Placental Characteristics and Complications

in

Monochorionic Twin Pregnancies

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CONTENTS

Chapter 1

General Introduction

General introduction

Monochorionic (MC) twin pregnancies are at high risk for perinatal mortality and morbidity compared to dichorionic (DC) twin pregnancies.[1] This excess of adversity is due to the unique placental angioarchitecture in MC twin pregnancies.[2] Almost all MC placentas have vascular anastomoses connecting the circulation of both fetuses. In most cases, inter-twin blood transfusion between the two fetuses is balanced. In some cases, inter-twin transfusion is unbalanced and complications may arise such as twin–twin transfusion syndrome (TTTS) or twin anemia-polycythemia sequence (TAPS).[2]

The number and type of anastomoses vary greatly between MC placentas. Scrutinized examination of placental angioarchitecture often provides indispensable information to understand and unravel the pathogenesis, even diagnosis of the specific complications in MC twins. Placental injection using colored–dye is a simple and accurate method to demonstrate the angioarchitecture in MC placentas.[3] In this thesis, we studied the placental characteristics (vascular anastomoses, umbilical cord insertions and placental share) and their associated clinical consequences in various types of complications exclusive for MC twins. In the Netherlands, the Leiden University Medical Center (LUMC) is the national referral center for all complicated MC twin pregnancies.

Monochorionic Placentas: analysis and characteristics

It is well recognized that vascular anastomoses are ubiquitous in MC placentas, but extremely rare in DC placentas. However, this has not been confirmed in studies with large cohort of MC and DC placentas. Importantly, DC placentas are rarely examined using colored–dye injection, preventing reliable comparison. Comparison of the angioarchitecture of MC versus DC twin placentas may help understand the differences in clinical outcome. In

addition, large studies in various subgroups of MC twin placentas are needed to determine the exact type and number of anastomoses, type of cord insertions and the occurrence of unequal placental sharing.

Placental characteristics in relation to specific complications

MC placentas are characterized by the vascular anastomoses connecting the fetal circulations. Three types of vascular anastomoses have been described, including arteriovenous (AV) anastomoses, arterioarterial (AA) anastomoses and venovenous (VV) anastomoses. The blood flow in AA and VV anastomoses is bidirectional whereas AV anastomoses carry unidirectional blood flow from one twin to its co-twin and may lead to the development of TTTS or TAPS. The role of the different types of anastomoses on the development of complications is not fully understood. Several studies reported a higher prevalence of VV anastomoses in TTTS placentas compared to no-TTTS placentas though the blood flow in VV anastomoses is thought to be freely bidirectional and should enable volume equilibrium within twin pairs.[4, 5] Other studies also related the presence of VV anastomoses to fetal demise due to sudden blood exchange in fetal venous circulations.[6] However, these results were not confirmed in others studies.[7-9] The discrepancies among these studies are partially due to the small sample size. In addition to vascular anastomoses, individual placental share is also closely related to clinical outcome in MC twin pregnancies.[10] Large placental share discordance can result in selective intrauterine growth discordance (sIUGR) substantially increasing perinatal morbidity and mortality in MC twin pregnancies.[10] Although larger placental share generally leads to a larger birth weight, several studies observed a different scenario in a subgroup of MC twins with TAPS where the anemic twin is usually the smaller twin, but often has a larger placental share

compared to its polycythemic co-twin.[11, 12] The association between fetal growth and corresponding placental share in MC twins with TAPS requires further evaluation.

Fetoscopic laser ablation of vascular anastomoses is the optimal treatment for TTTS or TAPS. However, residual anastomoses may be present in up to 33% of placentas treated with fetoscopic laser surgery.[13] The failure of treatment is partially due to the lack of data on anatomic localization of vascular anastomoses on placental surface. In another scenario where the umbilical cords of MC twins are quite close to each other (proximate cord insertions), fetoscopic laser surgery is challenged due to the difficulty in identifying vascular equator. However, the scientific definition of proximate cord insertions and associated impact on clinical outcome remains to be elucidate.

Histologic chorioamnionitis and early–onset neonatal sepsis after laser surgery for TTTS

Although fetoscopic laser surgery is well accepted as the optimal treatment for TTTS, intrauterine fetal interventions may also lead to (iatrogenic) premature rupture of the membranes, chorioamnionitis and premature delivery. The rate of clinical chorioamnionitis diagnosed based on clinical signs was reported to vary from 0 to 4%.[14-24] As a result of intrauterine infection and chorioamnionitis, neonates may develop early onset sepsis, which is one of the leading causes for neonatal morbidity and mortality. However, clinical chorioamnionitis is not equated to histologic chorioamnionitis which is a more reliable indicator for maternal and fetal inflammatory response.[25] Studies on the incidence and consequences of histological chorioamnionitis in MC twin pregnancies treated with fetoscopic laser surgery are lacking.

Outline of the thesis

Since the progress in understanding the angioarchitecture of MC placentas shed light on the management of MC twin pregnancies, the aim of this thesis was to further investigate the placental characteristics in relation to each unique complication of MC twin pregnancies.

Chapter 1 – General Introduction

Part I – Monochorionic Placentas: analysis and characteristics

Chapter 2 – Study on the specific characteristics of MC placentas compared to dichorionic placentas.

Chapter 3 – Comparison of the prevalence, size, number and localization of vascular anastomoses among various types of monochorionic placentas

Chapter 4 – Study on the definition, prevalence and angio-architecture of MC placentas with proximate cord insertions.

Part II – Placental characteristics in relation to TTTS and TAPS

Chapter 5 – Evaluation of the prevalence of VV anastomoses in a large cohort of TTTS compared to MC twin pregnancies without TTTS: a multicenter study.

Chapter 6 – Study on the fetal growth in MC twins with and without TAPS.

Chapter 7 – Comparison of outcome after fetoscopic laser surgery for TTTS with and without proximate cord insertion.

