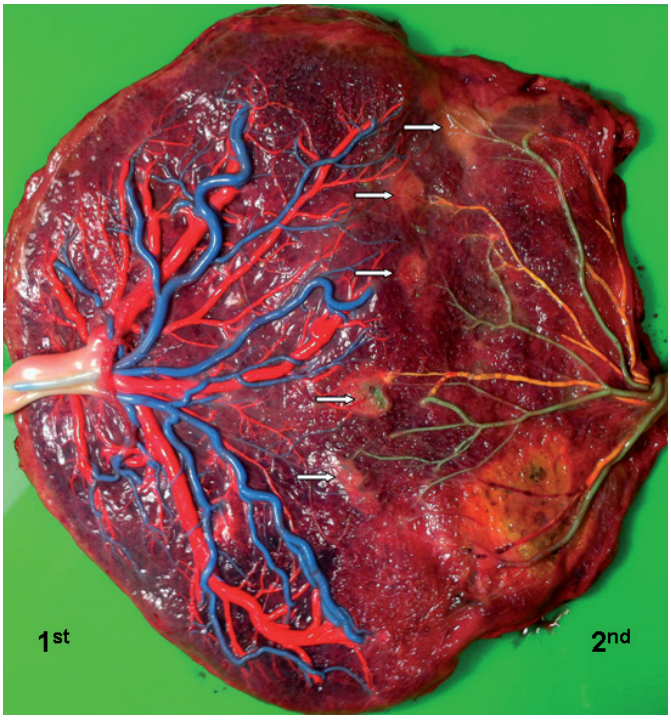

Figure 3: TTTS placenta treated with laser (gestational age at delivery: 29 weeks) with 5 laser spots (white arrows). No residual anastomoses were found.

tion with color dye. Most residual anastomoses are extremely small and localized near the placental margin.[24] The reported rate of residual anastomoses in several studies varies from 4 to 33%.[24-28] Discordances in rates of residual anastomoses may be due to different injection technique with varying accuracy as well as differences in laser technique. Recently, we conducted a randomized trial (Solomon trial; www.trialregister.nl, trial ID: NTR1245)) aimed at reducing the rate of residual anastomoses. In this trial, a coagulation line is drawn with the laser beam across the entire vascular equator from one placental margin to the other, instead of coagulating only the visible anastomoses (**Figure 4**). Hypothetically, this technique may be more effective in coagulating all vascular anastomoses, in particular the very small anastomoses which may be difficult to identify during fetoscopy. The results of this study are currently being evaluated.

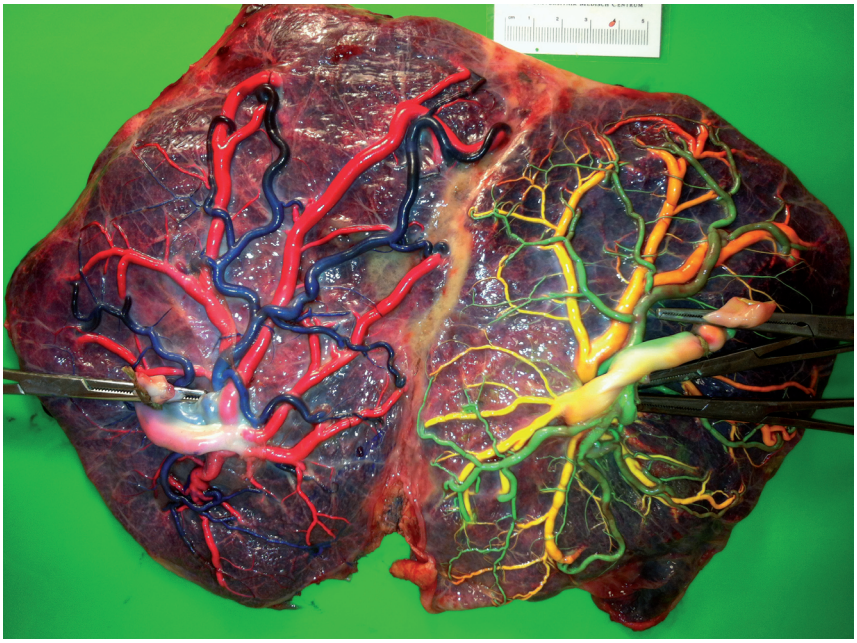

Figure 4: TTTS placenta treated with laser using the Solomon technique (gestational age at delivery: 37 weeks). No residual anastomoses were found. Note the laser line dividing completely the vascular equator.

B.3. TAPS placentas

Twin anemia-polycythemia sequence (TAPS) is also a form of chronic inter-twin transfusion, characterized by a large inter-twin difference in hemoglobin level without polyhydramnios-oligohydramnios sequence. TAPS may occur spontaneously or after laser surgery for TTTS due to small residual anastomoses. The incidence of spontaneous TAPS is approximately 3 to 5% and the incidence of post-laser TAPS ranges from 2% to 14%. [5, 23, 24]TAPS can be diagnosed antenatelly or postnatally. The antenatal diag-

nosis criteria are based on the Doppler ultrasound abnormalities showing an increased peak systolic velocity in the middle cerebral artery (MCA-PSV >1.5MoM) in the donor twin (indication of fetal anemia) and a decrease (MCA-PSV <1.0MoM) in the recipient twin (indication of polycythemia); the postnatal diagnostic criteria for TAPS require an inter-twin hemoglobin difference >8.0 g/dL, reticulocyte count ratio >1.7 and/or placenta with only small (diameter <1 mm) vascular anastomoses.[5]

TAPS placentas are characterized by the large difference in color between the plethoric share of the recipient and the pale share of the donor. In addition, TAPS placentas have typically only a few minuscule anastomoses.[29] We injected a total of 22 spontaneous TAPS placentas (**Figure 5**). The mean number of anastomoses per placentas was 3.8±2.2. The prevalence of AV (and VA) anastomoses, AA anastomoses and VV anastomoses was 100% (22/22), 14% (3/22) and 0% (0/22). The mean number of anastomoses in TAPS placentas is significant lower than in normal MC placentas. In addition, the diameter of AV and AA anastomoses is extremely small (mean 0.16±0.01mm). The small diameter of anastomoses and low rate of AA anastomoses in TAPS placentas probably leads to chronic inter-twin transfusion and insufficient compensation, but without the large volume imbalance as seen in TTTS. The incidence of velamentous cord insertion and placental territory discordance of TAPS placentas are similar to that of normal MC placentas. The results of sharing discordance and type of cord insertion in TAPS placentas in our cohort are shown in Table 1.

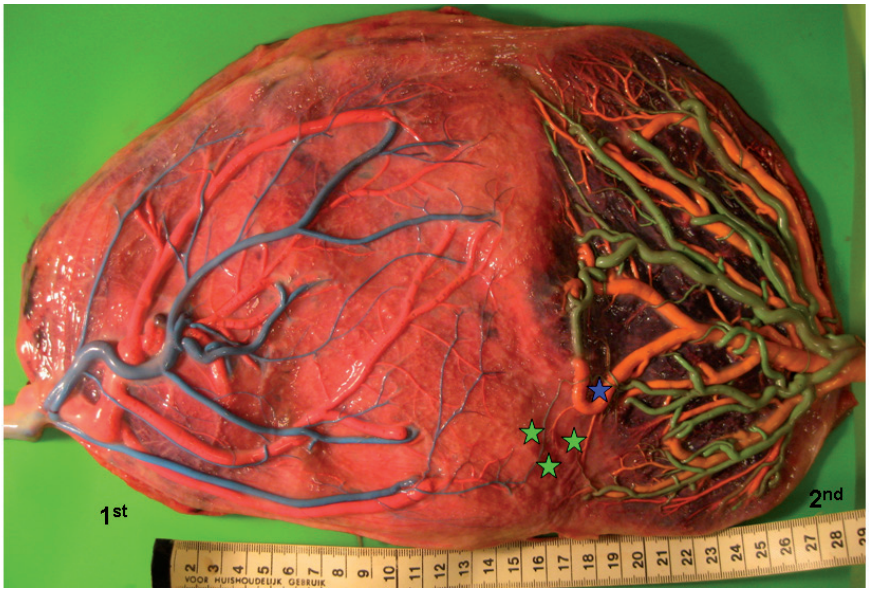

Figure 5: Spontaneous TAPS placenta (gestational age at delivery: 33 weeks) showing 3 small AV anastomoses (green stars) and 1 small AA anastomosis (blue star). Note the difference in color between the plethoric placental share of the recipient and the pale placental share of the donor.

B.4. sIUGR placentas

Selective intrauterine growth restriction (sIUGR) affects approximately 10- 20% of MC twin pregnancies compared to 8% in dichorionic twins.[30, 31] sIUGR results in an increased rate of mortality and morbidity among MC twins.[4, 31, 32] To evaluate the clinical outcome and association with placental anastomoses, sIUGR is classified into 3 types based on the characteristics of umbilical artery (UA) Doppler flow in the smaller twin: Type I (UA Doppler with positive diastolic flow), Type II (persistent absent or reversed end-diastolic flow) and Type III (intermittent absent or reversed end-diastolic flow).[33]

Placentas with sIUGR are characterized by the presence of a large AA anastomosis, large placental sharing discordance and higher rates of velamentous cord insertion.[3, 32, 34, 35] The mean number of anastomoses in sIUGR placentas is similar to normal MC placentas. However, nearly all the sIUGR placentas have one AA anastomosis with a significantly larger diameter compared to normal MC placentas. We injected a total of 73 sIUGR placentas (**Figure 6**). The mean number of anastomoses per placentas was 9.4±5.6. The prevalence of AV (and VA) anastomoses, AA anastomoses and VV anastomoses was 100% (73/73), 100% (73/73) and 23% (17/73). sIUGR is strongly associated with unequal placental sharing: a large placental share for the large twin and a small placental share for the growth restricted twin. The incidence of velamentous cord

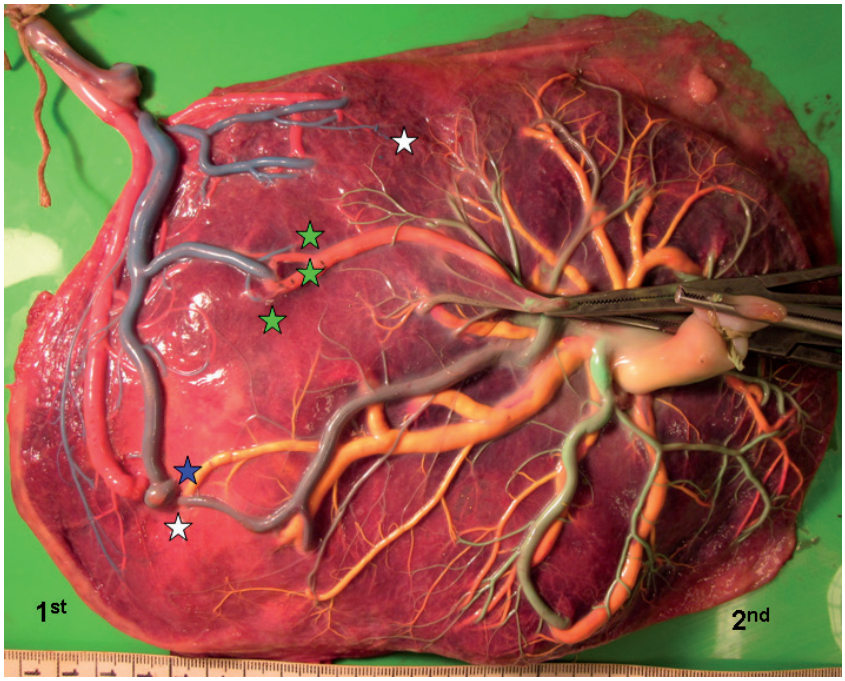

Figure 6: sIUGR placenta (gestational age at delivery: 29 weeks) showing 3 AV anastomoses (green stars), 2 VA anastomoses (white stars) and 1 large AA anastomosis (blue star). The growth restricted fetus (1st fetus) has a velamentous cord insertion and a small placental share (left side of the picture).

insertion in sIUGR placenta is up to 30%, which is significantly higher compared to normal MC placentas. The velamentous cord insertion belongs usually to the growth restricted fetus. The results of sharing discordance and type of cord insertion in sIUGR placentas in our cohort are shown in Table 1.

B.5. Monoamniotic (MA) placentas

Monoamniotic (MA) twins account for about 1% of MC pregnancies. MA twins share not only their placenta but also the amniotic sac. MA twins are diagnosed on ultrasound examination by the presence of a single amniotic sac and lack of an inter-twin septum.

MA placentas are characterized by the presence of large AA anastomoses, cords insertions that are close together and cord entanglement.[36, 37].[38] Inter-twin blood flow in MA twins is well compensated due to the large AA, leading to a lower incidence of TTTS (< 3%)[38] . On the other hand, the close insertion of umbilical cords also causes the ubiquitous cords entanglement. Cords entanglement is prone to the cord compression, which primarily contributes to the intrauterine fetal demise in MA twins.[37] We injected a total of 18 MA placentas (**Figure 7**). The mean number of anastomoses per placentas was 8.5±5.6. The prevalence of AV (and VA) anastomoses, AA anastomoses and VV anastomoses was 91% (38/43), 98% (41/43) and 43% (18/43). The results of sharing discordance and type of cord insertion in MA placentas in our cohort are shown in Table 1.

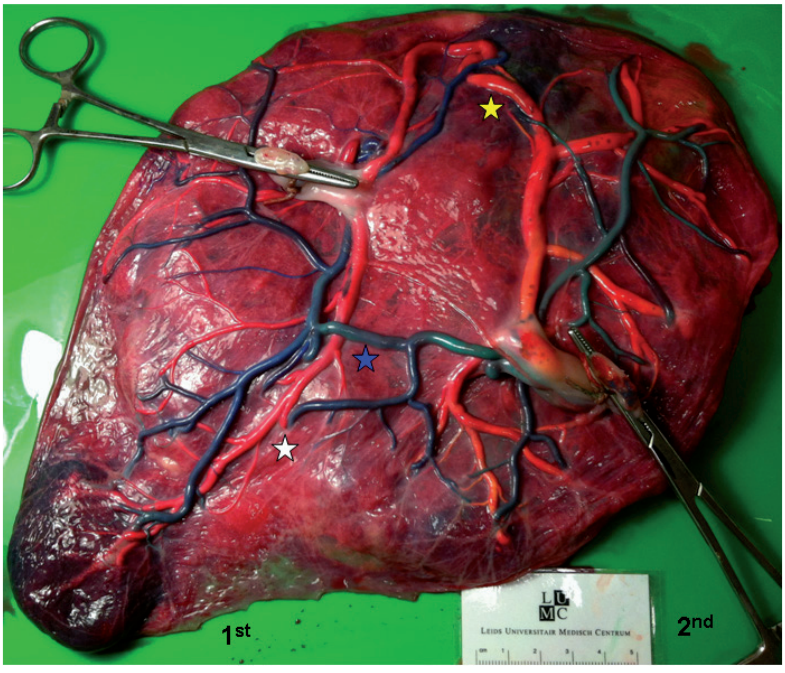

Figure 7: MA placenta with paracentral cord insertions (gestational age at delivery: 31 weeks) showing 1 AA anastomosis (blue star), 1 VV anastomosis (yellow star) and 1 VA anastomosis (white star).

B.6. TRAP placentas

Twin reversed arterial perfusion sequence (TRAP) complicates about 1 out of 35.000 pregnancies.[39] TRAP is defined as the blood flow pumped from one twin (referred to as the pump twin) into the other twin (referred to as the perfused twin).[6] The perfused twin is malformed without a functional heart (acardiac twin).[39] The pathogenesis of TRAP is related to the presence of a large AA anastomosis and returning back to the pumped twin via a large VV anastomosis (**Figure 8**). TRAP is diagnosed by the detection of color Doppler ultrasound showing the reversed blood flow in the umbilical artery.

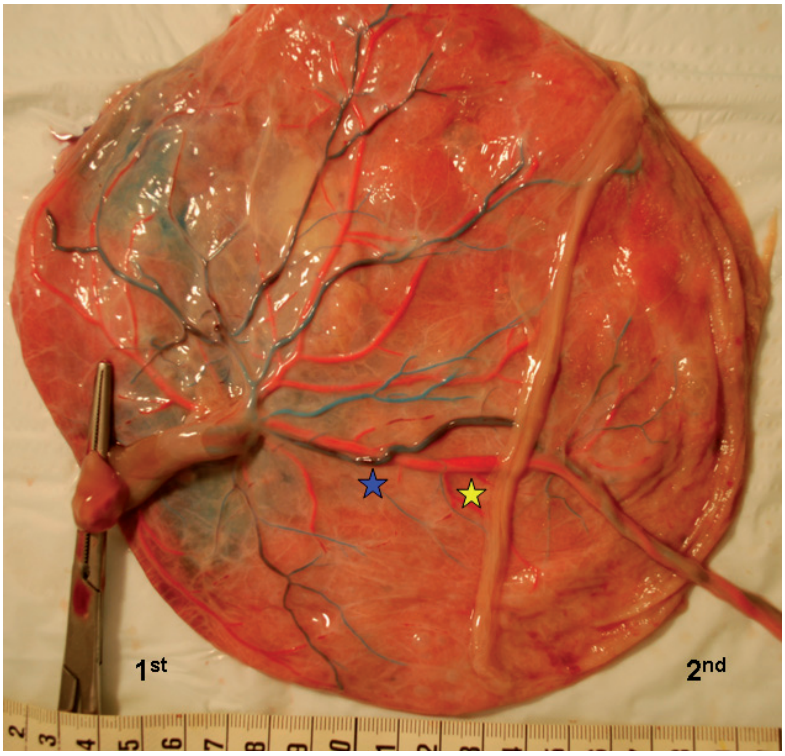

Figure 8: TRAP placenta (gestational age at delivery: 23 weeks) showing a large AA anastomosis (blue star) and VV anastomosis (yellow star). Placental share on the left-side of the picture belongs to the pump twin and on the right-side to the acardiac twin.