Part III – Chorioamnionitis and early onset sepsis in TTTS after laser surgery

Chapter 8 – Study on the incidence of intrauterine infection in TTTS managed with fetoscopic laser surgery compared to a control group of MC twins not treated with fetoscopic laser surgery.

Chapter 9 – Study on the early-onset neonatal sepsis after fetoscopic laser surgery for TTTS.

Part IV – Summary, General Discussion and Future perspectives

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PART I

Monochorionic Placentas: analysis and characteristics

Chapter 2

Comparison between monochorionic and dichorionic placentas with special attention to vascular anastomoses and placental share

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Abstract

Placental vascular anastomoses in twins lead to a shared circulation and may subsequently enable the development of severe complications such as twin–twin transfusion syndrome (TTTS) and twin anemia–polycythemia sequence (TAPS). The presence of vascular anastomoses has frequently and systematically been studied in monochorionic (MC) placentas, but only rarely in dichorionic (DC) placentas. The aim of this study was to compare the prevalence of vascular anastomoses and evaluate the sharing discordance in MC and DC placentas. All consecutive placentas of MC and DC twins delivered at the Leiden University Medical Center (The Netherlands) and Medical University of Warsaw (Poland) from 2012 to 2015 were routinely injected with colored–dye and included in the study. We excluded twin pregnancies treated with fetoscopic laser surgery. A total of 258 placentas were analyzed in this study, including 134 MC placentas and 124 DC placentas. Vascular anastomoses were present in 99% (133/134) MC placentas and 0% DC placentas ($p < .01$). Placental share discordance between MC twins was significantly larger compared to DC twins, 19.8 (interquartile range (IQR) 8.1-33.3) and 10.8 (IQR 6.2-19.0), respectively ($p<.01$). Vascular anastomoses–associated complications occurred in 16% (22/134) MC twins. Our findings show that vascular anastomoses are almost ubiquitous in MC placentas, but non-existent in DC placentas. In addition, unequal placental sharing appears to be more common in MC than in DC placentas.

Keywords: monochorionic placenta, dichorionic placenta, vascular anastomoses, unequal placental share

Monochorionic (MC) twins are at substantially increased risk of adverse outcome compared to dichorionic (DC) twins [1]. This excess of adversity in MC twins is mainly attributed to the complications resulting from connected circulation [2]. The vascular anastomoses are the anatomical basis for connected circulation within twin pairs . Three types of vascular anastomoses are reported in injection studies of MC placentas, namely arterio–arterial (AA) anastomoses, veno–venous (VV) anastomoses and arterio–venous (AV) anastomoses. The unidirectional blood flow in AV anastomoses enables volume disequilibrium, resulting in severe complications such as twin–twin transfusion syndrome (TTTS) and twin anemia–polycythemia (TAPS) [3]. The association between TTTS/TAPS and vascular anastomoses in MC twins has been extensively illustrated in placental injection studies [4-8]. In contrast, little is known on the vascular anastomoses in DC placentas due to lack of placental injection for DC placentas. In addition, placental share discordance is quite common in MC twins, leading to discordant fetal growth, even selective intrauterine growth restriction (sIUGR).[9, 10] Again, since placental injection is not routine practice for the examination of DC placenta, the placental share discordance in DC twins remains to be elucidated. The aim of this study is to compare the placental characteristics between a large cohort of MC and DC placentas using colored–dye injection.

Materials and methods

All placentas of twin pregnancies consecutively delivered at Leiden University Medical Center (The Netherlands) and Medical University of Warsaw (Poland) from September 2012 to December 2015 were eligible for this study. MC placentas treated with fetoscopic laser surgery were excluded. We also excluded twin placentas with single or double fetal demise, incomplete injection due to maceration, fixation in formalin and severe damage.

Chorionicity was evaluated during the 11-14 weeks' sonographic examination and was confirmed postnatally by macroscopic or microscopic histopathological evaluation. The type of umbilical cord insertion and number of umbilical vessels were recorded. Velamentous cord insertion was defined as the insertion of umbilical cord into the amniotic membrane instead of placental parenchyma. All twin placentas were injected according to the protocol published previously [11]. After injection, the type and number of vascular anastomoses were documented. Digital placental pictures were taken for various further computerized analysis, such as measurement of placental share and anastomostic size. Individual placental share was measured as the venous return area of each twin using Image J 1.45s (Image J, National Institute of Health, USA). Placental share difference was calculated as the larger placental share minus the smaller placental share. Placental share discordance was calculated using the following formula: (larger placental share - smaller placental share)/larger placental share × 100%. Part of the placental data were reported to describe a special type of AA and VV anastomoses, the so called partially–hidden AA and VV anastomoses [12].

The following perinatal variables were collected prospectively: TTTS, TAPS, sIUGR, gestational age at birth, birth weight, Hb levels at birth and delivery mode. Diagnosis of TTTS was based on the Eurofetus criteria ref. TAPS was defined as the diagnostic criteria proposed by Slaghekke et al ref. Birth weight discordance was calculated by the following formula: (larger twin - smaller twin)/larger twin × 100%. sIUGR was defined as a birth weight discordance of ≥ 25% [13]. Individual birth weight share was calculated by dividing the birth weight of each infant by the sum of the birth weights of both infants. Birth weight

share/placental share ratio was calculated by dividing the birth weight share by the corresponding placental share [10, 14].

Statistics

Kolmogorov–Smirnov test was adopted to assess the normality of continuous variables. Data were analyzed using chi-square, Fisher exact, Mann–Whitney or Student t tests, as appropriate. Spearman *r* was generated to evaluated the correlation between placental share and birth weight share. Statistical significance was considered if a p value was less than 0.05. Data were analyzed using GraphPad Prism v6.0 (GraphPad Software Inc. La Jolla, CA 92037 USA) and IBM SPSS Statistics 22.0® (IBM Corporation, Armonk, New York, USA).