B.7. Bipartite placentas

Nearly all MC placentas are composed of a single mass. However, in our cohort, 2% (13/608) of MC twins have two separate placental masses (so-called bipartite MC placenta) (**Figure 9**). Vascular anastomoses were detected in 69% (9/13)of bipartite placentas and TTTS occurred in 23% (3/13) of bipartite placentas.[41] Therefore, detection of two distinct placental masses on prenatal ultrasound or gross examination after delivery does not rule out monochorionicity.[40]

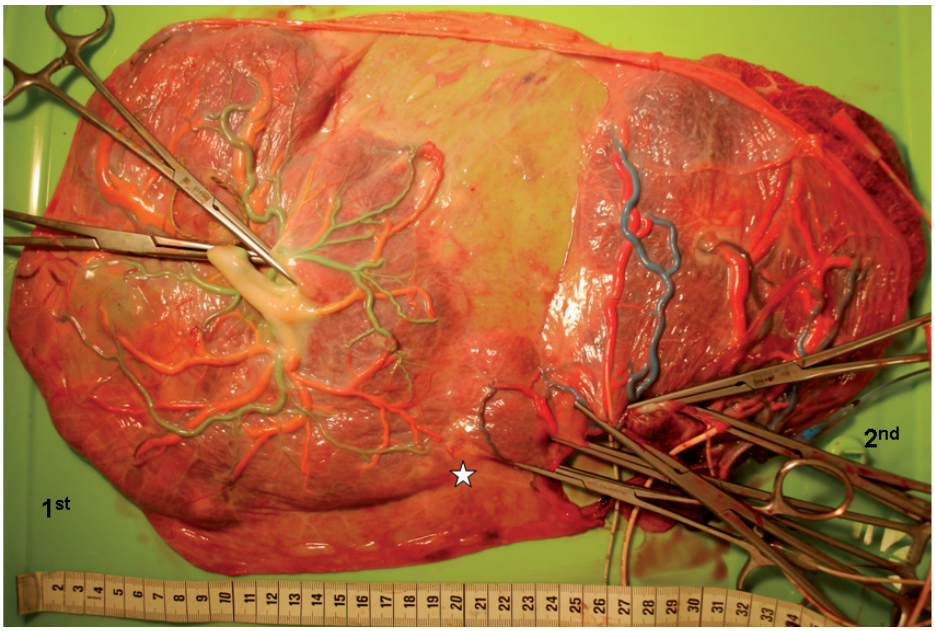

Figure 9: Bipartite placenta (gestational age at delivery: 36 weeks) showing two separate placental masses connected with each other through the amniotic membranes. The white star indicates a VA anastomosis near the placental margin.

CONCLUSIONS

Placental vascular anastomoses and sharing discordance result in several specific complications in MC pregnancies such as TTTS, TAPS, sIUGR, MA and TRAP. These complications primarily contribute to the risk of mortality and morbidity in MC twin fetuses and neonates. However, the pathogenesis of these disorders still needs to be elucidated. Routine examination and injected of all MC placentas can provide insight into the development of these complications and optimal management.

REFERENCES

[1] Dube J, Dodds L and Armson BA. Does chorionicity or zygosity predict adverse perinatal outcomes in twins? Am J Obstet Gynecol. 2002;186(3):579-83.

[2] Sebire NJ, Snijders RJ, Hughes K, Sepulveda W and Nicolaides KH. The hidden mortality of monochorionic twin pregnancies. Br J Obstet Gynaecol. 1997;104(10):1203-7.

[3] Lewi L, Cannie M, Blickstein I, Jani J, Huber A, Hecher K, Dymarkowski S, Gratacos E, Lewi P and Deprest J. Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. Am J Obstet Gynecol. 2007;197(6):587 e1-8.

[4] Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P and Deprest J. 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):514 e1-8.

[5] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP and Lopriore E. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther. 2010;27(4):181-90.

[6] Moore TR, Gale S and Benirschke K. Perinatal outcome of forty-nine pregnancies complicated by acardiac twinning. Am J Obstet Gynecol. 1990;163(3):907-12.

[7] Hack KE, Derks JB, Schaap AH, Lopriore E, Elias SG, Arabin B, Eggink AJ, Sollie KM, Mol BW, Duvekot HJ, Willekes C, Go AT, Koopman-Esseboom C, Vandenbussche FP and Visser GH. Perinatal outcome of monoamniotic twin pregnancies. Obstet Gynecol. 2009;113(2 Pt 1):353-60.

[8] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ and Oepkes D. Accurate and simple evaluation of vascular anastomoses in monochorionic placenta using colored dye. J Vis Exp. 2011;(55):e3208.

[9] Denbow ML, Cox P, Taylor M, Hammal DM and Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol. 2000;182(2):417-26.

[10] Bermudez C, Becerra CH, Bornick PW, Allen MH, Arroyo J and Quintero RA. Placental types and twin-twin transfusion syndrome. Am J Obstet Gynecol. 2002;187(2):489-94.

[11] Diehl W, Hecher K, Zikulnig L, Vetter M and Hackeloer BJ. Placental vascular anastomoses visualized during fetoscopic laser surgery in severe mid-trimester twin-twin transfusion syndrome. Placenta. 2001;22(10):876-81.

[12] Bajoria R. Vascular anatomy of monochorionic placenta in relation to discordant growth and amniotic fluid volume. Hum Reprod. 1998;13(1O):2933-40.

[13] Umur A, van Gemert MJ, Nikkels PG and Ross MG. Monochorionic twins and twin-twin transfusion syndrome: the protective role of arterio-arterial anastomoses. Placenta. 2002;23(2-3):201-9.

[14] de Villiers SF, Slaghekke F, Middeldorp JM, Walther FJ, Oepkes D and Lopriore E. Arterio-arterial vascular anastomoses in monochorionic placentas with and without twin-twin transfusion syndrome. Placenta. 2012;33(8):652-4.

[15] Sato Y and Benirschke K. Increased prevalence of fetal thrombi in monochorionic-twin placentas. Pediatrics. 2006;117(1):e113-7.

[16] Quintero RA. Twin-twin transfusion syndrome. Clin Perinatol. 2003;30(3):591-600.

[17] van Gemert MJ, Umur A, Tijssen JG and Ross MG. Twin-twin transfusion syndrome: etiology, severity and rational management. Curr Opin Obstet Gynecol. 2001;13(2):193-206.

[18] Slaghekke F, Kist WJ, Oepkes D, Middeldorp JM, Klumper FJ, Vandenbussche FP and Lopriore E. TAPS and TOPS: two distinct forms of feto-fetal transfusion in monochorionic twins. Z Geburtshilfe Neonatol. 2009;213(6):248-54.

[19] Mahieu-Caputo D, Meulemans A, Martinovic J, Gubler MC, Delezoide AL, Muller F, Madelenat P, Fisk NM and Dommergues M. Paradoxic activation of the renin-angiotensin system in twin-twin transfusion syndrome: an explanation for cardiovascular disturbances in the recipient. Pediatr Res. 2005;58(4):685-8.

[20] Senat MV, Deprest J, Boulvain M, Paupe A, Winer N and Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med. 2004;351(2):136-44.
[21] Simpson LL. Twin-twin transfusion syndrome. Am J Obstet Gynecol. 2013;208(1):3-18.
[22] Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Walther FJ and Vandenbussche FP. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. Am J Obstet Gynecol. 2007;196(2):159 e1-5.
[23] Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J and Ville Y. 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):796-803.
[24] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D and Vandenbussche FP. 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):66 e1-4.
[25] Lewi L, Jani J, Cannie M, Robyr R, Ville Y, Hecher K, Gratacos E, Vandecruys H, Vandecaveye V, Dymarkowski S and Deprest J. Intertwin anastomoses in monochorionic placentas after fetoscopic laser coagulation for twin-to-twin transfusion syndrome: is there more than meets the eye? Am J Obstet Gynecol. 2006;194(3):790-5.
[26] Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ and Vandenbussche FP. Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome. Placenta. 2007;28(2-3):204-8.
[27] Quintero RA, Martinez JM, Lopez J, Bermudez C, Becerra C, Morales W and Arroyo J. Individual placental territories after selective laser photocoagulation of communicating vessels in twin-twin transfusion syndrome. Am J Obstet Gynecol. 2005;192(4):1112-8.
[28] Quintero RA, Comas C, Bornick PW, Allen MH and Kruger M. Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol. 2000;16(3):230-6.
[29] Lopriore E, Deprest J, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP and Lewi L. Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstet Gynecol. 2008;112(4):753-8.
[30] Victoria A, Mora G and Arias F. Perinatal outcome, placental pathology, and severity of discordance in monochorionic and dichorionic twins. Obstet Gynecol. 2001;97(2):310-5.
[31] De Paepe ME, Shapiro S, Young L and Luks FI. Placental characteristics of selective birth weight discordance in diamniotic-monochorionic twin gestations. Placenta. 2010;31(5):380-6.
[32] Lewi L, Gucciardo L, Huber A, Jani J, Van Mieghem T, Done E, Cannie M, Gratacos E, Diemert A, Hecher K, Lewi P and Deprest J. Clinical outcome and placental characteristics of monochorionic diamniotic twin pairs with early- and late-onset discordant growth. Am J Obstet Gynecol. 2008;199(5):511 e1-7.
[33] Gratacos E, Lewi L, Munoz B, Acosta-Rojas R, Hernandez-Andrade E, Martinez JM, Carreras E and Deprest J. A classification system for selective intrauterine growth restriction in monochorionic pregnancies according to umbilical artery Doppler flow in the smaller twin. Ultrasound Obstet Gynecol. 2007;30(1):28-34.
[34] Lopriore E, Pasman SA, Klumper FJ, Middeldorp JM, Walther FJ and Oepkes D. Placental characteristics in growth-discordant monochorionic twins: a matched case-control study. Placenta. 2012;33(3):171-4.
[35] De Paepe ME, Shapiro S, Young L and Luks FI. Placental characteristics of selective birth weight discordance in diamniotic-monochorionic twin gestations. Placenta. 2010;31(5):380-6.
[36] Hack KE, van Gemert MJ, Lopriore E, Schaap AH, Eggink AJ, Elias SG, van den Wijngaard JP, Vandenbussche FP, Derks JB, Visser GH and Nikkels PG. Placental characteristics of monoamniotic twin pregnancies in relation to perinatal outcome. Placenta. 2009;30(1):62-5.
[37] Huber A and Hecher K. How can we diagnose and manage twin-twin transfusion syndrome? Best Pract Res Clin Obstet Gynaecol. 2004;18(4):543-56.

[38] Umur A, van Gemert MJ and Nikkels PG. Monoamniotic-versus diamniotic-monochorionic twin placentas: anastomoses and twin-twin transfusion syndrome. Am J Obstet Gynecol. 2003;189(5):1325-9.
[39] Diehl W and Hecher K. Selective cord coagulation in acardiac twins. Semin Fetal Neonatal Med. 2007;12(6):458-63.
[40] Machin GA. Why is it important to diagnose chorionicity and how do we do it? Best Pract Res Clin Obstet Gynaecol. 2004;18(4):515-30.
[41] Lopriore E, Sueters M, Middeldorp JM, Klumper F, Oepkes D and Vandenbussche FP. Twin pregnancies with two separate placental masses can still be monochorionic and have vascular anastomoses. Am J Obstet Gynecol. 2006;194(3):804-8.

Chapter 3

Velamentous cord insertion in monochorionic twins with or without twin-twin transfusion syndrome: does it matter?

T. Costa-Castro

S.F. de Villiers

N. Montenegro

M. Severo

D. Oepkes

A. Matias

E. Lopriore

Placenta, 2013 vol. 34(11) pp. 1053-8.

ABSTRACT

Objective: To study the association between velamentous cord insertion (VCI) and different outcomes in monochorionic twins with and without twin-twin transfusion syndrome (TTTS).

Methods: We recorded the cord insertion type in all consecutive monochorionic placentas examined in two tertiary medical centers. The association between VCI and several outcomes was estimated.

Results: A total of 630 monochorionic placentas (304 with and 326 without TTTS) were studied. The incidence of VCI in the TTTS and non-TTTS group was 36.8% and 35.9%, respectively (p=0.886). The presence of VCI in one twin was significantly associated with lower gestational age (GA) at birth (regression coefficient -1.31, confidence interval [CI] -2.07, -0,56), small for gestational age (SGA) (relative risk [RR] 1.20, 95% CI 1.04, 1.38), severe birth weight discordance (RR 2.39, 95% CI 1.66, 3.45) and intrauterine fetal demise (IUFD) (RR 1.80, 95% CI 1.26, 2.56). The prevalence of IUFD in monochorionic pregnancies without TTTS increased from 4.6% to 14.1% in the presence of VCI. In the TTTS group, the prevalence of IUFD was comparable in the absence or presence of VCI. In a similar way, GA at birth was significantly lower in the presence of VCI only in the non-TTTS group.

Conclusion: Our findings suggest that VCI is not associated with the development of TTTS but increases the risk of adverse outcomes. Both VCI and TTTS independently increase the prevalence of IUFD and lower GA at birth in a similar way, showing that VCI is an important indicator of adverse perinatal outcome in monochorionic twins.

Keywords

Monochorionic twins, twin-twin transfusion syndrome, velamentous cord insertion, severe birth weight discordance, intrauterine fetal demise

INTRODUCTION

Twin-twin transfusion syndrome (TTTS) is a complication of monochorionic twin pregnancies and results from unbalanced inter-twin blood transfusion via placental vascular anastomoses. Although vascular anastomoses are invariably found in almost all monochorionic placentas, only 10% will eventually develop TTTS [1;2]. Differences in angio-architecture, among those the absence of arterio-arterial anastomoses, are one of the major factors involved in the development of TTTS [1-4]. However, angio-architecture alone does not fully explain the pathophysiology of TTTS [1-4]. Several other hypotheses on the pathophysiology of TTTS have been proposed, including utero-placental insufficiency and paradoxic activation of fetal vasoactive and humoral factors [3;4].

Several authors found higher incidence of velamentous cord insertions (VCI) in TTTS placentas and hypothesized that VCI may lead to utero-placental insufficiency, subsequently establishing a vicious cycle resulting in the development of TTTS [5-8]. However, these hypotheses were mostly unsubstantiated or based on small studies [5-8]. Moreover, several recent reports show that the incidence of velamentous or marginal cord insertion is similar in monochorionic twins with and without TTTS [9-11]

The objective of this study was to estimate the incidence of VCI in a large group of monochorionic twins with and without TTTS and study outcomes associated with VCI.

MATERIAL AND METHODS

All consecutive placentas of monochorionic twin pregnancies examined at the University Medical Center of Porto (Portugal) and Leiden (The Netherlands) between June 2002 and September 2012 were included in this study. Monochorionicity was confirmed after delivery by gross examination of the dividing membrane and/or histopathological examination of the placenta and the dividing membrane. Placentas were divided in a group with TTTS and a group without TTTS. TTTS was diagnosed using standard antenatal ultrasound criteria [12]. Both University hospitals are tertiary medical centers for perinatal medicine. The Leiden University Medical Center is the national referral center for fetal therapy in the Netherlands, including laser treatment for TTTS. Most TTTS cases referred to Leiden were therefore treated with laser.

Part of the placentas (n=139) included in this study were already presented in a previous report [11].

During prenatal ultrasound in TTTS twin pairs, great care was taken to define which fetus, donor or recipient, would be born first. At delivery, umbilical cords were labeled to identify the first and second-born twin. The type of abnormal umbilical cord insertion, velamentous or marginal insertion (within 1 cm of placental margin), was recorded.

We studied the association of VCI with several outcomes, including gestational age (GA) at birth, small for gestational age (SGA), severe birth weight discordance (BWD) (> 25%), intrauterine fetal demise (IUFD) and neonatal mortality. In the TTTS group we analyzed the association of VCI with Quintero stage. The BW discordance (in %) was calculated as BW = [(BW larger fetus – BW smaller fetus)/BW larger fetus] × 100%. SGA was defined as a birth weight less than 10th percentile.

We excluded monochorionic twin pregnancies with twin anemia-polycythemia sequence, twin reversed arterial perfusion, monoamniotic twins and higher multiple pregnancies. Placentas with IUFD were excluded when placental maceration prohibited accurate evaluation of type of umbilical cord insertion and placental sharing.

The fetal level data was aggregated by pair of twins, counting the number of fetuses in the pair with an outcome (SGA, IUFD, Neonatal Mortality) or exposure (VCI).

Crude and adjusted relative risks (RR) and respective 95% confidences intervals (CI) were estimated by binomial generalized linear models with log link function and Bernoulli generalized linear models with log link function for the fetal and pair level dichotomous outcome variables, respectively.

Regression coefficients and proportional odds ratio and 95% CI were estimated by linear regression and ordinal regression for the pair level continuous (GA at birth) and ordinal variable (Quintero stage), respectively.

The interaction between TTTS and VCI with different outcomes was studied. The number of fetuses with several outcomes (SGA, IUFD, Neonatal Mortality) or exposure (VCI) was aggregated (0 versus 1 or 2 twins).

$P<0.05$ was considered to indicate statistical significance.

Statistical analyses were performed using the software R 2.12.1 and SPSS for Windows version 17.0 (SPSS, Inc., Chicago, Illinois, USA).

RESULTS

A total of 630 consecutive monochorionic diamniotic placentas were examined at our two centers and included in this study (monochorionic placentas with TTTS, n=304 and monochorionic placentas without TTTS, n=326).

The data required for this study could not be recorded completely for 67 placentas (38 in the TTTS-group and 29 in the non-TTTS group) because of placental maceration caused by intrauterine fetal demise (n=12 in TTTS group and n=3 in no-TTTS group) and loss or destruction of the placenta after delivery (n=26 in the TTTS-group and n=26 in the no-TTTS group). These 67 cases were excluded from further analysis.

Mean gestational age at birth in the TTTS group and non-TTTS group was 30 weeks (range: 15 to 38 weeks) and 33 weeks (range: 16 to 38 weeks) respectively. The mono-

chorionic pregnancies with TTTS were treated with fetoscopic laser coagulation (n=258), amniodrainage (n=24) or without intrauterine intervention (n=23). Baseline characteristics in both groups are presented in Table 1.

Table 1. Baseline characteristics in the 630 monochorionic diamniotic pregnancies

		non-TTTS group	TTTS group	P
		N (%)	N (%)	
		326 (51.7)	304 (43.3)	
Fetal level variable				
SGA (yes)				
	0 twins	130 (41.1)	107 (38.8)	0.470
	1 twin	121 (38.3)	119 (43.1)	
	both twins	65 (20.6)	50 (18.1)	
IUFD (yes)				
	0 twins	297 (91.7)	256 (84.5)	0.020
	1 twin	10 (3.1)	16 (5.3)	
	both twins	17 (5.2)	31 (10.2)	
Neonatal Mortality (yes)[1]				
	0 twins	281 (97.2)	232 (90.6)	0.003
	1 twin	7 (2.4)	18 (7.0)	
	both twins	1 (0.3)	6 (2.3)	
Pair level variable				
BW discordance				
	<25%	237 (76.7)	127 (82.5)	0.191
	>=25%	72 (23.3)	27 (17.5)	
GA at birth (weeks)		33.5 (3.9)	30.8 (4.8)	<0.001
Center				
	Netherlands	241 (73.9)	285 (93.8)	<0.001
	Portugal	85 (26.1)	19 (6.2)	
Quintero stage				
	1	Not applicable	49 (16.2)	---
	2		97 (32.0)	
	3		138 (45.5)	
	4		16 (5.3)	
	5		3 (1.0)	

TTTS, twin-twin transfusion syndrome; SGA, small for gestational age; IUFD, intrauterine fetal demise; BW, birth weight; GA gestational age.
[1]Analysis only for the pair of twins who did not have IUFD

The incidence of VCI per twin pair (one/ both fetuses) in the TTTS group and non-TTTS group was 36.8% (112/304)/ 3.9% (12/304) and 35.9% (117/326)/ 3.4% (11/326), respectively (p=0.886). Further details on the type of umbilical cord insertion in both

groups are presented in Table 2. Two examples of monochorionic placentas with VCI are shown in figure 1 and 2 (pictures are taken after colored dye injection).