Results

A cohort of 267 eligible twin placentas were examined at both centers during the study period, including 143 MC placentas and 124 DC placentas. Nine (3%) placentas were excluded due to incomplete injection. The remaining 134 MC placentas and 124 DC placentas were analyzed in this study. In the group of MC twins, 18 (13%) were complicated with TTTS (not treated with fetoscopic laser surgery), 8 cases (6%) with TAPS and 31 (23%) cases with growth discordance. Neither TTTS nor TAPS occurred in the group of DC twins whereas growth discordance occurred in 10% (12/124) of DC twins. Additional characteristics of two groups were shown in Table 1.

Vascular anastomoses were detected in 99% (133/134) MC placentas and 0% (0/124) DC placentas, respectively ($p < .01$).

Table 1 Baseline characteristics

	Monochorionic twins (n=134)	**Dichorionic twins (n=124)**	***p* value**
Gestational age at birth – wks	33.0 ± 4.1	34.5 ± 3.6	<.01
Birth weight – gr	1833 ± 762	2202 ± 725	<.01
Birth weight discordance – %	12.2 (6.8 -25.0)	9.8 (5.4-18.1)	.02
Birth weight discordance ≥ 25% – n (%)	31 (23)	12 (10)	<.01
Cesarean section – n (%)	88 (66)	44 (35)	<.01
Intertwin Hb difference at birth – g/dl	2.1 (.6-4.2)	1.5 (.3-3.4)	.03

Data was displayed as mean ± SD, median (IQR) or n (%).

Table 2 Comparison of placental angio–architecture between MC and DC placentas

	Monochorionic placentas (n=134)	**Dichorionic placentas (n=124)**	***p* value**
Placentas with vascular anastomoses – n (%)	133 (99)	0	<.01
Velamentous cord insertion – n (%)[a]	58 (22)	28 (11)	<.01
Placental share discordance – %	19.8 (8.1-33.3)	10.8 (6.2-19.0)	<.01
Unequal placental share ≥ 20% – n (%)	67 (50)	29 (23)	<.01
birth weight share/placental share ratio[b]	1.0 (.87-1.20)	1.0 (0.91-1.11)	.33

[a] Denotes to the presence of velamentous cord insertion per infant instead of twin pair. [b] Value was given as median (95%CI)

In the group of MC placentas, the frequency of AV anastomoses, AA anastomoses and VV anastomoses was respective 99% (133/134), 85% (114/134) and 28% (38/134). The median number of vascular anastomoses per MC placenta was 11 (interquartile 6-18). One percent (1/134) of MC placentas consisted of two separate placental mass (so-called bipartite MC placentas). In 44% (54/124) of DC placentas had two separate placental mass, whereas the rest of DC placentas were fused. Comparison of placental characteristics between MC and DC placentas were summarized in Table 2. Examples of MC and DC placentas after colored–dye injection are illustrated in Figure 1 and 2, respectively.

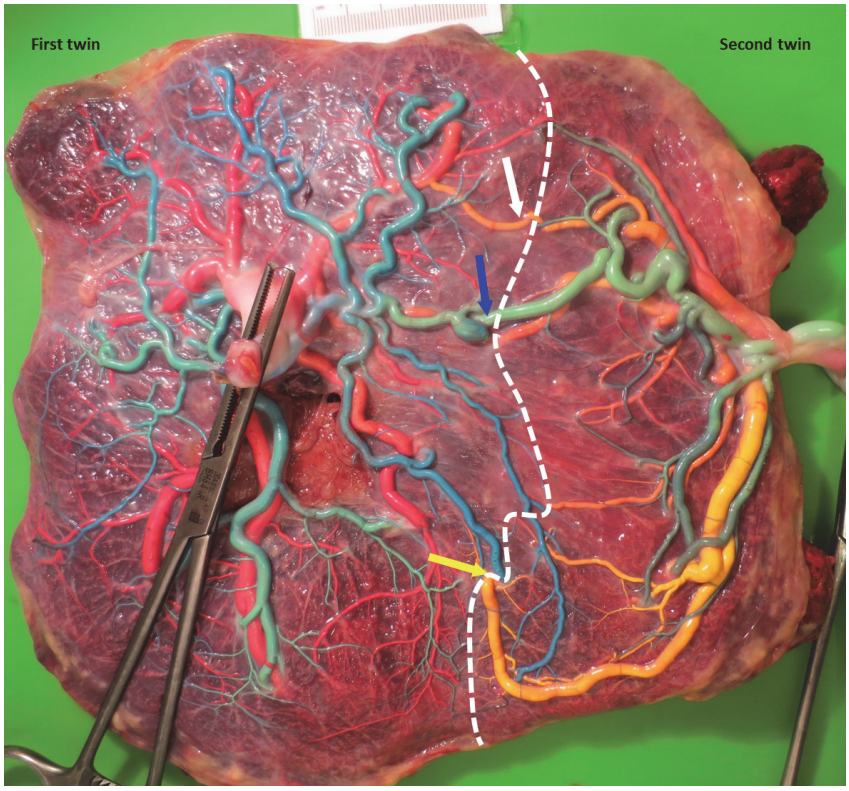

Figure 1: A monochorionic placenta after colored–dye injection. The blue, white and yellow arrows indicate the AA anastomoses, VV anastomosis and AV anastomoses, respectively. The white–dotted line indicated the vascular equator. The first twin had a placental share of 67% and the second twin 33%.

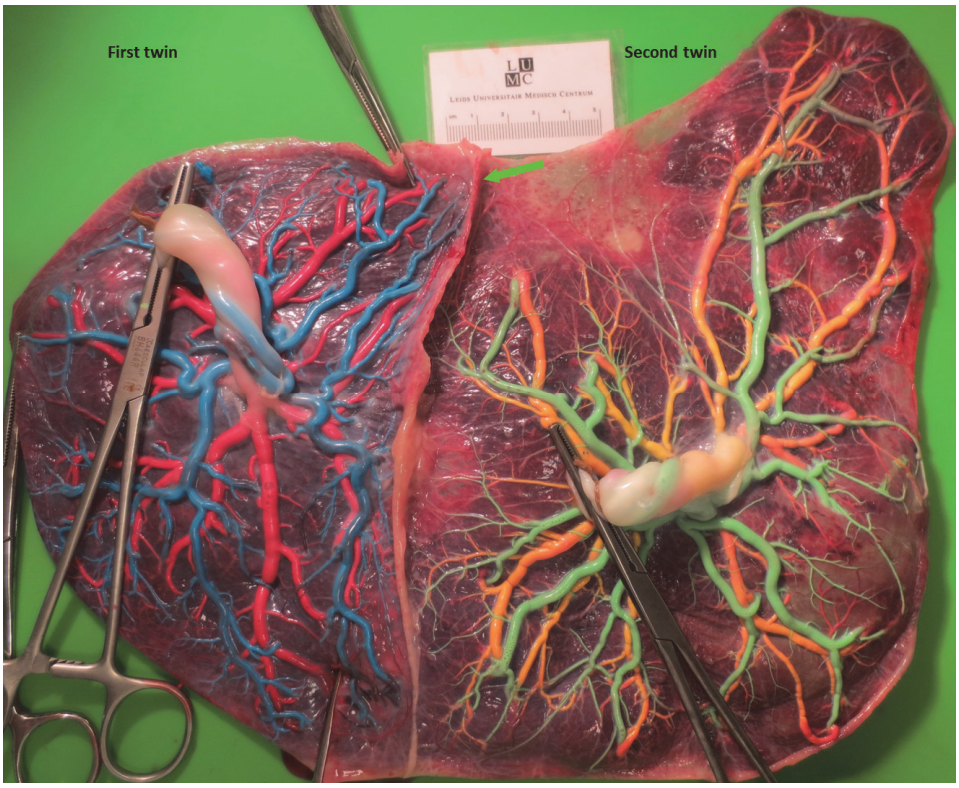

Figure 2: A dichorionic placenta after colored–dye injection. The two placental masses were fused. No vascular anastomoses were detected after injection. The green arrow indicates the inter–twin septum. The individual placental share in first and second twin was 39% and 61%.

We further related the individual placental share to birth weight share in MC and DC twins. We found that birth weight share was significantly associated with placental share in both MC twins (Spearman r = .64, 95% confidence interval .56-.71, $p < .01$, Figure 3) and DC twins (Spearman r = .32, 95% confidence interval .19-.44, $p < .01$, Figure 3).

Discussion

This is the first study to compare the angioarchitecture between MC and DC placentas using an accurate and reliable technique. We found that vascular anastomoses are almost always present in MC placentas but non-existent in DC placentas. As a result, hematological and

perinatal complications due to shared circulation by vascular anastomoses occur only in MC twins, but not in DC twins.

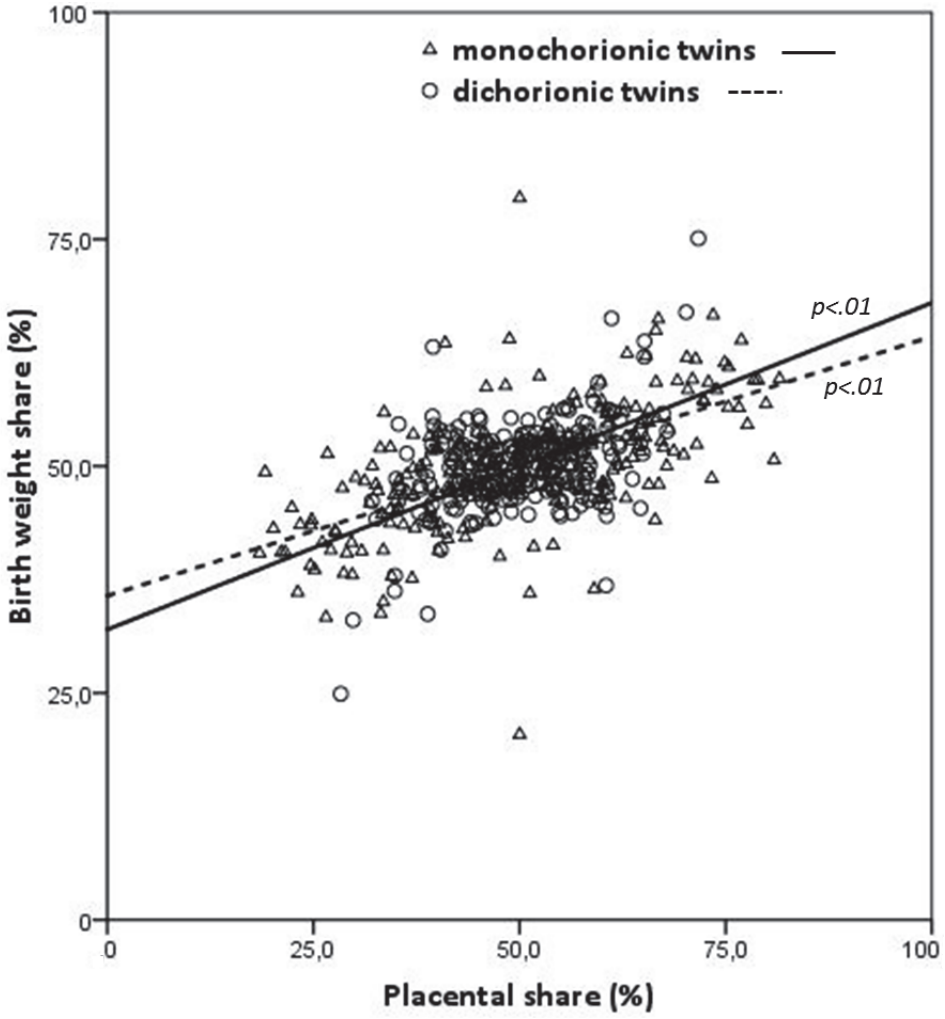

Figure 3: Correlation between placental share and birth weight in MC twins (Spearman r =.64; 95% confidence interval (CI): .56 to .71; *p*<.01) and DC twins (Spearman r = .32; 95% CI: .19 to .44; *p*<.01).