Table 2. Type of umbilical cord insertion in monochorionic pregnancies with and without Twin-twin transfusion syndrome

	non-TTTS group	TTTS group	P
	N (%)	N (%)	
	326 (51.7)	304 (43.3)	
Fetal level variable			
Velamentous cord insertion			
0 twins	198 (60.7)	180 (59.2)	0.886
1 twin	117 (35.9)	112 (36.8)	
both twins	11 (3.4)	12 (3.9)	
Marginal cord insertion			
0 twins	203 (62.3)	178 (58.6)	0.584
1 twin	94 (28.8)	99 (32.6)	
both twins	29 (8.9)	27 (8.9)	
Velamentous or marginal cord insertion			
0 twins	97 (29.8)	81 (26.6)	0.661
1 twin	171 (52.5)	169 (55.6)	
both twins	58 (17.8)	54 (17.8)	

TTTS, twin-twin transfusion syndrome

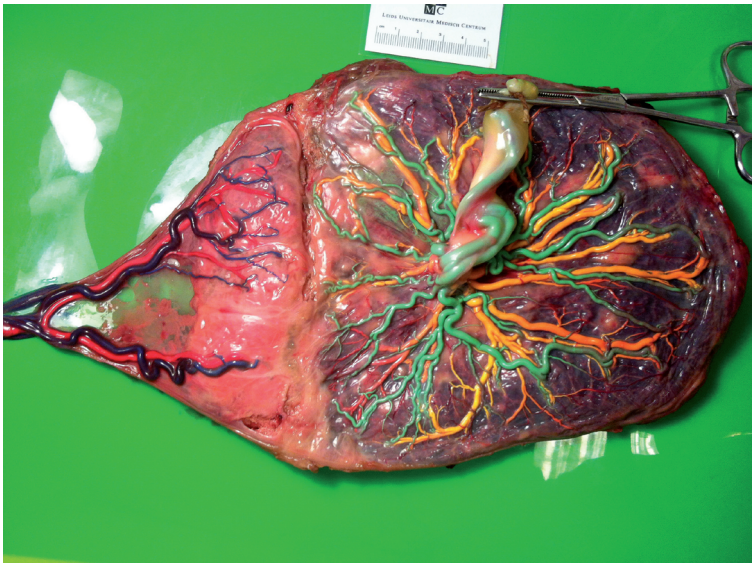

Figure 1: Monochorionic placenta with twin-twin transfusion syndrome (Quintero stage 2) treated with fetoscopic laser coagulation at 16 weeks gestational age. IUFD of ex-donor at 32 weeks. Ex-recipient twin delivered at 33 weeks.

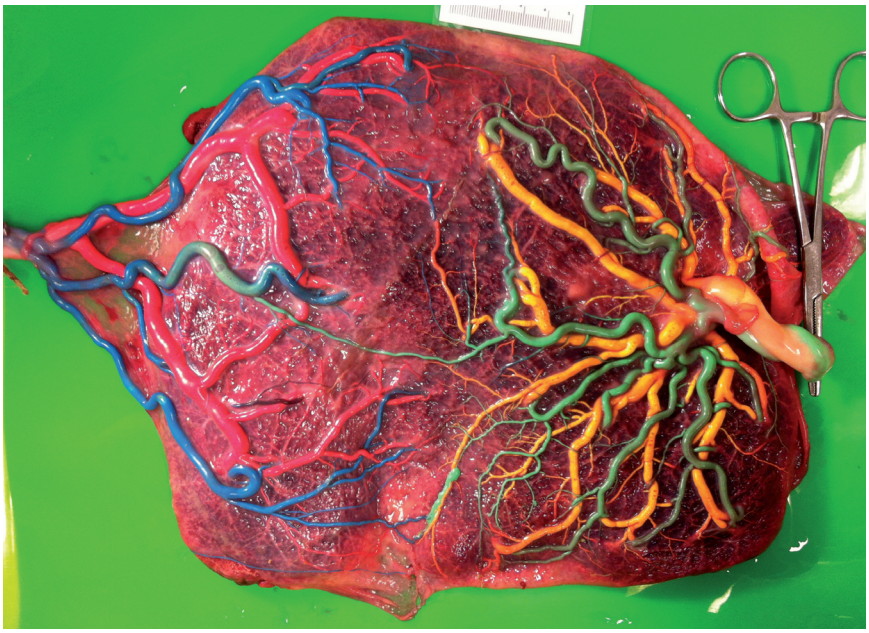

Figure 2: Monochorionic placenta with selective intrauterine growth restriction, delivered at 36 weeks gestation. Twin 1 delivered with 2040gr (placenta share on left side), twin 2 delivered with 2800gr.

Risk factor analysis showed that the presence of VCI in one twin was significantly associated with lower GA at birth (regression coefficient -1.46, CI -2.21, -0.07), SGA (RR 1.16 95% confidence interval [CI] 1.01, 1.34), severe BWD (RR 3.14 95% CI 1.97, 5.02) and IUFD (RR 1.76 95% CI 1.24, 2.94). We found no association between the presence of VCI and neonatal mortality (RR 1.25 95% CI 0.66, 2.34) or higher Quintero stage (proportional OR 1.02 95% CI 0.65, 1.58). The presence of VCI in both twins revealed a significant association with lower GA at birth (regression coefficient -2.24 CI -4.15, -0.32) and IUFD (RR 2.37 95% CI 1.10, 4.37), but not with SGA (RR 1.10 95% CI 0.74, 1.52) severe BWD (RR 2.60 95% CI 0.87, 7.77), neonatal mortality (RR 0.80 95% CI 0.04, 3.62) and higher Quintero stage (proportional OR 0.78 95% CI 0.27, 2.33). These associations continue to occur after adjustment for Center. Results of univariate and multivariate risk analysis are presented in Table 3.

Our results also showed significant interaction between TTTS and VCI when we considered IUFD and GA at birth. The prevalence of IUFD (in at least one twin) in monochorionic pregnancies without TTTS increased from 4.6% to 14.1% in the presence of VCI. In the TTTS group, the prevalence of IUFD was comparable in the absence or presence of VCI (15.1% and 16.1%, respectively). The GA at birth was similar in the non-TTTS group with VCI and the TTTS without VCI (32 and 31 weeks, respectively) compared with 34 weeks for the non-TTTS group without VCI. For this outcome as well, the GA at birth significantly lowers in the presence of VCI but only in the non-TTTS group (Table 4).

Table 3. Association between Velamentous cord insertion and several outcomes

OUTCOME		Crude RR (95%CI)	Adjusted RR (95%CI)[1]
SGA			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	1.16 (1.01, 1.34)	1.20 (1.04, 1.38)
	both twins with VCI	1.10 (0.74, 1.52)	1.20 (0.80, 1.61)
Severe BWD			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	3.14 (1.97, 5.02)	2.39 (1.66, 3.45)
	both twins with VCI	2.60 (0.87, 7.77)	2.06 (0.93, 4.59)
IUFD			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	1.76 (1.24, 2.94)	1.80 (1.26, 2.56)
	both twins with VCI	2.37 (1.10, 4.37)	2.46 (1.14, 4.56)
Neonatal Mortality[2]			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	1.25 (0.66, 2.34)	1.20 (0.63, 2.25)
	both twins with VCI	0.80 (0.04, 3.62)	0.74 (0.04, 3.40)
		Crude regression Coefficient (95%CI)	Adjusted regression Coefficient (95%CI) *
GA at birth (weeks)			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	-1.46 (-2.21, -0,07)	-1.31 (-2.07, -0,56)
	both twins with VCI	-2.24 (-4.15, -0.32)	-2.00 (-3.92, -0.09)
		Proportional Odds Ratio (95%CI)	Proportional Odds Ratio (95%CI)
Quintero stage[3]			
	0 twins with VCI	Ref	Ref
	1 twin with VCI	1.02 (0.65, 1.58)	0.94 (0.60, 1.46)
	both twins with VCI	0.78 (0.27, 2.33)	0.68 (0.23, 2.06)

RR, relative risk; VCI, velamentous cord insertion; SGA, small for gestational age; BWD, birth weight discordance; IUFD, intrauterine fetal demise; GA gestational age.

[1]Adjusted for Center

[2]Analysis only for the pair of twins who did not have IUFD

[3]Analysis only for the pair of twins who did have TTTS

Table 4. Interaction between Twin-twin transfusion syndrome and Velamentous cord insertion to Intrauterine fetal demise and Gestational age at birth (weeks)

	non-TTTS group		TTTS group		
IUFD	0 twins N (%)	at least 1 twin N (%)	0 twins N (%)	at least 1 twin N (%)	P for Interaction
With VCI					
0 twins	187 (95.4)	9 (4.6)	152 (84.9)	27 (15.1)	0.027
1/both twins	110 (85.9)	18 (14.1)	104 (83.9)	20 (16.1)	
	non-TTTS group		**TTTS group**		
GA at birth (weeks)	mean (SD)		mean (SD)		P for Interaction
With VCI					
0 twins	34.4 (2.8)		31.0 (4.7)		0.014
1/both twins	32.1 (4.9)		30.4 (5.6)		

TTTS, twin-twin transfusion syndrome; IUFD, intrauterine fetal demise; VCI, velamentous cord insertion; GA gestational age.

DISCUSSION

This is the largest study reporting on the difference in velamentous cord insertion and discordant placental share between monochorionic twin placentas with and without TTTS. We found that the rate VCI in monochorionic placentas with and without TTTS was almost identical. Our findings refute a possible causative relationship between VCI and the development of TTTS.

VCI are rare in singleton placentas (2%) and far more common in dichorionic (7%) and particularly in monochorionic twin placentas (12%) [13]. The high incidence of VCI in monochorionic twin placentas is thought to result from a “battle” for space between each twin’s placental shares, a competition process also called trophotropism [14-16]. VCI are associated with smaller placental mass and lower birth weights [14;15;17;18].

In monochorionic twinning, VCI have also been related to the development of TTTS. In a study of 38 monochorionic placentas, Fries *et al* [5] reported a significantly higher prevalence of VCI in TTTS placentas than in non-TTTS placentas, respectively 32% (7/22) and 9% (5/54) ($p<0.01$). In view of this finding, Fries *et al* proposed an etiologic role for VCI in the development of TTTS [5]. As a VCI can be easily compressed, Fries *et al* suggested that TTTS could result from hemodynamic instability due to reduced blood flow to the donor twin with a VCI. However, the number of placentas studied was small. Moreover, 3 of the 38 (8%) monochorionic pregnancies were monoamniotic. This probably represents a selection bias, as placental angio-architecture, type of umbilical cord insertion and incidence of TTTS are known to be different in monoamniotic and diamniotic monochorionic pregnancies [19]. In a study of 60 monochorionic placentas, Machin [6]

reported that roughly 30% of twins with velamentous or marginal cord insertion have TTTS, whereas only 6% (1/17) of twins without velamentous or marginal cord insertion develop TTTS. However, exact data on the number of placentas with TTTS was not mentioned. Contrarily, in a study of 58 monochorionic twin pregnancies, Bajoria reports similar frequencies of VCI in TTTS and non-TTTS twins (16% and 19% respectively) [10]. In a recent study of 89 consecutive monochorionic placentas, De Paepe *et al* also found a similar prevalence of velamentous or marginal cord insertion in TTTS and non-TTTS placentas (37% and 36% respectively) [9]. In another (unpublished) series of 90 monochorionic placentas, Taylor *et al* also found equally high incidence VCI in TTTS and non- TTTS placentas (53% and 52% respectively) [20]. In a study of 139 placentas, Lopriore *et al* also reported a similar incidence of VCI in the groups with and without TTTS (13% versus 14%, respectively) [11]. The wide range of reported incidence of VCI in the various studies is remarkable, ranging from 6% to 53%. There are several reasons that could explain the discrepancy in results, such as different ways of counting and reporting the data (VCI per pair or per fetus), subjectivity in defining VCI and small number of placentas included in some studies.

From the risk factor analysis, we found four outcomes to be associated with the presence of VCI in one twin, including lower GA at birth, SGA, severe BWD and IUFD. These associations continue to occur after adjustment for Center. We failed to find a correlation between the presence of VCI and neonatal mortality or higher Quintero stage.

In our study, the presence of VCI in one twin is significantly associated with SGA and severe BWD. This study is in agreement with previous studies showing that VCI are associated with smaller birth weights and severe BWD [6;14;15;18]. Up to 21% of monochorionic twin pregnancies are complicated by severe BWD even in the absence of TTTS (selective intrauterine growth restriction, sIUGR) [21]. While its pathogenesis remains incompletely understood the development of sIUGR is generally attributed to aberrant placental characteristics. The higher frequency of peripheral cord insertion and uneven placental sharing has been well documented [6;18;22]. These findings are in accordance with previous reports. However, our results show that the presence of VCI in both twins is not associated with severe BWD which seems easy to understand, but, surprisingly, the presence of VCI in both twins is not significantly associated with SGA status as well. To our knowledge, this is the first study which differentiates the presence of VCI in one and both fetuses during the analysis of different outcomes. More studies are necessary to understand these differences.

In this study we also found that the presence of VCI was significantly associated with increased IUFD. Perinatal mortality and morbidity is higher in monochorionic twin pregnancies than in dichorionic twin pregnancies [23]. The higher mortality in MC twins is attributed to the effects of placental morphologic characteristics. Placental vascular anastomoses, unequal placental sharing and abnormalities in umbilical cord insertions

are associated with fetal growth and BWD, the latter being a major contributor to unfavorable perinatal outcome in MC twins [18]. Placental cord insertion has been reported as an indicator for adverse perinatal outcome. [5;6;24] Our findings are in agreement with previous reports. However, in this study, the prevalence of IUFD increases in the presence of VCI, but only in the non-TTTS group. Our results showed a significant interaction between TTTS and VCI for IUFD. The prevalence of IUFD (in at least one twin) in monochorionic pregnancies without TTTS increased from 4.6% to 14.1% in the presence of VCI (in one or both twins). In the TTTS group, the prevalence of IUFD was comparable in the absence or presence of VCI (15.1% and 16.1%, respectively). In a similar way, the GA at birth significantly lowers in the presence of VCI but only in the non-TTTS group.

Antenatal surveillance in monochorionic twins aims to identify those pregnancies that are at an increased risk of complications. Therefore, the knowledge and addition of ultrasound predictors of adverse outcome (such as VCI) may be useful in risk stratification and management of twin pregnancies.

The value of ultrasound assessment in the determination of the umbilical cord insertion in first trimester has been examined in singleton and twin pregnancies. Di Salvo et al [25] compared prenatal ultrasound examination and postnatal histopathologic findings with respect to umbilical cord insertion with an overall sensitivity and specificity of 69% and 100%, respectively. In a prospective study, Sepulveda et al [26] determined that VCI could be determined reliably with prenatal ultrasound in 99% of cases.

Several possible limitations to our study are acknowledged. The number of placentas that did not undergo examination because of placental maceration caused by IUFD raises the possibility of selection bias. More studies are necessary to clarify this issue. In a future research perspective, a prospective study using ultrasound in the determination of the umbilical cord insertion at first trimester would be useful in the determination of the incidence of VCI in monochorionic twins with and without TTTS. The fact that all placental examinations were not carried out in a single laboratory is another potential source of bias. However, the nature of the placental examination variable that was studied here (umbilical cord insertion) should limit any potential bias in this regard.

In conclusion, our findings show that the frequency of VCI is similar in TTTS and non-TTTS monochorionic twins, challenging the notion of a causative relationship with the development of TTTS. However, in this study, VCI increases the risk of several adverse outcomes, such as SGA status, severe BWD, IUFD and lower GA at birth. We also found that both VCI and TTTS independently increase the prevalence of IUFD and lower GA at birth in a similar way, showing that VCI is an important indicator of adverse perinatal outcome in monochorionic twins. This point underscores the need for first trimester prenatal detection of VCI and increased surveillance in these twins, even in the absence of TTTS.

REFERENCE LIST

[1] Huber A, Hecher K. How can we diagnose and manage twin-twin transfusion syndrome? Best Pract Res Clin Obstet Gynaecol 2004;18:543-56.

[2] Lewi L, Van Schoubroeck D, Gratacos E, Witters I, Timmerman D, Deprest J. Monochorionic diamniotic twins: complications and management options. Curr Opin Obstet Gynecol 2003;15:177-94.

[3] Lopriore E, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Twin-to-twin transfusion syndrome: from placental anastomoses to long-term neurodevelopmental outcome. Curr Pediatr Rev 2005;1:191-203.

[4] Galea P, Jain V, Fisk NM. Insights into the pathophysiology of twin-twin transfusion syndrome. Prenat Diagn 2005;25:777-85.

[5] Fries MH, Goldstein RB, Kilpatrick SJ, Golbus MS, Callen PW, Filly RA. The role of velamentous cord insertion in the etiology of twin-twin transfusion syndrome. Obstet Gynecol 1993;81:569-74.

[6] Machin GA. Velamentous cord insertion in monochorionic twin gestation. An added risk factor. J Reprod Med 1997;42:785-89.

[7] Saunders NJ, Snijders RJ, Nicolaides KH. Therapeutic amniocentesis in twin-twin transfusion syndrome appearing in the second trimester of pregnancy. Am J Obstet Gynecol 1992;166:820-24.

[8] Bruner JP, Anderson TL, Rosemond RL. Placental pathophysiology of the twin oligohydramnios-polyhydramnios sequence and the twin-twin transfusion syndrome. Placenta 1998;19:81-6.

[9] De Paepe ME, DeKoninck P, Friedman RM. Vascular distribution patterns in monochorionic twin placentas. Placenta 2005;26:471-75.

[10] Bajoria R. Vascular anatomy of monochorionic placenta in relation to discordant growth and amniotic fluid volume. Hum Reprod 1998;13:2933-40.

[11] Lopriore E, Sueters M, Middeldorp M, et al. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. Am J Obstet Gynecol 2007;196:159.e1-159.e5.

[12] Wittmann BK, Baldwin VJ, Nichol B. Antenatal diagnosis of twin transfusion syndrome by ultrasound. Obstet Gynecol 1981;58:123-27.