The vascular anastomoses and associated consequences in MC twins have been well studied. In accordance with previous placental injection studies, this study shows that the presence of vascular anastomoses in MC placentas is quite common [10, 15-17]. In contrast, the presence of vascular anastomoses in DC placentas has not systematically been studied with colored–dye injection. However, several case reports have reported on DC twins with

placental vascular anastomoses [18-23]. In these reports, vascular anastomoses were inspected when associated complications were suspected, such as TTTS, TAPS and twin reversed arterial perfusion (TRAP). Nevertheless, Robertson et al. reported a paucity of vascular anastomoses in DC placentas with fused mass [15].Thus, the vascular anastomoses in the general population of DC twins remains uncertain. In this study, we consecutively examined a large cohort of DC placentas with colored–dye injection and did not detect any vascular anastomoses. This disparity in vascular anastomoses between MC and DC placentas may be due to the distinct embryological process. In DC twins, a prerequisite for the formation of vascular anastomoses is that the chorionic vessels of one twin pass through the chorion and amnion of both twins into the placental territory of the co–twin. This process may be not only hampered by mechanical factors, but also be inhibited by the chemical factors in amnion [24].

In this study, we found that birth weight was strongly associated with placental share in both MC and DC twins. Our findings support the theory proposed by Salafia et al. that the growth relationship between birth weight and placental weight is comparable between MC twins and DC twins [25]. Interestingly, unequal placental share appears to be less frequent in DC twins than MC twins despite of the common existence of inter–twin competition for space and nutrition in both types of twins. Several studies argue that the blastocyst allocated to each twin is disequilibrated during the twining process of monochorionic twins, leading to different growth potential within twin pairs [26]. In addition, implantation into an unfavorable milieu of one twin may also play a role in the increased frequency of unequal placental share in MC twins given the higher prevalence of velamentous cord insertion indicative of insufficient placentation [27].

This study has several limitations. One is the selection bias due to the referral nature of our centers. Twin pregnancies referred to our centers usually underwent a complicated course, especially MC twins. Since vascular anastomoses and unequal placental share are significantly related to the adverse outcome in MC twins [28], the findings on MC twins in this study may be overestimated. However, the prevalence of TTTS, TAPS and sIUGR detected in the MC twin cohort in this study is comparable to the expected prevalence in an unselected cohort of MC twins [2]. Another possible limitation is that individual placental share in DC twins may not well represent the size of individual placental mass. Placentometric studies show that many aspects of placental gross morphology are associated with fetal growth, including area of placental surface and placental weight [29]. Unfortunately, the weight of individual placental mass was not measured in this study. Finally, minuscule vascular anastomoses have also been discovered underneath the placental surface using a casting technique with latex injection. In this study, placental casting was not performed and the presence of deep-hidden anastomoses was not evaluated [30].

In conclusion, vascular anastomoses are extremely rare (and almost non-existent) in DC placentas, but ubiquitous in MC placentas. In addition, unequal placental sharing appears to occur more frequently in MC twin placentas. The two placental characteristics are responsible for the increased risk of perinatal complications associated with MC twinning.

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Chapter 3

Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas

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Abstract

Introduction: Most monochorionic (MC) twin pregnancies have an uncomplicated course, but some develop severe complications including selective intrauterine growth restriction (sIUGR), twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS). The underlying pathogenesis of these various complications is associated with the ubiquitous presence of vascular anastomoses in MC placentas.

Methods: The aim of this study was to estimate the prevalence, number, size and localization of the anastomoses in sIUGR, TTTS and TAPS placentas compared to normal MC placentas using color dye injection. We excluded MC twin pregnancies treated with fetoscopic laser surgery or selective feticide.

Results: A total of 235 placentas fulfilled the inclusion criteria: 126 normal MC, 47 TTTS, 46 sIUGR and 16 spontaneous TAPS. Median number of anastomoses in normal MC, sIUGR, TTTS and TAPS placentas was 8 (IQR: 4-12), 8 (IQR: 5-14), 7 (IQR: 5-11) and 4 (IQR: 3-5), respectively. The prevalence of arterio-arterial (AA) anastomoses in normal MC, sIUGR, TTTS and TAPS placentas was 96%, 98%, 47% and 19%, respectively. We found AV anastomoses to be evenly distributed along the vascular equator in all MC placentas except in TAPS cases, where anastomoses were mostly localized near the margin. We also found that, in sIUGR and TTTS placentas, AA anastomoses tended to be at the center of the placenta.

Conclusion: The present study shows that the prevalence, size, number and localization of the various types of anastomoses differ between normal MC, sIUGR, TTTS and TAPS placentas.

1. Introduction

All monochorionic (MC) placentas have vascular anastomoses connecting the circulation of the two fetuses. Three different types of anastomoses may be present: arterioarterial (AA), venovenous (VV) and arteriovenous (AV) anastomoses. The former two are superficial with bidirectional blood flow, whereas AV anastomoses occur at deep capillary level within shared cotyledon and allow only unidirectional blood flow. [1]Vascular anastomoses may lead to severe complications in MC twin pregnancies including selective intrauterine growth restriction (sIUGR) (incidence 11-21%), twin-twin transfusion syndrome (TTTS) (incidence 9%) and spontaneous twin anemia-polycythemia sequence (TAPS) (incidence 3-5%).[2-5] Several differences in placenta angioarchitecture have been reported between the various subgroups of MC placentas. In general, sIUGR placentas are characterized by a large sharing discordance, a high prevalence of velamentous cord insertion and large AA anastomoses, TTTS placentas are characterized by a low prevalence of AA anastomoses, whereas TAPS placentas have typically only few minuscule AV anastomoses and a low prevalence of AA anastomoses. [3, 5-8] Although the type, number and size of these anastomoses may vary between the different subgroups of MC twin placentas, not much is know on the localization of the anastomoses along the vascular equator. The aim of this study was to estimate the prevalence, number, size and localization of the anastomoses in sIUGR, TTTS and TAPS placentas compared to normal MC placentas using color dye injection.