[13] Sato Y, Benirschke K. Increased prevalence of fetal thrombi in monochorionic-twin placentas. Pediatrics 2006;117:e113-e117.

[14] Loos RJ, Derom C, Derom R, Vlietinck R. Birthweight in liveborn twins: the influence of the umbilical cord insertion and fusion of placentas. BJOG 2001;108:943-48.

[15] Benirschke K, Masliah E. The placenta in multiple pregnancy: outstanding issues. Reprod Fertil Dev 2001;13:615-22.

[16] Machin GA. Why is it important to diagnose chorionicity and how do we do it? Best Pract Res Clin Obstet Gynaecol 2004;18:515-30.

[17] Victoria A, Mora G, Arias F. Perinatal outcome, placental pathology, and severity of discordance in monochorionic and dichorionic twins. Obstet Gynecol 2001;97:310-15.

[18] Hanley ML, Ananth CV, Shen-Schwarz S, Smulian JC, Lai YL, Vintzileos AM. Placental cord insertion and birth weight discordancy in twin gestations. Obstet Gynecol 2002;99:477-82.

[19] Umur A, van Gemert MJ, Nikkels PG. Monoamniotic-versus diamniotic-monochorionic twin placentas: anastomoses and twin-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1325-29.

[20] Jain V, Fisk NM. The twin-twin transfusion syndrome. Clin Obstet Gynecol 2004;47:181-202.

[21] Ortibus E, Lopriore E, Deprest J, et al. The pregnancy and long-term neurodevelopmental outcome of monochorionic diamniotic twin gestations: a multicenter prospective cohort study from the first trimester onward. Am J Obstet Gynecol 2009;200:494.e1-494.e8

[22] De Paepe ME, Shapiro S, Young L, Luks FI. Placental characteristics of selective birth weight discordance in diamniotic-monochorionic twin gestations. Placenta 2010;31:380-6

[23] Hack KE, Derks JB. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. BJOG 2008;115: 58–67
[24] Hack KE, Nikkels PG, Koopman-Esseboom C, Derks JB, Elias SG, Van Gemert MJ, Visser GH. Placental characteristics of monochorionic diamniotic twin pregnancies in relation to perinatal outcome. Placenta 2008;29:976–81
[25] Di Salvo DN, Benson SB, Laing FS, Brown DL, Frates MC, Doubilet PM. Sonographic evaluation of the placental cord insertion site. AJR Am J Roentgenol 1998;170:1295-8
[26] Sepulveda W, Rojas I, Robert JA, Schnapp C, Alcalde JL. Prenatal detection of velamentous insertion of the umbilical cord: a prospective color Doppler ultrasound study. Ultrasound Obstet Gynecol 2003;21:564–9

Chapter 4

Arterio-arterial vascular anastomoses in monochorionic placentas with and without twin-twin transfusion syndrome

S.F. de Villiers

F. Slaghekke

J.M. Middeldorp

F.J. Walther

D. Oepkes

E. Lopriore

Placenta, 2012 vol. 33(8) pp. 652-4.

ABSTRACT

We performed a matched case-control study to analyze the placental angioarchitecture, in particular the diameter of arterio-arterial (AA) anastomoses in monochorionic placentas from pregnancies with twin-twin transfusion syndrome (TTTS) compared to a control group of uncomplicated monochorionic placentas. Placental angioarchitecture was analyzed using colored dye injection. AA anastomoses were detected in 37% (14/38) of TTTS placentas versus 91% (209/228) in control placentas ($p < 0.001$). The median diameter of AA anastomoses in the group with and without TTTS was 1.9 mm and 2 mm, respectively ($p = 0.711$). In conclusion, our findings show that AA anastomosis occur less frequently in TTTS placentas, supporting the concept of the protective role of AA anastomoses in TTTS. However, the size of the AA anastomosis, when present, does not appear to influence the pathophysiology of the disease.

INTRODUCTION

Twin-twin transfusion syndrome (TTTS) is a severe complication of 9% of monochorionic twin pregnancies [1]. It is caused by chronic imbalance in intertwin blood flow through the placental vascular anastomoses and leads to oligohydramnios in the donor twin and polyhydramnios in the recipient twin [2,3]. Untreated TTTS is associated with high mortality and morbidity rates [2,3]. The pathogenesis of TTTS is not yet clearly understood. Previous studies have shown that arterio-arterial (AA) anastomoses are significantly less common in TTTS placentas [4,5]. AA anastomoses are therefore thought to have a protective effect against TTTS by creating an equilibrating shift and compensating for hemodynamic imbalances [5,6].

The objective of this study was to compare the diameter of AA anastomoses in monochorionic (MC) twin placentas with and without TTTS to determine if the size of the AA anastomosis could play a role in the pathophysiology of TTTS.

METHODS

All TTTS placentas examined at our center between June 2002 and March 2012 were included in this study. For the purpose of this study we excluded placentas treated with fetoscopic laser coagulation.

Each MC placenta examined at our center is routinely injected with colored dye according to a previously described protocol [7]. After colored dye injection, placentas are photographed in a plain view, and digital pictures are saved for computer analysis. Data on placenta angioarchitecture, including the number and type of anastomoses, the percentage of placental territory and the type of umbilical cord insertion are prospectively entered in a dedicated database. The diameter of AA anastomoses was the primary outcome. This was measured with Image Tool for Windows version 3.0 (Image tool, San Antonio, Texas, USA, http://ddsdx.uthscsa.edu/dig/itdesc.html).

Each TTTS placenta with an AA anastomosis was compared with three control placentas from uncomplicated MC twin pregnancies (with an AA anastomosis). The uncomplicated control MC pregnancies were identified from a dedicated database in which all monochorionic twins delivered at our hospital are registered and were the next twin pregnancy with a matched gestational age at birth (± 1 week gestational age).

Diagnosis of TTTS was based on internationally accepted standardized antenatal ultrasound criteria [8] and staged according to the staging system from Quintero [9].

Results of continuous and categorical variables were analyzed using the Mann-Whitney U test and Fisher exact test. A p-value <0.05 was considered to indicate statistical

significance. All statistical data were analyzed using SPSS statistics version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 41 TTTS placentas (not treated with laser) were examined at our centre. Of these, three were excluded due to damage (n = 2) or contamination with formaldehyde (n = 1). Of the remaining 38 TTTS placentas, AA anastomoses were detected in 14 (37%) placentas. Quintero stage at diagnosis in the 14 included placentas was stage 1: n=6 (43%), stage 2: n=2, (14%); stage 3: n=5 (36%) and stage 4: n=1 (7%). Median Quintero stage at diagnosis in the study group was 2 (interquartile range (IQR): 1-3) and was similar to the TTTS group without AA anastomosis (median 2, IQR: 1-3)(p= .79). Eight TTTS pregnancies (57%) were treated with serial amnioreduction and 6 (43%) were managed expectantly.

Of the 228 uncomplicated MC placentas injected at our center during the same study period, 209 (91%) had an AA anastomosis. The rate of AA anastomosis in TTTS placentas was significantly lower than in the control group of uncomplicated MC placentas (37% (14/38) versus 91% (209/228); $p < 0.001$). We were able to match each TTTS placenta with an AA anastomosis (n = 14) with 3 control MC placentas (n = 38), except for three cases. Two TTTS cases delivered at 17 weeks' and 23 weeks' gestation could only both be matched with 2 control MC placentas and 1 TTTS case delivered at 20 weeks' gestation could only be matched with 1 control MC placenta. Mean gestational age at delivery in the TTTS group and control group was 28.4 weeks (± 5.7) and 29.1 weeks (± 5.4), respectively ($p = 0.549$).

The median diameter of AA anastomoses in the group with and without TTTS was 1.9 mm (range: 0.3-4.2) and 2.0 mm (range: 0.7-4.6), respectively ($p = 0.711$). The diameter of the AA anastomoses was ≤ 1 mm in 4 of the TTTS placentas (29%, 4/14) and in 6 (16%, 6/38) of the placentas without TTTS ($p = 0.428$). Further details on the placental characteristics in both groups are shown in Table 1. An example of an injected TTTS placenta with an AA anastomosis is shown in figure 1.

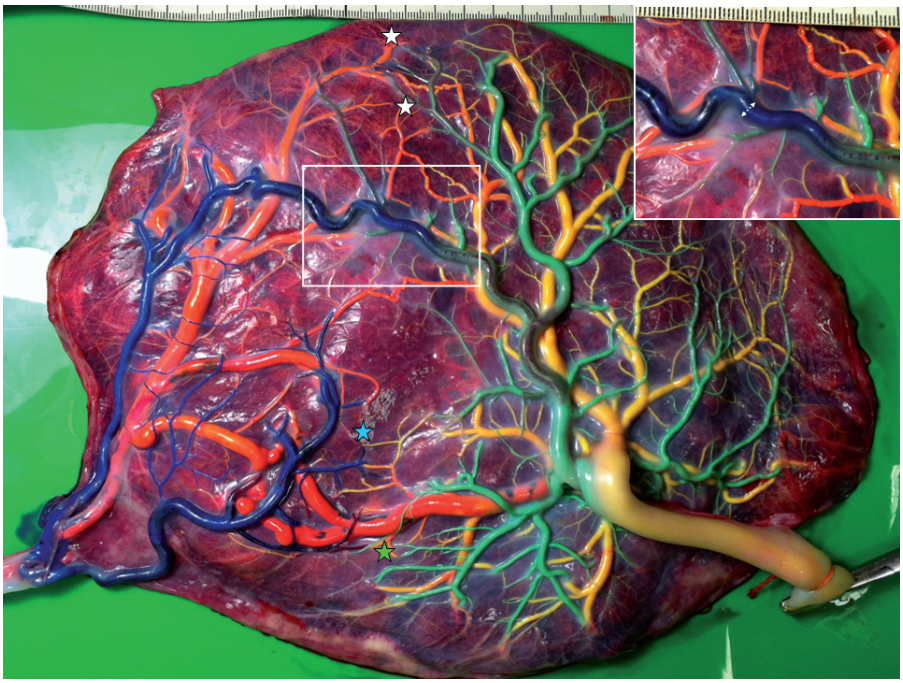

Figure 1: Monochorionic placenta delivered at 29 weeks gestation after stage 1 TTTS. Arteries are injected with blue or green dye and veins are injected with orange or yellow dye. A large arterio-arterial anastomosis (diameter 4.3mm) is visible in the middle of the placenta (detail of the anastomosis is shown in the top-right corner). The green star indicates a veno-venous anastomosis, the white stars indicate arterio-venous anastomoses and the blue star shows a veno-arterial anastomosis.

Table 1: Placental characteristics in MC placentas with and without TTTS

	MC placentas with TTTS (n=14)	MC placentas without TTTS (n=38)	p-value
Number of anastomoses per placenta[a]	7 (2-18)	7 (3-26)	0.963
VV anastomoses present – (%)	7 (50)	7 (18)	0.035
AV anastomoses present – (%)	13 (93)	38 (100)	0.269
Diameter of AA anastomosis (mm)[a]	1.9 (0.3-4.2)	2.0 (0.7-4.6)	0.711
Placental share discordance %[a]	19.5 (0.4-50)	17.7 (0-68)	0.663
Unequal placental sharing >20% – n (%)	7 (50)	11 (29)	0.197
Velamentous cord insertion – n (%)[b]	12 (43)	20 (26)	0.150
Marginal cord insertion – n (%)[b]	4 (14)	14 (18)	0.774
Velamentous or marginal cord insertion – n (%)[b]	16 (57)	32 (42)	0.190

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

DISCUSSION

This study shows that AA anastomoses occur less frequently in TTTS placentas than in uncomplicated MC control placentas, 37% (14/38) versus 91% (209/228) ($p < 0.001$). These findings are in accordance with several other studies showing the paucity of AA anastomoses in TTTS [4-6]. Our findings support therefore concept of protective role of AA anastomosis against the development of TTTS [4-6]. Several studies have shown that the bidirectional and low-resistance aspect of AA anastomoses may help equilibrate and compensate for hemodynamic inter-twin imbalances [4-6].

However, our results show that when an AA anastomosis is present, its size is not significantly different than AA anastomoses in control MC placentas, suggesting that the size of the AA anastomosis has no major role in the development of TTTS. The similar AA anastomosis diameter in the TTTS group and control group is in contrast to our recent findings of placentas with and without twin anemia-polycythemia sequence (TAPS) [10]. In TAPS, the diameter of AA anastomoses (when present) is significantly smaller compared to the AA diameter in a control group of uncomplicated MC placentas. The small size of the AA anastomosis may thus play a role in the development of TAPS by inhibiting adequate compensatory mechanisms [11].

Although both TTTS and TAPS result from of unbalanced feto-fetal transfusion, the two forms differ significantly in terms of pathogenesis and placental angioarchitecture [12]. We previously hypothesized that TAPS may primarily result from slow inter-twin blood transfusion, whereas TTTS is probably a more complex disease resulting from imbalanced inter-twin blood transfusion in combination with imbalanced hormonal regulation [12].

Our results should be interpreted with care due to the retrospective nature of the study and a possible selection bias. Although all cases fulfilled the criteria for TTTS, there was an overrepresentation of stage 1 cases (6/14), probably due to the fact that most TTTS cases diagnosed at our center with higher Quintero stages (>1) are treated primarily with laser surgery. Since it is not possible to measure the diameter of AA anastomoses in placentas treated with laser surgery, we had to exclude these cases. Additional studies on the AA diameter in another TTTS cohort should therefore be repeated, and performed in a country where laser treatment is not routinely available.

In conclusion, our findings show that AA anastomoses occur less frequently in TTTS placentas, supporting the concept of the protective role of AA anastomoses in TTTS. However, the size of the AA anastomosis, when present, does not appear to influence the pathophysiology of the disease.

REFERENCE LIST

[1] Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-8.

[2] Lewi L, Van Schoubroeck D, Gratacos E, Witters I, Timmerman D, Deprest J. Monochorionic diamniotic twins: complications and management options. Curr Opin Obstet Gynecol 2003;15:177-94.

[3] Lopriore E, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Twin-to-twin transfusion syndrome: from placental anastomoses to long-term neurodevelopmental outcome. Curr Pediatr Rev 2005;1:191-203.

[4] Taylor MJ, Denbow ML, Duncan KR, Overton TG, Fisk NM. Antenatal factors at diagnosis that predict outcome in twin-twin transfusion syndrome. Am J Obstet Gynecol 2000;183:1023-8.

[5] Denbow ML, Cox P, Taylor M, Hammal DM, Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol 2000;182:417-26.

[6] Umur A, van Gemert MJ, Nikkels PG, Ross MG. Monochorionic twins and twin-twin transfusion syndrome: the protective role of arterio-arterial anastomoses. Placenta 2002;23:201-9.

[7] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ, Oepkes D. Accurate and Simple Evaluation of Vascular Anastomoses in Monochorionic Placenta using Colored Dye. J Vis Exp 2011;e3208

[8] Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-44.

[9] Quintero RA. Twin-twin transfusion syndrome. Clin Perinatol 2003;30:591-600.

[10] de Villiers S, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D, Lopriore E. Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. Placenta 2012;33:227-9.

[11] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-90.

[12] Slaghekke F, Kist WJ, Oepkes D, Middeldorp JM, Klumper FJ, Vandenbussche FP, Lopriore E. TAPS and TOPS: two distinct forms of feto-fetal transfusion in monochorionic twins. Z Geburtshilfe Neonatol 2009;213:248-54.

Chapter 5

Correlation between veno-venous anastomoses, TTTS and perinatal mortality in monochorionic twin pregnancies

S.F. de Villiers

D.P. Zhao

D. Cohen

E.W. van Zwet

T. Duan

D. Oepkes

E. Lopriore

Placenta 2015, vol. 36(5) pp 603-6.

ABSTRACT

Introduction: The clinical significance of veno-venous (VV) anastomoses in monochorionic (MC) placentas remains inconclusive and controversial. The purpose of this study was to investigate the correlation between the presence of VV anastomoses and clinical outcome in a large cohort of MC twin pregnancies.

Methods: All MC placentas injected with colored dye from 2002 to 2014 were included in the study. We excluded MC pregnancies managed with fetoscopic laser surgery.

Results and Discussion: A total of 384 MC placentas were analyzed. VV anastomoses were detected in 27% (104/384) of MC placentas. The prevalence of twin-twin transfusion syndrome (TTTS) in MC placentas with VV anastomoses was significantly higher compared to MC placentas without VV anastomoses, 20% (21/104) versus 10% (29/280), respectively (P=.01). The overall perinatal mortality in MC twins with and without VV anastomoses was 16% versus 10%, respectively (P=.02). Risk factor analysis showed the presence of VV anastomoses was associated with perinatal mortality (P=.02; odds ratio (OR): 1.76; 95% confidence interval (CI): 1.11-2.79), but was not an independent risk factor for perinatal mortality (P=.26, OR: 0.66; 95% CI: 0.33-1.35) in MC twin pregnancies. However, VV anastomoses was associated with and was an independent risk factor for TTTS (P=.00, OR: 3.59; 95% CI: 1.72-7.47). VV anastomoses-related perinatal mortality may be due to the high rate of TTTS in MC twins with VV anastomoses.

Conclusion: The presence of VV anastomoses is correlated with TTTS and perinatal mortality, but is not an independent risk factor for perinatal mortality in MC twin pregnancies.

INTRODUCTION

Monochorionic (MC) twin pregnancies have an increased risk of adverse outcome as a result of the ubiquitous vascular anastomoses in MC placentas. There are three types of vascular anastomoses: arteriovenous (AV), arterioarterial (AA) and venovenous (VV) anastomoses. The AV anastomoses are formed at the capillary level within shared cotyledon and carry unidirectional blood from one twin to the other, which may result in chronic fetal transfusion, such as twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) [1]. The AA and VV anastomoses are respectively created by direct connection of arteries and veins from each twin. Thus, the blood flowing in AA and VV is bidirectional and is theoretically involved in the equilibrium of inter-twin blood distribution. AA anastomoses are rare in TTTS placentas. This has led to the theoretical idea that AA anastomoses may have a protective role against the development of TTTS, which has been substantiated by a computer model [2-5]. In contrast, a few studies reported that the presence of VV anastomoses was more common in TTTS placentas and could facilitate the development of TTTS [6, 7]. Furthermore, a previous study related VV anastomoses to increased perinatal mortality [2]. However, this was not detected in other studies [8-10]. Therefore, the correlation between VV anastomoses and clinical outcome in MC twin pregnancies remains inconclusive and controversial. This study aimed to investigate the influence of VV anastomoses on clinical outcome (including the development of TTTS and perinatal mortality) based on analysis of a large cohort of MC twin pregnancies.

MATERIALS AND METHODS

All MC placentas were consecutively examined at Leiden University Medical Center between July 2002 and November 2014. We excluded MC twins managed with fetoscopic laser surgery where the vascular anastomoses were iatrogenically photocoagulated. We also excluded MC placentas due to severe damage, maceration and fixation in formalin preventing colored-dye injection. At our center, MC placentas were routinely injected with color dye according to a previously published protocol [11]. After injection, the type and number of vascular anastomoses were recorded. High-resolution pictures were also perpendicularly taken for post-hoc computer-based measurements using Image J 1.45s (Image J, National Institute of Health, USA). The type of umbilical cord insertion was categorized as velamentous, marginal and (para-) central in each twin. Placental share was delineated as the venous return of each twin. Placental share difference was calculated by the larger placental share minus the smaller placental share.

MC twin placentas were classified into 5 groups, including 1) uncomplicated MC; 2) TTTS; 3) TAPS; 4) growth discordance; 5) monoamniotic (MA). TTTS was defined

according to the Eurofoetus criteria: polyhydramnios (deepest vertical pocket ≥ 8cm before 20 weeks of gestation or ≥ 10cm after 20 weeks of gestation) in the recipient and oligohydramnios (deepest vertical pocket ≤ 2cm) in the donor [12]. Diagnosis of TAPS is based on prenatal ultrasound findings or postnatal hematologic criteria as described before [13]. Growth discordance was defined as inter-twin birth-weight discordance ≥ 25%. Monoamnioticity was determined based on the ultrasound examination in first trimester showing absence of an inter-twin membrane. Birth weight discordance was calculated by the following formula: (larger twin birth weight – smaller twin birth weight) / larger twin birth weight x 100%. The respective clinical data were recorded in a SPSS-based database, such as occurrence of fetal loss (≤24 weeks gestational age) and fetal demise (> 24 weeks gestational age), neonatal mortality, gestational age at birth and birth weight.

Statistics

Continuous variables were compared using unpaired *t* test or *Mann-Whitney U* test, where appropriate. Categorical variables were analyzed using Chi-square or Fisher's exact test, as appropriate. The following potential risk factors for perinatal mortality were studied in an univariable regression model (binary regression), including TTTS, birth weight discordance, velamentous cord insertion and presence of VV anastomoses. Those factors significantly associated with perinatal mortality were further entered into a multivariable regression model (generalized estimating equation) to evaluate their independency in relation to perinatal mortality. Similar univariable and multivariable regression model analyses were performed with TTTS as the outcome. The results of correlation and risk factor analysis were displayed as odds ratio (OR) and 95% confidence interval (CI). A *P* value <.05 was accepted as statistical significance. All statistical analysis was performed using SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 411 MC placentas not managed with invasive fetal therapy were examined during this study period. Twenty-seven (7%) MC placentas were excluded due to maceration, severe damage and fixation in formalin. The resulting 384 cases (93%) were analyzed in this study, including 104 cases (27%) with VV anastomoses and 280 cases (73%) without VV anastomoses. The group of MC placentas with VV anastomoses included 54 (52%) uncomplicated MC, 21 (20%) TTTS, 1 (1%) TAPS, 20 (19%) growth discordance and 8 (8%) MA placentas. Cases with TTTS included in this study was staged according to Quintero system: 20 (40%) stage I, 7 (14%) stage II, 17 (35%) stage III, 3 (6%) stage IV and 3 (6%) stage V. The perinatal mortality rate in the cases with TTTS was 13% (5/40) in the cases with stage I, 43% (6/14) in cases with stage II, 47% in cases with stage III,

11% in cases with stage IV and 67% (4/6) in cases with stage V. Characteristics of MC placentas with and without VV anastomoses were summarized in Table 1. An example of a colored-dye injected MC placenta with VV anastomoses is shown in Figure 1.

Table 1. Placental characteristics of MC placentas with and without VV

	MC placentas with VV (n=104)	MC placentas without VV (n=280)	*p*-value
AA anastomoses present – n (%)	90 (87)	225 (80)	.16
Number of AV anastomoses – n [a]	8 (4-12)	6 (4-10)	.13
Total number of anastomoses per placenta – n [a]	9 (6-13)	7 (4-11)	.00
Velamentous cord insertion – n (%) [b]	50 (24)	131 (23)	.86
Placental share difference – % [a]	18 (9-34)	22 (9-34)	.38

[a] **Data** was displayed as median (IQR).
[b] **Denotes** the cord insertion of per fetus.

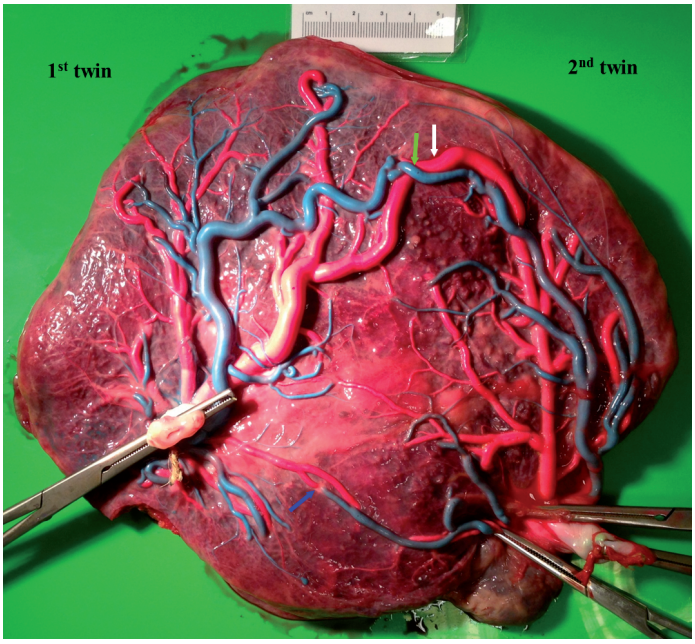

Figure 1: A TTTS (Quintero stage 3) monochorionic placenta managed expectantly and delivered at 32^{+4} weeks gestation. The 2nd twin is the ex-donor twin. After delivery, injection with colored dye (blue or green for arteries and pink or yellow for veins) was given to display the vascular anastomoses. The white and green arrows denote the VV and AA anastomosis, respectively. AV anastomoses are indicated with a blue arrow.

Mean gestational age at delivery in MC twins with VV anastomoses was similar to that of MC twins without VV anastomoses, 32.4±4.4 weeks versus 32.4±4.6 weeks (p=.72), respectively. Mean birth weight of MC twins with VV anastomoses was comparable to that of MC twins without VV anastomoses, 1896±780 grams versus 1939±739 grams (p=.49), respectively. The occurrence of overall fetal demise was significantly increased in MC twins with VV anastomoses compared with cases without VV anastomoses, 13% (28/208) versus 7% (41/560), respectively (p=.01; OR: 1.97; 95% CI: 1.18 to 3.28). Additional comparison of clinical outcome between MC twin pregnancies with and without VV anastomoses was presented in Table 2.

Table 2. Clinical outcome of MC twins with VV anastomoses compared with MC twins without VV anastomoses

	MC placentas with VV (n=104)	**MC placentas without VV (n=280)**	***p*-value**	OR (95% CI)
Early preterm (≤32weeks) - n (%)	42 (40)	113 (40)	1.00	1.00 (.63 – 1.58)
Growth discordance – n (%)	20 (19)	63 (23)	.21	.82 (.47 – 1.44)
TTTS – n (%)	21 (20)	29 (10)	.01	2.19 (1.19 – 4.05)
Fetal loss ≤ 24 weeks – n (%)	9 (9)	17 (6)	.37	1.47 (.63 – 3.40)
Fetal demise > 24 weeks – n (%) [a]	10 (5)	7 (1)	.00	3.99 (1.50 – 10.63)
Overall fetal demise – n (%) [a]	28 (13)	41 (7)	.01	1.97 (1.18 – 3.28)
Neonatal mortality – n (%) [a]	6 (3)	15 (3)	.88	1.08 (.41 – 2.82)
Overall perinatal mortality – n (%) [a]	34 (16)	56 (10)	.02	1.76 (1.11 – 2.78)

95% CI: 95% confidence interval.
[a] Refers to as the number of fetuses.

Univariable analysis indicated TTTS, birth weight discordance, velamentous cord insertion and VV anastomoses were associated with perinatal mortality (Table 3). These risk factors were subsequently included in the multivariable regression model (Table 3). We found that the presence of VV anastomoses was not an independent risk factor for perinatal mortality in MC twin pregnancies (p=.26, OR: .66; 95% CI: 0.33-1.35).

Univariable analysis showed that the presence of VV anastomoses was associated with and is an independent risk factor for TTTS (p=.00, OR: 3.59; 95% CI: 1.72-7.47) (Table 4). Inversely, AA anastomoses were associated with the prevention of TTTS (p=.00, OR: .08; 95% CI: .04-.16).

Table 3. Analysis of potential risk factors of perinatal mortality

Risk factors	Perinatal mortality (n=90)	Survivals (n=678)	*p* value	Univariate OR (95% CI)	Multivariate OR (95% CI)	*p* value
TTTS present	32/100 (32%)	68/100 (68%)	.00	4.95 (2.54-9.66)	5.0 (2.17 – 11.54)	.00
TTTS absent	58/668 (9%)	610/668 (91%)	-	-	-	-
Birth weight discordance - % [a]	20 (12-33%)	14 (6-25%)	.00	1.04 (1.03-1.06)	1.04 (1.02-1.07)	.00
Velamentous cord insertion present	36/181 (20%)	145/181 (80%)	.00	2.45 (1.55-3.88)	1.90 (1.17-3.07)	.01
Velamentous cord insertion absent	54/587 (9%)	533/587 (91%)	-	-	-	-
VV anastomoses present	34/208 (16%)	174/208 (84%)	.02	1.76 (1.11-2.79)	0.66 (0.33-1.35)	.26
VV anastomoses absent	56/560 (10%)	504/560 (90%)	-	-	-	-

[a] Data was displayed as median (IQR).

Table 4. Analysis of potential risk factors of TTTS

Risk factors	TTTS (n=50)	Non-TTTS (n=334)	*p* value	Univariate OR (95% CI)	Multivariate OR (95% CI)	*p* value
AA anastomoses, present	22/50 (44%)	294/334 (88%)	.00	.08 (.05-.13)	.08 (.04-.16)	.00
AA anastomoses, absent	28/50 (56%)	40/334 (12%)	-	-	-	-
Velamentous cord insertion present	27/50 (54%)	138/334 (41%)	.01	1.90 (1.18-3.06)	1.91 (.99-3.67)	.05
Velamentous cord insertion absent	23/50 (46%)	196/334 (59%)	-	-	-	-
VV anastomoses, present	21/50 (42%)	83/334 (25%)	.00	3.59 (2.15-6.02)	3.59 (1.72-7.47)	.00
VV anastomoses, absent	29/50 (58%)	251/334 (75%)	-	-	-	-

DISCUSSION

This is the first study in which we looked into more detail in the association between VV anastomoses and clinical outcome in a large cohort of MC twin pregnancies. Our previous placental studies focused mainly on the role of AA anastomoses, abnormal cord insertions and placental share [14-16]. This study shows that the presence of VV anastomoses decreases perinatal survival rate, but is not an independent risk factor for perinatal mortality in MC twin pregnancies. Furthermore, this study shows that the presence of VV anastomoses is associated with TTTS.

The presence of AV, AA and VV anastomoses in MC placentas connects the two circulations and causes hemodynamic changes. The presence of AV and AA anastomoses in relation to clinical pictures has been well studied both *in vivo* and *in vitro* [1, 17-19]. In contrast, the role of VV anastomoses has thus far been paid little attention, which is partially due to the paucity of VV anastomoses (present in approximately 25% MC placentas). In a small cohort, Denbow et al. related VV anastomoses to decreased perinatal survival [2], which is in accordance with our findings in this study. However, this detrimental effect was not detected in other small studies [8-10]. Although the prevalence of VV anastomoses is similar in these studies (17% to 25%), the constitution of various types of MC twins was not shown in these studies, preventing further comparison.

It is not clear why the presence of VV anastomoses contributes to perinatal mortality.. A previous study indicated VV anastomoses played a role in the development of TTTS, the most important cause of perinatal mortality in MC twins [7]. Consistently, in this study the group of MC twins with VV anastomoses has a higher proportion of TTTS (20%) compared to the group of MC twins without VV anastomoses where the prevalence of TTTS (10%), which is comparable to that (9%) reported by Lewi et al. in a prospective cohort [20]. Furthermore, it is hypothesized that inter-twin sudden transfusion can occur through VV anastomoses. These hemodynamic changes in shared venous system may prompt the development of TTTS [7], although severe sudden blood loss may also result in fetal demise prior to the clinical presentation of TTTS."[21]. Nevertheless, this hemodynamic change cannot be distinguished during ultrasound examination due to the unexpected timing of occurrence and lack of recognizable sonographic pattern [18].

This study further evaluates whether VV anastomoses is an independent risk factor of perinatal mortality in MC twin gestations. In monochorionic twins, TTTS, discordant growth and velamentous cord insertion are strongly associated with perinatal mortality [20, 22, 23]. These factors coupled with VV anastomoses were used to establish a multiple regression model. Interestingly, we found the presence of VV anastomoses was not independently associated with perinatal mortality in MC twin gestations. This implies that VV anastomoses-related perinatal mortality may be explained primarily by the high rate of TTTS, rather than by the presence or absence of VV anastomoses.. VV anastomoses

are, however, independently associated with the presence of TTTS in MC twins. This substantiates the theory that VV anastomoses facilitate the development of TTTS.

One limitation of our study, besides its retrospective nature, is the selection bias as not all MC placentas were included. However, we minimized the exclusion to 7% of all MC placentas examined during this study period. Another potential limitation of our study is that most TTTS cases referred to our center are managed with fetoscopic laser surgery. Therefore, the subgroup of TTTS placentas not treated with fetoscopic laser surgery in this study may not be representative of the general population of TTTS. In particular a slightly higher prevalence of VV anastomoses (42%, 21/50) was present compared with that reported in previous studies (from 16% to 38%) [2, 6, 8]. Nevertheless, the higher prevalence of VV anastomoses in our cohort may be also due to the advances in injection studies. It is noticed that some VV anastomoses are quite small and can be obscured by the paired artery running over it. The colored-dye injection can solve this problem because of the mixture of different colored dyes when VV anastomoses are present. Finally, our cohort is not large enough to investigate the effect of VV anastomoses on clinical outcome by excluding the confounding from AA anastomoses. The role of AA anastomoses in equilibrating the inter-twin blood distribution has been well demonstrated. In the vast majority of cases (90%), VV anastomoses coincide with the concomitant presence of AA anastomoses, with the exception of the subgroup of TTTS placentas (50%) [8]. Further investigation is required to show whether the hemodynamic change caused by VV anastomoses can be reflected by the alteration of sonographic pattern of AA anastomoses. The elegant method described to detect the hemodynamics in AA anastomoses may be useful to clarify this interaction between VV and AA anastomoses [18, 19]. To date, the presence of VV anastomoses cannot be detected directly in a reliable way during prenatal assessment. The information reported in this study has therefore no direct clinical implications but may help elucidate and understand the implications and the role of VV anastomoses.

In conclusion, our study demonstrated that the presence of VV anastomoses was correlated with perinatal mortality, but was not an independent risk factor for perinatal mortality in MC twin gestations. VV anastomoses are, however, independent risk factors for the development of TTTS. Further studies are needed to investigate the interaction of hemodynamic changes between AA and VV anastomoses.

REFERENCES

[1] Lopriore E, van den Wijngaard JP, Middeldorp JM, Oepkes D, Walther FJ, van Gemert MJ and Vandenbussche FP. Assessment of feto-fetal transfusion flow through placental arterio-venous anastomoses in a unique case of twin-to-twin transfusion syndrome. Placenta. 2007;28(2-3):209-11.

[2] Denbow ML, Cox P, Taylor M, Hammal DM and Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol. 2000;182(2):417-26.

[3] Diehl W, Hecher K, Zikulnig L, Vetter M and Hackeloer BJ. Placental vascular anastomoses visualized during fetoscopic laser surgery in severe mid-trimester twin-twin transfusion syndrome. Placenta. 2001;22(10):876-81.

[4] Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D and Lopriore E. Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta. 2013;34(7):589-93.

[5] Umur A, van Gemert MJ, Nikkels PG and Ross MG. Monochorionic twins and twin-twin transfusion syndrome: the protective role of arterio-arterial anastomoses. Placenta. 2002;23(2-3):201-9.

[6] De Paepe ME, Shapiro S, Greco D, Luks VL, Abellar RG, Luks CH and Luks FI. Placental markers of twin-to-twin transfusion syndrome in diamniotic-monochorionic twins: A morphometric analysis of deep artery-to-vein anastomoses. Placenta. 2010;31(4):269-76.

[7] Zhao DP, Cohen D, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D and Lopriore E. The role of veno-venous anastomoses in twin-twin transfusion syndrome. Placenta. 2014;35(5):334-6.

[8] Hack KE, Nikkels PG, Koopman-Esseboom C, Derks JB, Elias SG, van Gemert MJ and Visser GH. Placental characteristics of monochorionic diamniotic twin pregnancies in relation to perinatal outcome. Placenta. 2008;29(11):976-81.

[9] Hack KE, van Gemert MJ, Lopriore E, Schaap AH, Eggink AJ, Elias SG, van den Wijngaard JP, Vandenbussche FP, Derks JB, Visser GH and Nikkels PG. Placental characteristics of monoamniotic twin pregnancies in relation to perinatal outcome. Placenta. 2009;30(1):62-5.

[10] Nikkels PG, Hack KE and van Gemert MJ. Pathology of twin placentas with special attention to monochorionic twin placentas. J Clin Pathol. 2008;61(12):1247-53.

[11] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ and Oepkes D. Accurate and simple evaluation of vascular anastomoses in monochorionic placenta using colored dye. J Vis Exp. 2011;(55):e3208.

[12] Senat MV, Deprest J, Boulvain M, Paupe A, Winer N and Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Eng J Med. 2004;351(2):136-44.

[13] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP and Lopriore E. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther. 2010;27(4):181-90.

[14] Zhao D, Slaghekke F, Middeldorp JM, Duan T, Oepkes D and Lopriore E. Placental share and hemoglobin level in relation to birth weight in twin anemia-polycythemia sequence. Placenta. 2014;35(12):1070-4.

[15] de Villiers SF, Slaghekke F, Middeldorp JM, Walther FJ, Oepkes D and Lopriore E. Arterio-arterial vascular anastomoses in monochorionic placentas with and without twin-twin transfusion syndrome. Placenta. 2012;33(8):652-4.

[16] Costa-Castro T, De Villiers S, Montenegro N, Severo M, Oepkes D, Matias A and Lopriore E. Velamentous cord insertion in monochorionic twins with or without twin-twin transfusion syndrome: Does it matter? Placenta. 2013;34(11):1053-8.