2. Methods

We included in this retrospective study all MC placentas examined at our center, the Leiden University Medical Center, between June 2002 and October 2012. All MC placentas are routinely injected with colored dye and subsequently photographed and stored for further

analysis on computer. Detailed injection protocol has been described previously.[9] We excluded all MC placentas treated with fetoscopic laser coagulation of the vascular anastomoses or selective feticide. Cases with twin reversed arterial perfusion (TRAP) sequence, fetal demise and higher order MC twins were also excluded. Placentas were also excluded because of contamination by formalin or severe damage preventing adequate placental injection. Lastly, placentas were excluded if the picture did not include a measuring-tape or if the quality of the placenta pictures was insufficient to allow reliable measurement of the size and localization of anastomoses. Part of placental data was reported in previous studies.[6, 10]

We divided the MC placentas into 4 groups: 1.) normal MC; 2.) sIUGR; 3.) TTTS; and 4.) spontaneous TAPS. sIUGR was defined as discordance in birth weight ≥ 25%. Discordance of birth weight was calculated according to following formula: (larger twin – smaller twin)/larger twin x100%. TTTS was diagnosed after ultrasonographical manifestations of polyhydramnios (deepest vertical pocket ≥ 8cm) in the recipient sac and oligohydramnios (deepest vertical pocket ≤ 2cm) in the donor sac.[11] TAPS was diagnosed based on prenatally diagnostic criteria (MCA-PSV >1.5 MoM in the donor and MCA-PSV <1.0 MoM in the recipient) and/or postnatally diagnostic criteria (Inter-twin hemoglobin difference >8.0 g/dl, *and* at least one of the following: Reticulocyte count ratio >1.7 and placenta with only small (diameter <1 mm) vascular anastomoses).[4]

Measurements of anastomoses: Measurements of the size and localization of the anastomoses were performed using Image J 1.45s (Image J, National Institute of Health, USA).The caliber of the artery was recorded to measure the size of the AV anastomoses. The measurement of the AV was done within 1 cm of the end of the artery and its connection to the corresponding vein. The measurement of the AA and VV was done exactly in the middle

of the connection between the arterial or venous branches on either side, where the vascular equator was determined to be, by visual inspection.

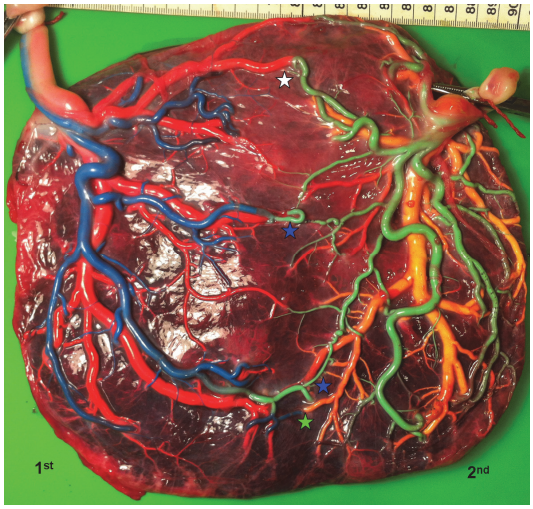

Figure 1 Normal MC placenta (gestational age at delivery: 28 weeks) showing several AV and VA anastomoses and 2 AA anastomoses.

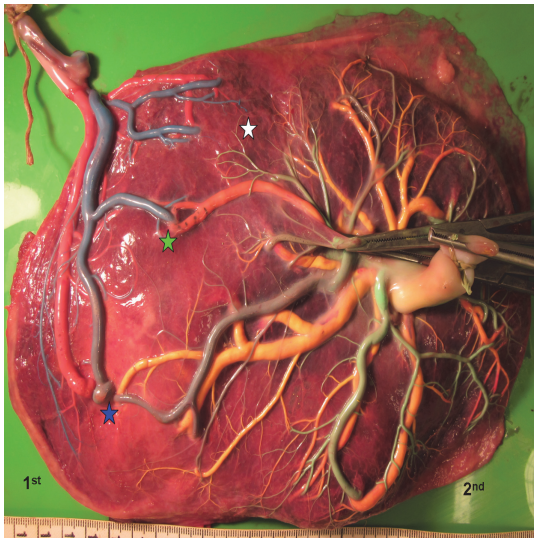

Figure 2 sIUGR placenta (gestational age at delivery: 29 weeks) showing several AV and VA anastomoses and 1 large AA anastomosis.

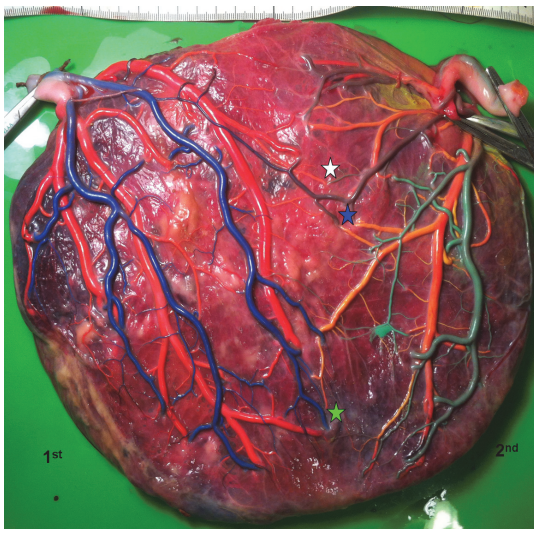

Figure 3 TTTS placenta treated with amnioreduction (gestational age at delivery: 33 weeks) showing several AV and VA anastomoses and 1 AA anastomosis.

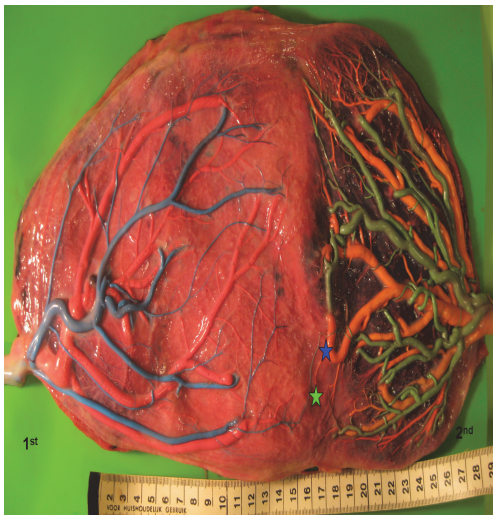

Figure 4 Spontaneous TAPS placenta (gestational age at delivery: 33 weeks) showing several small AV anastomoses and 1 small AA anastomosis.

Statistical analysis: Chi-square test or Fisher's exact test was applied to analyze categorical variables, as appropriate. For comparison of continuous variables, independent-samples t test or Mann-Whitney *U* test was used. Chi-Square Goodness-of-Fit test was used to show the trend of anastomostic distribution in various types of placenta. A *P*-value <0.05 was considered to indicate statistical significance. We performed statistical analysis using SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA).

After measuring the total length of the vascular equator, we calculated its radius. The localization of anastomoses was recorded as the ratio of their distance and radius (distance/radius) as previously reported.[12] Briefly, we divided the placental plate from each placental edge to the center into 5 equal parts along the vascular equator. These parts were then classified as Localization 1 (L1) up to Localization 5 (L5), where L1 was at the margin of the placenta and L5 at the center of the placenta.

3. Results

3.1. Patient and clinical data

A total of 235 MC placentas fulfilled our inclusion criteria, including 126 (54%) normal MC, 46 (19%) sIUGR, 47 (20%) TTTS and 16 (7%) TAPS placentas. In the TTTS group, 43% (20/47) were stage 1, 28% (13/47) were stage 2, 23% (11/47) were stage 3 and 6% (3/47) were stage 4. Examples of normal MC, sIUGR, TTTS and TAPS placentas after color dye injection were shown in Figure 1-4. Baseline characteristics in the 4 subgroups of MC twin pregnancies were shown in Table 1. Mean gestational age (GA) at birth in normal MC, sIUGR, TTTS and TAPS was 33.9±3.4, 33.0±3.5, 27.6±5.7 and 32.9±2.2, respectively. Compared with normal MC pregnancies, GA at birth of TTTS was significantly lower (*P*<0.01). No difference in GA at birth was found between normal MC and sIUGR or TAPS. Birth weight discordance in the groups with sIUGR, TTTS and TAPS was significantly larger than that of normal MC (*P*<0.01,

P<0.01 and *P*=0.03, respectively). The rate of cesarean delivery in TTTS was similar to normal MC pregnancies, but the rate of cesarean delivery in sIUGR and TAPS was significantly higher compared to normal MC pregnancies.

Table 1 Baseline characteristics.

	Normal MC (n=126)	sIUGR (n = 46)	TTTS (n = 47)	TAPS (n = 16)	P1	P2	P3
GA at birth-wks	33.9 ± 3.4	33.0 ± 3.5	27.6 ± 5.7	32.9 ± 2.2	.1	<.01	.29
BW - g	2167 ± 633	1784± 743	1293±827	1796±450	<.01	<.01	<.01
BWD - %[a]	10 (4-15)	32 (25-54)	15 (8-19)	16 (8-26)	<.01	<.01	.03
Cesarean - n (%)	37 (29)	29 (61)	17 (36)	7 (44)	<.01	.5	<.01

Results are shown as mean ± SD. GA: gestational age; BWD: birth weight discordance; P1: normal MC vs sIUGR; P2: normal MC vs TTTS; P3: normal MC vs TAPS. a Denotes median (range).

3.2. Overall anastomoses: number and size

The median number of anastomoses was similar in normal MC, sIUGR and TTTS placentas: 8 (interquartile range (IQR): 4-12), 8 (IQR: 5-14), 7 (IQR: 5-11), respectively, but was significantly lower in TAPS placentas 4 (IQR: 3-5) (*P*<0.01). The median diameter of anastomoses in TTTS and TAPS placentas was significantly smaller compared to normal MC placentas (0.4 (IQR: 0.3-0.6) vs 0.5 (IQR: 0.3-0.9), *P*<0.01 and 0.1 (IQR: 0.1-0.2) vs 0.5 (IQR: (0.3-0.9), *P*<0.01). In contrast, the median diameter of anastomoses in sIUGR placentas were significantly larger than in normal MC placentas (0.6 (IQR: 0.4-1.2) vs 0.5 (IQR: 0.3-0.9), *P*<0.01) (Table 2).

3.3. AV anastomoses: prevalence, number and size

The prevalence of AV anastomoses was similar in the 4 groups: 98%, 100%, 96% and 100%, respectively. The median number of AV anastomoses in normal MC, sIUGR and TTTS

placentas was 7 (IQR: 3-10), 6 (IQR: 4-13) and 6 (IQR: 4-10), respectively and was significantly lower in TAPS placentas 4 (IQR: 2-5) (P<0.01) (Table 2). The median diameter of AV anastomoses in TAPS was significantly smaller compared to normal MC (0.1 (IQR: 0.1-0.2) vs 0.4 (IQR: 0.3-0.7, P<0.01), but significantly larger in sIUGR compared to normal MC (0.5 (IQR: 0.4-0.9) vs 0.4 (IQR: 0.3-0.7), P<0.01) (Table 2).