[17] Denbow ML, Taylor M, Cox P and Fisk NM. Derivation of rate of arterio-arterial anastomotic transfusion between monochorionic twin fetuses by Doppler waveform analysis. Placenta. 2004;25(7):664-70.

[18] Denbow ML, Cox P, Talbert D and Fisk NM. Colour Doppler energy insonation of placental vasculature in monochorionic twins: absent arterio-arterial anastomoses in association with twin-to-twin transfusion syndrome. Br J Obstet Gynaecol. 1998;105(7):760-5.

[19] Taylor MJ, Denbow ML, Tanawattanacharoen S, Gannon C, Cox PM and Fisk NM. Doppler detection of arterio-arterial anastomoses in monochorionic twins: feasibility and clinical application. Hum Reprod. 2000;15(7):1632-6.
[20] Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P and Deprest J. 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).
[21] Lewi L, Deprest J and Hecher K. The vascular anastomoses in monochorionic twin pregnancies and their clinical consequences. American Journal of Obstetrics and Gynecology. 2013;208(1):19-30.
[22] Gratacos E, Carreras E, Becker J, Lewi L, Enriquez G, Perapoch J, Higueras T, Cabero L and Deprest J. Prevalence of neurological damage in monochorionic twins with selective intrauterine growth restriction and intermittent absent or reversed end-diastolic umbilical artery flow. Ultrasound Obstet Gynecol. 2004;24(2):159-63.
[23] Machin GA. Velamentous cord insertion in monochorionic twin gestation - An added risk factor. J Reprod Med. 1997;42(12):785-9.

Part III

TAPS

Chapter 6

Short communication:

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

S.F. de Villiers

F. Slaghekke

J.M. Middeldorp

F.J. Klumper

F.J. Walther

D. Oepkes

E. Lopriore

Placenta, 2012 vol. 33(3) pp. 227-9.

ABSTRACT

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

INTRODUCTION

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

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

METHODS

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

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

The primary outcome was the diameter of the AA anastomoses. Diameters of the AA anastomoses and individual placental territories were measured using Image Tool for Windows version 3.0 (Image Tool, San Antonio, Texas, USA, http://ddsdx.uthscsa.edu/dig/itdesc.html). Part of the placental data reported in this study was included previously in a case report on AA anastomoses in TAPS [1,4].

Results of continuous and categorical variables were analyzed using the Mann Whitney U test and Fisher exact test. A p-value <0.05 was considered to indicate statistical

significance. All statistical data were analyzed using SPSS statistics version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

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

The median diameter of AA anastomoses in the group with and without TAPS was 0.4mm (range: 0.3 – 0.6) and 2.2mm (range: 0.7 - 3.5), respectively (p = 0.01). The diameter of the AA anastomosis was ≤ 1mm in each TAPS placenta whereas only 2 AA anastomoses in the control group had a diameter ≤ 1 mm (22.2%, 2/9), leading to a sensitivity of 100% (3/3) and a specificity of 77.8% (7/9). A total of 11 AV anastomoses were detected in the 3 TAPS cases. The direction of the AVs in the TAPS cases was mostly (64%, 7/11) from the anemic fetus to the polycythemic one. Further details on placental characteristics in both groups are shown in Table 1. An example of a TAPS placenta with a small AA anastomosis is shown in Figure 1.

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

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

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

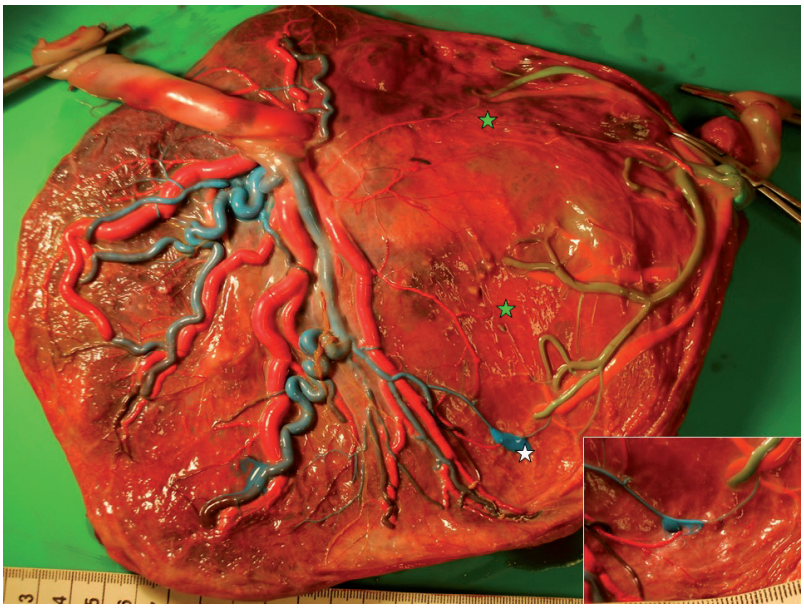

Figure 1: TAPS placenta after colored dye injection (blue and green for arteries, pink and orange for veins) with a small AA anastomosis (white star)(diameter of anastomosis: 0.3mm. Detail of the AA anastomosis is shown in the bottom-right corner. The two green stars indicate two small AV anastomoses.

DISCUSSION

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

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

AA anastomoses are bidirectional anastomoses and are thought to have protective properties against hemodynamic imbalance caused by AV anastomoses [8-10], as confirmed by a mathematical computer model [11]. However, the blood flow through a minuscule AA anastomosis is probably extremely low and insufficient to allow for adequate equilibration of the blood volumes between both twins. Inter-twin blood flow volume and velocity is known to be strongly correlated to the diameter of an anastomosis, according to

Pouiseuille's equation. Flow resistance depends linearly upon the viscosity and the length of a vessel, but depends to the fourth power upon the radius. Minuscule AA anastomoses may thus have a high flow resistance and thus fail to prevent the development of TAPS.

In conclusion, AA anastomoses in placentas with TAPS are rare. When present, the diameter of the AA anastomosis is small (≤ 1mm), subsequently inhibiting adequate compensatory mechanisms and allowing development of TAPS.

REFERENCE LIST

[1] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-90.

[2] Lopriore E, Deprest J, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Lewi L. Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstet Gynecol 2008;112:753-8.

[3] Weingertner AS, Kohler A, Kohler M, Bouffet N, Hunsinger MC, Mager C, Hornecker F, Neumann M, Schmerber E, Tanghe M, Viville B, Favre R. Clinical and placental characteristics in four new cases of twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2010;35:490-4.

[4] van Meir H, Slaghekke F, Lopriore E, van Wijngaarden WJ. Arterio-arterial anastomoses do not prevent the development of twin anemia-polycythemia sequence. Placenta 2010;31:163-5.

[5] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ, Oepkes D. Accurate and Simple Evaluation of Vascular Anastomoses in Monochorionic Placenta using Colored Dye. J Vis Exp 2011;5;e3208

[6] Lopriore E, van den Wijngaard JP, Middeldorp JM, Oepkes D, Walther FJ, van Gemert MJ, Vandenbussche FP. Assessment of feto-fetal transfusion flow through placental arterio-venous anastomoses in a unique case of twin-to-twin transfusion syndrome. Placenta 2007;28:209-11.

[7] Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP. Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47-51.

[8] Denbow ML, Cox P, Taylor M, Hammal DM, Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol 2000;182:417-26.

[9] Umur A, van Gemert MJ, Nikkels PG. Monoamniotic-versus diamniotic-monochorionic twin placentas: anastomoses and twin-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1325-9.

[10] Bajoria R, Wigglesworth J, Fisk NM. Angioarchitecture of monochorionic placentas in relation to the twin-twin transfusion syndrome. Am J Obstet Gynecol 1995;172:856-63.

[11] Umur A, van Gemert MJ, Nikkels PG, Ross MG. Monochorionic twins and twin-twin transfusion syndrome: the protective role of arterio-arterial anastomoses. Placenta 2002;23:201-9.

Chapter 7

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

S.F. de Villiers

F. Slaghekke

J.M. Middeldorp

F.J. Walther

D. Oepkes

E. Lopriore

Placenta, 2013 vol. 34(5) pp. 456-9.

ABSTRACT

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

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

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

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

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

INTRODUCTION

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

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

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

METHODS

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

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

Each monochorionic placenta examined at our center is routinely injected with colored dye according to a previously described protocol [11]. After colored dye injection, placentas are photographed in a plain view, and digital pictures are saved for computer analysis. Data on placenta angioarchitecture, including the number, type and size of anastomoses and the percentage of placental territory are recorded and entered in a dedicated database.

We also recorded the type of abnormal umbilical cord insertion, velamentous or marginal insertion (within 1 cm of the placental margin). Combination insertions are the combination of cord insertions of one placenta. The term peripheral is used in this context to indicate both marginal and velamentous insertions. The insertion-diameter ratio is the ratio between the distance between the two cord insertions and the maximum diameter of the placenta.

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

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

RESULTS

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

In the post-laser TAPS group, 5/27 (18.5%) pregnancies were treated with intrauterine transfusion (IUT) or intraperitoneal transfusion. In the spontaneous TAPS group, 3/16 (18.8%) pregnancies were treated with intrauterine transfusion or intraperitoneal transfusion. Median gestational age at delivery was 33.5 weeks (interquartile range (IQR): 31-35 weeks) and 33 weeks (IQR: 29-34 weeks) in the spontaneous TAPS group and post-laser TAPS group, respectively (p = 0.038). Median intertwin hemoglobin difference in the spontaneous and post-laser TAPS group was 14.2g/dL and 12.2g/dL, respectively (p=0.784). Further details on the baseline characteristics are shown in Table 1.

A total of 124 anastomoses were detected. Of all anastomoses, the vast majority 96% (119/124) had a diameter <1mm. Median total number of anastomoses in the spontaneous TAPS group and post-laser TAPS group was 4 anastomoses (IQR: 3-5) and 2

anastomoses (IQR: 1-3), respectively (p=0.003). AV anastomoses were present in all (43/43) placentas. AA anastomoses were present in only 14% (6/43) of TAPS placentas and were detected in 18.8% (3/16) of spontaneous TAPS placentas compared to 11.1% (3/27) of post-laser TAPS placentas.

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

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

[a]value given as median (IQR)

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

In the spontaneous TAPS group, we found that in 75% (12/16) of placentas, AV anastomoses from donor to recipient were accompanied by VA anastomoses in opposite direction (from recipient to donor) or by bidirectional AA anastomosis. In the post-laser TAPS group, this combination occurred in only 37% (10/27) of cases. Compensating VA anastomoses or AA anastomoses occurred significantly more frequently in the spontaneous TAPS group than post-laser TAPS group (75% vs 37%, p< 0.01).

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

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

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

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

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

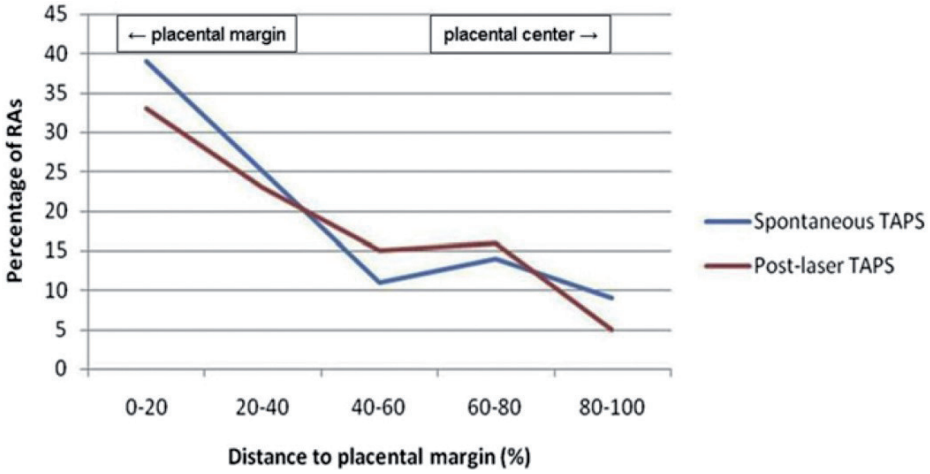

Figure 1: Distance of the anastomoses in relation to the margin of the placenta.

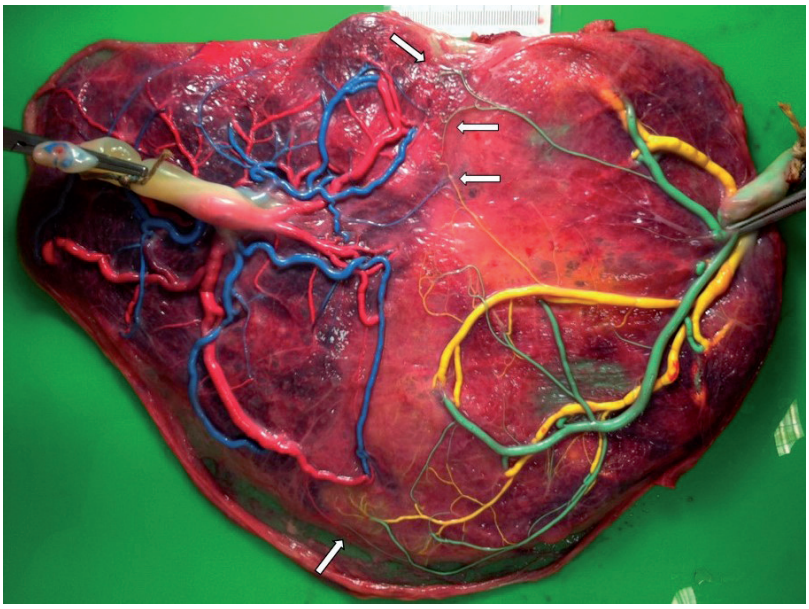

Figure 2: Spontaneous TAPS placenta after colored dye injection (blue or green for arteries and pink or yellow for veins). The placenta share on the right side of the picture belongs to the anemic donor and the placenta share on the left side belongs to the recipient. The white arrows indicate the AV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

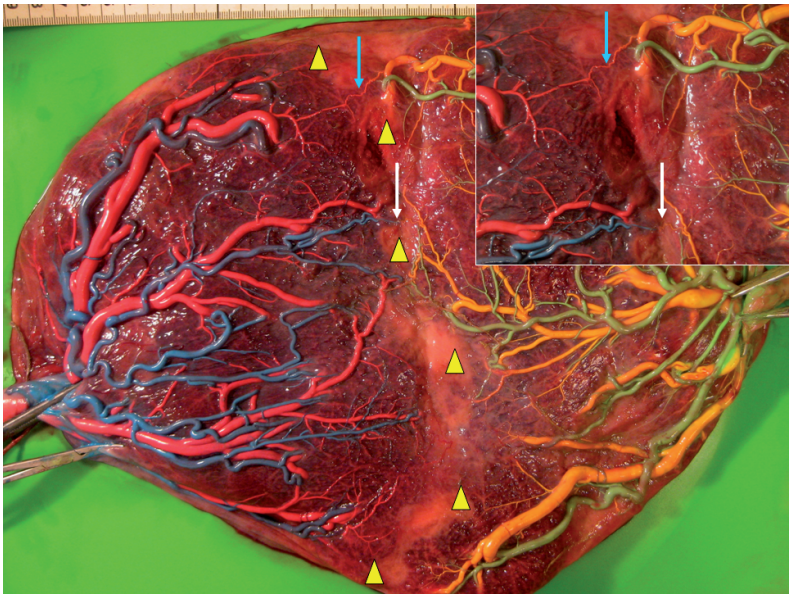

Figure 3: Post-laser TAPS placenta after colored dye injection lasered at 24 weeks gestation and delivered at 37 weeks gestation. The placenta share on the right side of the picture belongs to the anemic donor and the placenta share on the left side belongs to the recipient. The white arrow indicates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

DISCUSSION

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

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

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

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

This study also shows that (residual) anastomoses (RA) in post-laser TAPS cases are usually found on the margin of the placenta, as previously also reported [10]. Interestingly, we found the same trend of higher rates of anastomoses nearer to the placental margin compared to the placental center in the spontaneous TAPS group. The increased rate of residual anastomoses in the post-laser group is thought to result from increased technical difficulties during fetoscopy to accurately visualize the complete vascular equator. The

reason for the increased rate of anastomoses near the placental margin in the spontaneous TAPS group is not known. Whether this unequal spread of anastomoses differs from other monochorionic placentas with or without TTTS is not known either, as the localization of anastomoses has not yet been studied in monochorionic placentas.

The optimal management of TAPS is not clear and includes expectant management, intrauterine blood transfusion and fetoscopic intervention to coagulate the vascular anastomoses [1,2,8,15-17]. The clinical implication of the findings in this study is that if fetoscopic laser coagulation is envisaged, most of the few anastomoses in post-laser TAPS and spontaneous TAPS cases are found near the margins of the placenta. Since these anastomoses are extremely small and difficult to detect, laser coagulation of the complete equator (Solomon technique) [2] would probably be the preferred laser technique. However, more studies (ideally randomized controlled trials) are required to determine the best treatment intervention in TAPS cases.

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

REFERENCE LIST

[1] Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J, Ville Y. 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;194:796-803.

[2] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-90.

[3] Lopriore E, Oepkes D. Fetal and neonatal haematological complications in monochorionic twins. Semin Fetal Neonatal Med 2008;13:231-8.

[4] Lopriore E, Deprest J, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Lewi L. Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstet Gynecol 2008;112:753-8.

[5] de Villiers S, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D, Lopriore E. Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. Placenta 2012;33:227-9.

[6] Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-8.

[7] Gucciardo L, Lewi L, Vaast P, Debska M, De CL, Van MT, Done E, Devlieger R, Deprest J. Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438-42.

[8] Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R, Crombleholme T. Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201:417

[9] Rustico MA, Lanna MM, Faiola S, Schena V, Dell'avanzo M, Mantegazza V, Parazzini C, Lista G, Scelsa B, Consonni D, Ferrazzi E. Fetal and maternal complications after selective fetoscopic laser surgery for twin-to-twin transfusion syndrome: a single-center experience. Fetal Diagn Ther 2012;31:170-8.

[10] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;

[11] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, van Lith JM, Walther FJ, Oepkes D. Accurate and Simple Evaluation of Vascular Anastomoses in Monochorionic Placenta using Colored Dye. J Vis Exp 2011;e3208

[12] van den Wijngaard JP, Lewi L, Lopriore E, Robyr R, Middeldorp JM, Vandenbussche FP, Devlieger R, Deprest J, Ville Y, van Gemert MJ. Modeling Severely Discordant Hematocrits and Normal Amniotic Fluids After Incomplete Laser Therapy in Twin-To-Twin Transfusion Syndrome. Placenta 2006;

[13] Lopriore E, van den Wijngaard JP, Middeldorp JM, Oepkes D, Walther FJ, van Gemert MJ, Vandenbussche FP. Assessment of feto-fetal transfusion flow through placental arterio-venous anastomoses in a unique case of twin-to-twin transfusion syndrome. Placenta 2007;28:209-11.

[14] Lopriore E, van den Wijngaard JP, Pasman SA, Oepkes D, Walther FJ, van Gemert MJ, Vandenbussche FP. Quantification of feto-fetal transfusion rate through a single placental arterio-venous anastomosis in a monochorionic twin pregnancy. Placenta 2009;30:223-5.

[15] Weingertner AS, Kohler A, Kohler M, Bouffet N, Hunsinger MC, Mager C, Hornecker F, Neumann M, Schmerber E, Tanghe M, Viville B, Favre R. Clinical and placental characteristics in four new cases of twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2010;35:490-4.

[16] Herway C, Johnson A, Moise K, Moise KJ, Jr. Fetal intraperitoneal transfusion for iatrogenic twin anemia-polycythemia sequence after laser therapy. Ultrasound Obstet Gynecol 2009;33:592-4.

[17] Groussolles M, Sartor A, Connan L, Vayssiere C. Evolution of middle cerebral artery peak systolic velocity after a successful laser procedure for iatrogenic twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2012;39:354-6.

Part IV

Discussion and Summery

Chapter 8

General discussion and future perspectives

S.F. de Villiers

Great strides have been made in recent years to improve our understanding of the intertwin transfusion syndromes associated with monochorionic (MC) pregnancies. Placental studies with colored dye injection have proved essential to the understanding of the pathophysiology of the various complications and the detection of new disorders in MC twin pregnancies. Both twin-to-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) develop from a net imbalance of blood flow mainly over arterio-venous (AV) anastomoses. However, there are striking differences in the placental angioarchitecture and therefore in the amount of blood flow which results in the different clinical presentations.

This thesis presents a number of studies on the placental angioarchitecture of both TTTS and TAPS to investigate the pathogenesis of these diseases.

In *Chapter 2* we give a detailed description of the technique of placental dye-colored injection. This technique is of paramount importance in studying the placental angioarchitecture of MC placentas. Further a review of literature is given regarding the differences in placental architecture between various types of MC placentas, including normal MC placentas, TTTS placentas (with and without laser treatment), TAPS placentas, selective intrauterine growth restriction (sIUGR) placentas, monoamniotic placentas, twin reversed arterial perfusion sequence (TRAP) placentas and bipartite placentas.

Twin-to-twin transfusion syndrome (TTTS)

Extensive placental research during the last two decades has led to a clearer understanding of the pathogenesis of TTTS. The ubiquitous presence of deep, unidirectional arterio-venous (AV) anastomoses is the main cause of unbalanced blood flow and the development of TTTS. TTTS cannot develop without these anastomoses. Another crucial finding is related to the role of arterio-arterial (AA) anastomoses. Various studies have shown that AA anastomoses occur less frequently in TTTS placentas compared to uncomplicated MC placentas and imply that AA anastomoses play a protective role in preventing the development of TTTS [1,2]. AA anastomoses are bidirectional and have a low resistance. It is believed that they can compensate for hemodynamic imbalances by creating an equilibrating shift. Another important aspect of MC placentas, besides the type of vascular anastomoses, is related to the type of umbilical cord insertions. Controversy persists as to whether velamentous cord insertions (VCI) play a role in the development of TTTS. Several authors showed an increased rate of VCI in TTTS and suggested that VCI may lead to TTTS because of utero-placental insufficiency caused by VCI [3-6]. However, other studies contradicted those findings [7-9]. The main limitations of these previous studies was the small sample size. We therefore set up a large multicenter study, with enough power to detect significant differences and determine the role of VCI in TTTS compared to normal MC placentas (*Chapter 3*). In this study of 304 TTTS placentas and 326 normal MC placentas, VCI was detected in 36.8% of placentas with TTTS versus

35.9% without TTTS (p = 0.886). The presence of VCI in one twin was associated with small for gestational age twin and severe birth weight discordance, as is to be expected. In the normal MC group an increase in intrauterine fetal demise was seen from 4.6% to 14.1% (p = 0.027), as well as a decrease in gestational age at birth in the presence of VCI. Our study suggests that VCI is not associated with the development of TTTS, invalidating previous hypotheses. Both VCI and TTTS are independent risk factors for intrauterine fetal demise and lower gestational age at birth.

Although the protective role of AA anastomoses has largely been demonstrated, it is still not clear why TTTS may still develop, in a minority of cases, in the presence of an AA anastomosis. We hypothesized that the protective role of AA anastomoses could be related to the diameter of this vessel: a smaller AA anastomosis could, hypothetically, be less likely to equilibrate the intertwin difference of blood than a larger anastomosis due to the higher resistance. We studied the incidence and diameter of AA anastomoses in TTTS placentas in *Chapter 4*. In accordance with previous studies, we found a lower incidence of AA anastomoses in TTTS placentas than in normal MC placentas (37% and 91%, respectively, p < 0.001). However, we found no significant difference in the diameter of AA anastomoses in the TTTS group and the group without TTTS. A potential limitation of our study was the small sample size and possible lack of significant power. In a recent study we repeated the evaluation described here above but this time with a larger set of placentas. In this second larger study the diameter of the AA anastomoses was found to be smaller than in the control group of uncomplicated MC placentas, contesting our previous findings [10]. A larger diameter of the AA anastomosis allows a greater volume of blood to pass through because of a decreased vascular resistance (Poiseuille's law) and therefore could allow for equilibration of the hemodynamic imbalances. Further studies, preferably also by other research groups, are necessary to verify these findings.

Although the role of AA and AV anastomoses in the development of TTTS has been widely investigated, the role of VV anastomoses remains unclear and has received very little attention. VV anastomoses, in analogy to AA anastomoses, are superficial anastomoses and allow bidirectional flow. However, venous blood pressure is lower than arterial blood pressure. This could, hypothetically, lead to blood transfusion as a result of the influence of external factors such as the position of the fetuses on the venous blood pressure. In an effort to unravel the role of veno-venous (VV) anastomoses in MC placentas and specifically in TTTS we performed a single center study focusing on these VV anastomoses (*Chapter 5)*. The prevalence of VV anastomoses is significantly higher in TTTS placentas than in normal MC placentas, 37% and 7% respectively (p < 0.01). This suggests that VV anastomoses may support the development of TTTS [11]. In MC placentas with VV anastomoses the overall perinatal mortality was 16% versus 10% in the group without VV anastomoses (p = 0.02). The presence of VV anastomoses is associated with perinatal mortality, but is not an independent risk factor. However, the

presence of VV anastomoses was associated with and was an independent risk factor for TTTS ($p < 0.01$, OR: 3.59; 95% CI: 1.72-7.47). VV anastomoses-related perinatal mortality may be due to the high rate of TTTS (and its complications) in MC twins with VV anastomoses. This study is limited by its relatively small sample size. At present we are conducting a multicenter study with 5 other centers to confirm our conclusions. As shown in these and other studies, the type and size of anastomoses may play an important role in the development of TTTS. However, other placental factors remain to be elucidated such as the importance of placental share. Differences in placental share are known to be related to the development of sIUGR but could also play a role, in combination with additional factors, in the development of TTTS. In addition, most studies reported here above, analyzed MC placentas on a macroscopic level, but very few attempts have been made at studying the characteristics of TTTS placentas at a microscopic level. In one study looking at possible microscopic differences, deep-hidden anastomoses were found using casting techniques to evaluate chorionic-plate anastomoses. However, no inter-twin hemoglobin (Hb) difference was found between placentas with and without deep-hidden anastomoses. Deep-hidden anastomoses are believed to have no clinical consequences [12].

Although it would seem that the placental angioarchitecture plays the largest role, the development of TTTS is most probably also related to hormonal factors. On this front more research is necessary to obtain a fuller picture of the influence of vasoactive and hormonal factors, including the renin-angiotensin system, insulin-like growth factor (IGF)-II, leptin, and endothelin-1. It has been hypothesized that the release of vasoactive substances and oliguria in the donor could cause hypertension and renal damage, while transfer of these substances could cause hypertension in the recipient [13]. However, when related to the expected blood pressure for birth weight, donors had a lower than expected blood pressure and recipients a higher [14]. It would be interesting to look further at the blood pressure and possibly at the relative renal function of MC twins with TTTS.

Lastly, one of the most important roles of placenta injection studies with color dye is related to the detection of residual anastomoses in TTTS placentas treated with laser. This is important for a number of reasons, including, to evaluate the success of the operation and the operator performance as a type of quality control. This is not only necessary for the outcome of a single operation, but also to improve the techniques of fetal surgery in general and eventually create a benchmark or standard of practice [15].

Twin anemia-polycythemia sequence (TAPS)

In 2007, our research group discovered and described for the first time a new disease in MC twins and used the term ‘twin anemia-polycythemia sequence’. Since then our understanding of this new type of chronic feto-fetal transfusion has increased dramatically and in 2010 diagnostic and staging criteria were proposed [16]. TAPS, whether it

occurs spontaneously or iatrogenically after laser coagulation, is based on an imbalance in blood flow through a few minuscule AV anastomoses. On average spontaneous TAPS placentas have only 5 anastomoses compared to 10 in uncomplicated MC placentas [17]. The small size and small number of these anastomoses allow a very slow transfer of blood from the donor to the recipient, without the associated hormonal imbalance as seen in TTTS and, most importantly, without the development of polyhydramnios in the recipient and oligohydramnios in the donor. The lack of fluid imbalance implies that TAPS pregnancies do not result in premature contractions due to symptomatic polyhydramnios as often seen in TTTS. TAPS may go undetected unless Doppler ultrasound measurements are performed to detect fetal anemia and polycythemia.

In the first small cases series with TAPS placentas, all initial cases were characterized by the absence of AA anastomoses, which seemed to be a striking and unique finding. However, as TAPS started to become better known and the detection of TAPS cases started to increase, new findings emerged. One of these findings was that in sporadic cases, small AA anastomoses were detected, suggesting that TAPS may also develop in the presence of an AA anastomosis. To accurately evaluate the incidence of AA anastomoses, we analyzed our placental data after several years of prospective registration. In addition, we measured the diameter of the AA anastomoses (*Chapter 6*). This study was performed in a cohort of MC pregnancies with spontaneous TAPS. The incidence of AA anastomoses in spontaneous TAPS placentas was 20% (3/15). The median diameter of the AA anastomosis when present was 0.4 mm in the TAPS placentas and 2.2 mm in the normal MC placentas ($p = 0.01$). The significantly higher rate of AA anastomoses in uncomplicated MC placentas supports the hypothesis that AA anastomoses have a protective effect and prevent not only the development of TTTS but also of TAPS. In the sporadic cases in which TAPS develops in the presence of an AA anastomosis, the anastomosis is very small, ≤ 1 mm preventing intertwin blood equilibration.

Since TAPS may occur spontaneously as well as after laser treatment for TTTS, we hypothesized that the angioarchitecture in these two different forms of TAPS may not be similar. We therefore compared the placental characteristics of spontaneous TAPS and iatrogenic post-laser TAPS (*Chapter 7)*. We found that post-laser TAPS placentas were characterized by the presence of significantly fewer anastomoses compared to the spontaneous TAPS group, with a median of 2 and 4 anastomoses, respectively ($p = 0.003$). Interestingly, in both groups the majority of anastomoses were detected close to the placental margin. In the post-laser group this is thought to be because of increased technical difficulties to visualize the entire vascular equator, especially toward the edge of the placenta. However, in spontaneous TAPS, the reason for the majority of anastomoses to be close to the placental margin is as yet unknown. A more recent study from our group has shown that this appears only in TAPS placentas and that AV anastomoses are usually evenly distributed along the vascular equator in other MC placentas [10]. AA

anastomoses were detected in 14% of all TAPS placentas and all AA anastomoses were minuscule, ≤ 1mm.

Another interesting aspect of TAPS placentas is related to the placental sharing. In MC twin pregnancies in general, discrepancies in placental share lead to similar discrepancies in birth weight. The twin infant with a lower placental share often has more impaired growth and a lower birth weight, whereas the co-twin with the larger placental share has a higher birth weight. In contrast, with TAPS, birth weights seemed to be inversely related to the placental shares. To investigate this intriguing finding, we recently evaluated the placental share and hemoglobin level in TAPS twins in regards to birth weight. Interestingly, hemoglobin (Hb) level at birth was associated with birth weight, but placental share was not associated with birth weight. In other words, the recipient twin with the larger Hb level often had a larger birth weight but a smaller placental share, whereas the donor twin with the lower hemoglobin level had a larger placental share. This would seem to indicate that fetal growth in TAPS is determined by feto-fetal blood transfusion and not by placental share [18]. One could speculate that this chronic anemia and the resulting loss of nutrients as well as the associated chronic hypoxia could lead to diminished fetal growth. However the conversely larger placental share has yet to be explained. One explanation is that the chronic anemia, chronic hypoxia and loss of nutrients stimulate expansion of the placental territory and therefore a relatively larger placental share. Another explanation is that there is a form of selection bias at play and that in TAPS cases where the donor has a smaller placenta share there is also an increased risk of intra uterine mortality. Cases with fetal demise in utero lead to maceration of the placenta which were excluded from the study. We therefore only studied cases with double fetal survival which might explain the underreporting of donor twin with smaller placental shares.

Another element with regards to TAPS is the striking difference in color between the dark and plethoric placental share of the recipient and the pale placental share of the donor. This seems, intuitively, to be related to the large difference in Hb content in both placental shares. Microscopic confirmation of this explanation has not been published yet. The striking difference in color may also play a role as an additional diagnostic criteria for TAPS. The diagnosis of TAPS is based on the large Hb difference (>8g/dL) in association with at least one of the following criteria: intertwin reticulocyte count ratio > 1.7 and/or placenta injection studies showing only minuscule anastomoses. However, in various cases reticulocyte counts are not determined and placentas are not injected. In these cases, confirmation of the striking color difference could play an additional role. More research is needed to determine if the color difference can be measured easily and objectively and prove if this is indeed characteristic of TAPS.

Future research can focus on other placental characteristics of TAPS placentas which may or may not influence the pathogenesis and that have not yet been determined, e.g. the role of VV anastomoses and the type of umbilical cord insertion.

As our understanding of TAPS and TTTS continues to grow, so too are we better able to treat Exciting solutions are being investigated, especially with regards to the optimal treatment for TAPS. Further trials must determine if fetoscopic laser surgery in TAPS should be considered as the treatment of choice in TAPS, in analogy with TTTS.

The success of fetoscopic laser surgery in improving outcome in TTTS and possibly also in TAPS, has a drawback in terms of placenta examination and investigation. Since the goal of fetal surgery in these cases is to coagulate all anastomoses, the angioarchitecture in the majority of TTTS and TAPS cases is disrupted. We therefore foresee that since the vast majority of TTTS and TAPS placentas will be treated with laser surgery, evaluation of the vascular anastomoses will only be possible in the minority of untreated cases. To further investigate the role of these anastomoses in untreated placentas, international multicenter collaboration must be initiated.

It is therefore of paramount importance that all centers routinely perform placental injection studies with colored dye. This is not only of advantage to the center itself, but will also enable multicenter participation. To achieve this the placenta should be refrigerated in a sealed container and should not be frozen or fixed in formalin. This renders the placenta unviable for placental injection studies. The placenta should be examined preferably in the first few days, maximally within a week. The vessels should then be carefully catheterized for injection with contrasting colors of dye. Standard meticulous notes of the different anastomoses and types of cord insertions, as well as photographic evidence to later determine the diameter of anastomoses and placental share, will enable future research.

Hopefully, close collaboration between fetal surgeons, neonatologists and pathologists with experience in placental injection studies will further improve our understanding of TTTS and TAPS.

REFERENCE LIST

[1] Taylor MJ, Denbow ML, Duncan KR, Overton TG, Fisk NM. Antenatal factors at diagnosis that predict outcome in twin-twin transfusion syndrome. Am J Obstet Gynecol 2000;183:1023-8.

[2] Denbow ML, Cox P, Taylor M, Hammal DM, Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol 2000;182:417-26.

[3] Fries MH, Goldstein RB, Kilpatrick SJ, Golbus MS, Callen PW, Filly RA. The role of velamentous cord insertion in the etiology of twin-twin transfusion syndrome. Obstet Gynecol 1993;81:569-74.

[4] Machin GA. Velamentous cord insertion in monochorionic twin gestation. An added risk factor. J Reprod Med 1997;42:785-89.

[5] Saunders NJ, Snijders RJ, Nicolaides KH. Therapeutic amniocentesis in twin-twin transfusion syndrome appearing in the second trimester of pregnancy. Am J Obstet Gynecol 1992;166:820-24.

[6] Bruner JP, Anderson TL, Rosemond RL. Placental pathophysiology of the twin oligohydramnios-polyhydramnios sequence and the twin-twin transfusion syndrome. Placenta 1998;19:81-6.

[7] De Paepe ME, DeKoninck P, Friedman RM. Vascular distribution patterns in monochorionic twin placentas. Placenta 2005;26:471-75.

[8] Bajoria R. Vascular anatomy of monochorionic placenta in relation to discordant growth and amniotic fluid volume. Hum Reprod 1998;13:2933-40.

[9] Lopriore E, Sueters M, Middeldorp M, et al. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. Am J Obstet Gynecol 2007;196:159.e1-159.e5.

[10] Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D et al. Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta 2013; 34(7):589-593.

[11] Zhao DP, Cohen D, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D et al. The role of veno-venous anastomoses in twin-twin transfusion syndrome. Placenta 2014; 35(5):334-336.

[12] van den Wijngaard JP, Lopriore E, van der Salm SM, Schaap AH, Vandenbussche FP, Deruiter MC et al. Deep-hidden anastomoses in monochorionic twin placentae are harmless. Prenat Diagn 2007; 27(3):233-239.

[13] Gratac+¦s E, Ortiz JU, Martinez JM. A Systematic Approach to the Differential Diagnosis and Management of the Complications of Monochorionic Twin Pregnancies. Fetal Diagnosis and Therapy 2012; 32(3):145-155.

[14] Mercanti I, Boivin A, Wo B, Vlieghe V, Le RC, Audibert F et al. Blood pressures in newborns with twin-twin transfusion syndrome. J Perinatol 2011; 31(6):417-424.

[15] Peeters SH, Devlieger R, Middeldorp JM, DeKoninck P, Deprest J, Lopriore E et al. Fetal surgery in complicated monoamniotic pregnancies: case series and systematic review of the literature. Prenat Diagn 2014; 34(6):586-591.

[16] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ et al. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010; 27(4):181-190.

[17] De VS, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D et al. Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. Placenta 2012; 33(3):227-229.

[18] Zhao D, Slaghekke F, Middeldorp JM, Duan T, Oepkes D, Lopriore E. Placental share and hemoglobin level in relation to birth weight in twin anemia-polycythemia sequence. Placenta 2014; 35(12):1070-1074.

Summary

S.F. de Villiers

There are two types of twins. Twins in which each fetus has a separate placenta (dichorionic) and twins in which the fetuses share a placenta (dichorionic). Nearly one quarter of all twins have a dichorionic placenta. These monochorionic twins have an increased risk of complications during the pregnancy compared with dichorionic twins. This is as a result of the ubiquitous placental anastomoses that occur in monochorionic placentas, which can cause unbalanced net blood flow and different forms of chronic feto-fetal transfusions including, 1. twin-to-twin transfusion syndrome (TTTS) and 2. twin anemia-polycythemia sequence (TAPS). In TTTS the donor twin becomes hypovolemic and therefore oliguric with too little amniotic fluid in the amniotic sac (oligohydramnios). In contrast the recipient twin becomes hypervolemic and therefore polyuric and with too much amniotic fluid (polyhydramnios). TAPS can occur spontaneously or iatrogenically after fetoscopic laser coagulation (post-laser TAPS). In TAPS the donor twin becomes anemic while the recipient twin becomes polycythemic, but there is no sign of twin oligo-polyhydramnios sequence (TOPS) as seen in TTTS. In recent years our knowledge of the diagnosis and optimal management of these diseases has greatly increased, however challenges remain in our understanding of the pathogenesis.

This thesis presents a number of articles studying the placental angioarchitecture of both TTTS and TAPS to investigate the pathogenesis of these diseases.

In chapter 2 we give a detailed description of the technique of placental dye-colored injection. This technique is of paramount importance in studying the placental angioarchitecture of monochorionic placentas. Further a review of literature is given regarding the differences in placental architecture between various types of monochorionic placentas between normal monochorionic placentas and different types of monochorionic placentas complicated by TTTS placentas (with and without laser treatment), TAPS, and selective intrauterine growth restriction (sIUGR).

In chapters 3 to 5 we investigate various aspects of monochorionic placentas with and without TTTS. In chapter 3 we studied velamentous cord insertion in TTTS compared with normal monochorionic placentas. Previously, several studies reported a higher incidence of velamentous cord insertion in TTTS and suggested a pathophysiological role of velamentous cord insertion in the development of TTTS. In contrast to these studies, we found the rate of velamentous cord insertion to be nearly identical and was detected in 36.8% of placentas with TTTS versus 35.9% without TTTS ($p = 0.886$). We found that the presence of velamentous cord insertion in one twin is significantly associated with small for gestational age for that twin as well as a severe birth weight discordance. Surprisingly, we also found that the presence of velamentous cord insertion in both twins is not associated with small for gestational age. Furthermore, the presence of velamentous cord insertion was associated with an increased risk of intrauterine fetal demise (IUFD), with an increase from 4.6% to 14.1% ($p = 0.027$) in the normal monochorionic group in, as well as a decrease in gestational age (GA) at birth. The hypothesis that velamentous cord

insertion play a role in the development of TTTS is not supported by this study, however the association between velamentous cord insertion other risk factors e.g. intrauterine fetal demise and lower gestational age at birth is supported.

In chapter 4 the incidence and diameter of AA anastomoses in TTTS placentas were studied. In accordance to previous studies, we found a lower incidence of arterio-arterial (AA) anastomoses in TTTS placentas (37%) compared with normal monochorionic placentas (91%; $p < 0.001$). This supports the theory that AA anastomoses have a protective role in the development of TTTS by creating an equilibrating shift and compensating for hemodynamic imbalances. Interestingly, in this study we found no significant difference in the diameter of AA anastomoses in the group with TTTS and the group without TTTS. This study has a limitation in that a small number of only non-lasered placentas could be studied.

In chapter 5 we investigate the role of VV anastomoses in monochorionic placentas and specifically TTTS. The clinical significance of bidirectional VV anastomoses in MC placentas has remained largely inconclusive. Some studies have shown an increase in perinatal mortality associated with VV anastomoses. In our study, VV anastomoses were detected in 27% of all monochorionic placentas. We found that the risk of development TTTS was higher in the group with VV anastomoses (20%) than in the group without VV anastomoses (10%). A clear reason for this is not yet recognized. A possible hypothesis is that the development of TTTS can be prompted by hemodynamic changes in the shared venous system, via inter-twin transfusion. Further analysis showed that the presence of VV anastomoses was associated with and was an independent risk factor for TTTS ($p < 0.01$; OR: 3.59; 95% CI: 1.72-7.47). The presence of VV anastomoses was also associated with an increased risk of perinatal mortality in the monochorionic placenta group with VV anastomoses was 16% versus 10% in the group without VV anastomoses ($p = 0.02$). The presence of VV anastomoses is associated with perinatal mortality, but is not an independent risk factor.

In chapters 6 and 7 we studied various aspects of placentas with and without TAPS. In chapter 6 the incidence and diameter of AA anastomoses were studied with regards to spontaneously occurring TAPS. The incidence of AA anastomoses in TAPS placentas was 20%. The median diameter of the AA anastomosis when present was 0.4 mm in the TAPS placentas and 2.2 mm in the normal monochorionic placentas ($p = 0.01$). These findings support the hypothesis that AA anastomoses may have a protective effect against the development of TTS as well as against the development of TAPS. When TAPS develops in the presence of an AA anastomosis, the anastomosis is very small, ≤ 1 mm, and therefore contributes less to inter-twin equilibration.

In chapter 7 the placental characteristics of spontaneous TAPS and iatrogenic post-laser TAPS were studied. We found fewer anastomoses in the post-laser TAPS group than in the spontaneous TAPS group, with a median of 2 and 4 anastomoses respectively

(p = 0.003). AA anastomoses were detected in 14% of all TAPS placentas and all AA anastomoses were minuscule (diameter ≤ 1mm). Furthermore, we found that in both groups the majority of anastomoses were detected close to the placental margin. In the post-laser group this is thought to result from increased technical difficulties during fetoscopy, but the explanation for this in the spontaneous TAPS group is unknown and needs further study.

We conclude that placental injection studies are of great importance to improve our understanding of TAPS and TTTS. Further research and international collaboration to perform larger studies are crucial to xxx lacunas in our knowledge. Better understanding of the pathogenesis of TTS and TAPS will lead to improvement in the treatment of TTTS and TAPS, both of which are associated with one of the highest mortality rates in perinatal history.

Samenvatting

S.F. de Villiers

Er bestaan twee soorten tweelingen. Tweelingen waarbij iedere foetus een eigen moederkoek heeft (dichoriale placenta), en tweelingen waarbij de twee foetussen één moederkoek delen (monochoriale placenta). Ongeveer een kwart van alle tweelingen hebben een gezamenlijke placenta. Deze monochoriale tweelingen lopen veel meer risico's op complicaties dan dichoriale tweelingen tijdens de zwangerschap. Dit heeft te maken met het feit dat bij vrijwel alle monochoriale placenta's vaatverbindingen aanwezig zijn. Deze verbindingen, ook wel vaatanastomosen genoemd, kunnen leiden tot een ongebalanceerde bloeddoorstroming en verschillende vormen van chronische foeto-foetale transfusie waaronder 1. tweeling-transfusie syndroom (TTS) en 2. tweeling anemie-polycythemie sequentie (TAPS). In TTS heeft de gevende foetus (donor) relatief te weinig bloedvolume (hypovolemie) en daardoor een lage urineproductie (oligurie) en te weinig vruchtwater (oligohydramnion). Dit in tegenstelling tot de ontvangende foetus (recipiënt of ontvanger) die via de vaatanastomosen te veel bloed ontvangt, en hierdoor te veel bloedvolume heeft (hypervolemie) met een hoge urineproductie (polyurie) en te veel vruchtwater (polyhydramnion). TAPS kan spontaan ontstaan of iatrogeen optreden na fetoscopische lasercoagulatie (post-laser TAPS). In TAPS wordt de gevende foetus (donor) anemisch (bloedarmoede), terwijl de ontvangende foetus juist polycythemisch wordt (te veel rode bloedcellen) zonder een verschil in vruchtwatervolume zoals bij TTS. In de afgelopen jaren, is onze kennis over de diagnostiek en optimale behandeling van deze ziekten sterk toegenomen, maar verschillende aspecten omtrent de pathogenese moeten nog steeds opgehelderd worden.

Dit proefschrift presenteert een aantal studies die de placenta vaatanastomosen in zowel TTS en TAPS bestuderen om de pathogenese van deze aandoeningen de onderzoeken.

In hoofdstuk 2 geven wij een gedetailleerde beschrijving van de techniek om een placenta op te spuiten met kleurstof. Deze techniek is van groot belang om vaatanastomosen bij monochoriale placenta's nauwkeurig te kunnen bestuderen. Verder wordt een overzicht van de literatuur geven over de verschillen in de placenta architectuur tussen normale monochoriale placenta's en verschillende types van monochoriale placenta's gecompliceerd door TTS (met en zonder laserbehandeling), TAPS, en selectieve intrauteriene groeivertraging (sIUGR).

In hoofstukken 3 tot en met 5 onderzochten we verschillende karakteristieken van monochoriale placenta's met en zonder TTS. In hoofdstuk 3 onderzochten we de incidentie van velamenteuze navelstreng insertie in TTS in vergelijking met normale monochoriale placenta's. Eerdere studies rapporteerden een hogere incidentie van velamenteuze navelstreng insertie in TTS en suggereerden en pathofysiologische rol in de ontwikkeling van TTS. In tegenstelling tot eerdere studies, vonden wij een vrijwel identieke percentage velamenteuze navelstreng inserties in placenta's met TTTS (36.8%) en zonder TTS (35.9%) ($p = 0.886$). We vonden wel dat de aanwezigheid van velamenteuze navelstreng

insertie in een foetus geassocieerd is met een laag geboortegewicht voor de zwangerschapsduur en ernstige verschil in geboortegewicht tussen de foetussen. Verrassenderwijs vonden we dat de aanwezigheid van een velamenteuze navelstreng insertie bij beide foetussen niet geassocieerd is met een laag geboortegewicht voor de zwangerschapsduur. Een velamenteuze navelstreng insertie was verder geassocieerd met een verhoogd risico op intra-uteriene vruchtdood (toename van 4.6% tot 14.1%, p=0.027) en een afname van de zwangerschapsduur bij geboorte.

De hypothese dat velamenteuze navelstreng inserties een rol spelen in de ontwikkeling van TTS wordt derhalve niet ondersteund door onze studie, maar de associatie tussen velamenteuze inserties en andere risico's zoals laag geboortegewicht en verhoogde mortaliteit wordt wel bevestigd.

In hoofdstuk 4 onderzochten we de incidentie en diameter van arterio-arteriële anastomosen in TTS placenta's. In overeenstemming met eerdere studies vonden we een lagere incidentie van AA-anastomosen in TTS (37%) in vergelijking met normale monochoriale placenta's (91%; $p < 0.001$). Deze bevinding ondersteunt de theorie dat AA-anastomosen een beschermende rol kunnen spelen in de ontwikkeling van TTS door het creëren van een equilibrium en het compenseren van hemodynamische onevenwichtigheden. Interessant is dat we geen significant verschil vonden in de diameter van AA-anastomosen in de groep met of zonder TTS. Deze studie kende echter een aantal belangrijke beperkingen zoals het feit dat we alleen niet-gelaserde TTS placenta's konden onderzoeken, en dat we derhalve maar een kleine groep hebben kunnen bestuderen.

In hoofdstuk 5 onderzochten we de rol van veno-veneuze (VV) anastomosen in monochoriale placenta's en specifiek in TTS. De klinische betekenis van deze bi-directionele VV-anastomosen blijft grotendeels onduidelijk. Sommige studies tonen een associatie tussen perinatale sterfte en VV-anastomosen. In onze studie werden VV-anastomosen gedetecteerd in 27% van alle monochoriale placenta's. Het risico op ontstaan van TTS was hoger bij aanwezigheid van VV anastomosen (20%), dan wanneer er geen VV anastomosen aanwezig waren (10%). Een duidelijke verklaring hiervoor is niet bekend. Een hypothese zou kunnen zijn dat TTS aangezet wordt door hemodynamische veranderingen in het gedeelde veneuze systeem via bloedtransfusie tussen foetussen. Verdere analyse toonde aan dat de aanwezigheid van VV-anastomosen een onafhankelijke risicofactor voor TTS ($p <0.01$; OR: 3,59; 95% CI: 1,72-7,47). De aanwezigheid van VV-anastomosen was ook geassocieerd met een verhoogde risico op perinatale sterfte (16% in de groep met VV-anastomosen versus 10% in de groep zonder, ($p = 0.02$), maar dit was geen onafhankelijke risicofactor.

In hoofdstukken 6 en 7 onderzochten we verschillende karakteristieken van placenta's met en zonder TAPS. In hoofdstuk 6 onderzochten we de incidentie en diameter van AA-anastomosen bij spontaan optredende TAPS. De incidentie van AA-anastomosen in TAPS placenta's was 20%. De mediane diameter van deze AA-anastomosen was 0,4

mm in de TAPS placenta's en 2,2 mm in normale monochoriale placenta's (p = 0.01). Onze bevindingen ondersteunen de hypothese dat AA-anastomosen niet alleen een beschermend effect tegen het ontstaan van TTS maar ook tegen het ontstaan van TAPS. Wanneer TAPS alsnog ontstaat in de aanwezigheid van een AA-anastomose, de diameter van dit vat is dan zeer klein, ≤ 1 mm en draagt waarschijnlijk minder goed bij tot het in evenwicht houden van de twee circulaties.

In hoofdstuk 7 werden de placenta kenmerken van spontane TAPS en (iatrogene) post-laser TAPS bestudeerd. We vonden minder anastomosen in de post-laser TAPS groep dan in de spontane TAPS groep, met een mediaan van 2 en 4 anastomosen (p = 0.003). AA-anastomosen werden gedetecteerd in 14% van alle TAPS placenta's en alle AA-anastomosen waren wederom minuscuul (diameter ≤ 1mm). We vonden ook dat de meeste anastomosen dicht bij de rand van de placenta werden gedetecteerde. In het post-laser groep wordt dit toegeschreven aan de verhoogde technische uitdaging tijdens fetoscopische laserbehandeling, maar de verklaring voor de spontane TAPS groep is onbekend en moet verder onderzocht wordt.

Tot slot, concluderen wij dat nauwkeurige placenta injectie studies van groot belang zijn om de pathogenese van TTS en TAPS verder te ontrafelen. Verder onderzoek en internationale samenwerking om grotere studies te kunnen verrichten zijn cruciaal om lacunes in onze kennis verder te kunnen vullen. Meer kennis over de pathogenese van TTS en TAPS zal kunnen leiden tot verbetering van de behandeling en uitkomst bij deze ziekten. Ondanks belangrijke vooruitgang in de behandeling van TTS en TAPS, zijn beide ziekten, anno 2016, nog steeds geassocieerd met een van de hoogste sterftepercentages in de perinatale geneeskunde.

Part V

Appendices

LIST OF ABBREVIATIONS

AA	Arterio-arterial
AV	Arterio-venous
BW	Birth Weight
BWD	Birth Weight Discordance
DA	Diamniotic
DC	Dichorionic
GA	Gestational Age
Hb	Hemoglobin
IGF	Insulin-like Growth Factor
IUFD	Intrauterine Fetal Demise
IUT	Intrauterine Transfusion
LUMC	Leiden University Medical Centre
MA	Monoamniotic
MC	Monochorionic
MCA-PSV	Middle Cerebral Artery Peak Systolic Velocity
MoM	Multiples of the Mean
PET	Partial Exchange Transfusion
PPROM	Preterm Prelabor Rupture Of Membranes
RA	Residual Anastomoses
SGA	Small for Gestational Age
sIUGR	selective Intrauterine Growth Restriction
TAPS	Twin Anemia-Polycythemia Sequence
TOPS	Twin Oligo-Polyhydramnios Sequence
TRAP	Twin Reversed Arterial Perfusion
TTTS	Twin-to-Twin Transfusion Syndrome
UA	Umbilical Artery
VA	Veno-arterial
VCI	Velamentous Cord Insertion
VV	Veno-venous

PUBLICATIONS

Correlation between veno-venous anastomoses, TTTS and perinatal mortality in monochorionic twin pregnancies. de Villiers SF, Zhao DP, Cohen D, van Zwet EW, Duan T, Oepkes D, Lopriore E. Placenta 2015, vol. 36(5) pp 603-6.

Monochorionic twin placentas: Injection technique and analysis. de Villiers SF, Zhao DP, Oepkes D, Lopriore E. Diagnostico Prenatal 2014, vol. 25(2) pp. 35–42.

Velamentous cord insertion in monochorionic twins with or without twin-twin transfusion syndrome: Does it matter? Costa-Castro T, de Villiers SF, Montenegro N, Severo M, Oepkes D, Matias A, Lopriore E. Placenta, 2013 vol. 34(11) pp. 1053-8.

Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D, Lopriore E. Placenta. 2013 vol. 34(7) pp. 589-93.

Placental characteristics in monochorionic twins with spontaneous versus post-laser twin anemia-polycythemia sequence. de Villiers SF, Slaghekke F, Middeldorp JM, Walther FJ, Oepkes D, Lopriore E. Placenta, 2013 vol. 34(5) pp. 456-9.

Arterio-arterial vascular anastomoses in monochorionic placentas with and without twin-twin transfusion syndrome. de Villiers SF, Slaghekke F, Middeldorp JM, Walther FJ, Oepkes D, Lopriore E. Placenta, 2012 vol. 33(8) pp. 652-4.

Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. de Villiers SF, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D, Lopriore E. Placenta, 2012 vol. 33(3) pp. 227-9.

CURRICULUM VITAE

Suzanne de Villiers was born on 20th of February 1988 in Johannesburg, South Africa. She completed her secondary education at Bloemhof Girls High in 2006, and the following year commenced her medical studies at Tygerberg Hospital, University of Stellenbosch. In 2010 she transferred to the Leids Universitair Medisch Centre (LUMC), Leiden University where she obtained her Bachelors and then her Masters in Medicine in 2015. In parallel to her medical studies, she started a research trajectory with the Neonatology department of the LUMC, which has culminated in this thesis. Currently she is in her second year of pediatric specialization at the Centre Hospitalier Universitaire Vaudois (CHUV), in Switzerland.

ACKNOWLEDGEMENTS

It has been a long journey from the first moment I got the idea to dabble in research (thank you Enrico), to that happy point when articles became a printed thesis.

It's not a journey I've taken alone.

This thesis is dedicated to *all the people* who have helped make it a reality and especially all participating twins and their parents.