3.4 AA and VV anastomoses: prevalence and size

The prevalence of AA anastomoses was similar in normal MC and sIUGR placentas (96% and 98%, respectively), and was significantly lower in TTTS and TAPS placentas (47% and 19%) (Figure 5 and Table 2). Median diameter of AA anastomoses in TTTS was significantly smaller in comparison with normal MC placentas (0.6 (IQR: 0.4-1.2) vs 1.7 (IQR: 1.0-2.5), P<0.01), but larger in sIUGR placentas (2.2 (IQR: 1.5-3.1) vs 1.7 (IQR: 1.0-2.5), P=0.04) (Table 2). The prevalence of VV anastomoses in normal MC, sIUGR, TTTS and TAPS placentas was low (28%, 28%, 32% and 0%, respectively) (Figure 5 and Table 2).

3.5. Localization of anastomoses

In normal MC, sIUGR and TTTS placentas, AV anastomoses were evenly localized along the vascular equator (Table 3 and Figure 6).

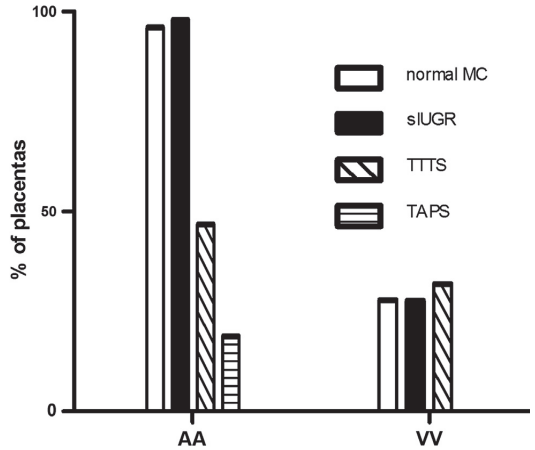

Figure 5 Prevalence of AA and VV in the various subgroups of MC placentas

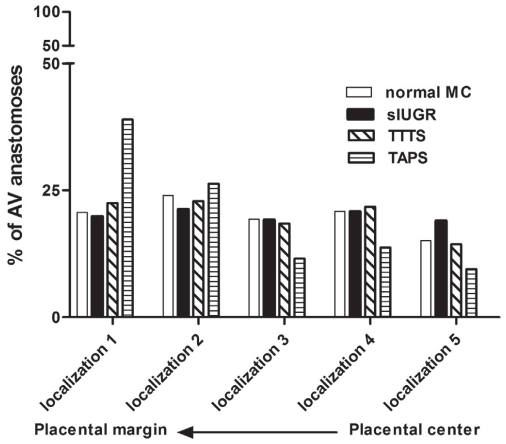

Figure 6 Localization of AV anastomoses in the various subgroups of MC placentas.

Table 2 Prevalence, number and size of anastomoses in the 4 groups of MC placentas.

	Normal MC (n=126)	sIUGR (n=46)	TTTS (n=47)	TAPS (n=16)	P_1 Value	P_2 Value	P_3 Value
Overall no. of anastomoses*	8 (4-12)	8 (5-14)	7 (5-11)	4 (3-5)	0.67	0.88	<0.01
Overall diameter of all anastomoses –mm*	0.5 (0.3-0.9)	0.6 (0.4-1.2)	0.4 (0.3-0.6)	0.1 (0.1-0.2)	0.01	<0.01	<0.01
Placentas with AV anastomoses - n (%)	124 (98)	46 (100)	45 (96)	16 (100)	1.00	0.30	1.00
No. of AV anastomoses per placenta*	7 (3-10)	6 (4-13)	6 (4-10)	4 (2-5)	0.74	0.84	<0.01
AV diameter – mm*	0.4 (0.3-0.7)	0.5 (0.4-0.9)	0.4 (0.3-0.6)	0.1 (0.1-0.2)	<0.01	0.07	<0.01
Placentas with AA anastomoses - n (%)	121 (96)	46 (100)	22 (47)	3 (19)	1.00	<0.01	<0.01
AA diameter – mm*	1.7 (1.0-2.5)	2.2 (1.5-3.1)	0.6 (0.4-1.2)	0.3 (0.2-0.4)	0.04	<0.01	0.02
Placentas with VV anastomoses - n (%)	35 (28)	13 (28)	15 (32)	0 (0)	1.00	0.58	—
VV diameter – mm*	2.8 (2.0-4.7)	2.4 (1.6-4.5)	1.1 (0.3-3.5)	—	0.85	0.08	—

*Results are shown as median (IQR). P1: normal MC vs sIUGR; P2: normal MC vs TTTS; P3: normal MC vs TAPS

In TAPS placentas, most AV anastomoses localized near the margin of the placenta (Table 3 and Figure 6). We found a trend towards an increased rate of localization of AA anastomoses towards the center of the placenta in sIUGR and TTTS placentas (Table 3 and Figure 7). In contrast, the localization of the few AA anastomoses in the TAPS group was nearer to the placental margin (Table 3 and Figure 7). Detailed information on the localization of AV anastomoses and AA anastomoses is presented in Table 3.

4. Discussion

The specific complications of MC pregnancies are associated with unique inter-twin placental angioarchitecture. The present study shows that the prevalence, size, number and localization of the various types of anastomoses differ between normal MC, TTTS sIUGR,

Table 3 Detailed trend of localization of AV and AA anastomoses in various type of placenta.

		margin ◄——► Center					
		L1	L2	L3	L4	L5	*P* value
Normal MC	AV - %	21	24	19	21	15	.70
	AA - %	16	20	24	24	16	.53
sIUGR	AV - %	20	21	19	21	19	1.0
	AA - %	6	21	28	30	15	<.01
TTTS	AV - %	23	23	18	22	14	.54
	AA - %	9	3	18	36	33	<.01
TAPS	AV - %	39	26	12	14	9	<.01
	AA - %	67	0	0	33	0	<.01

L1: localization 1; L2: localization 2; L3: localization 3; L4: localization 4; L5: localization 5.
Chi-Square Goodness-of-Fit test: df=4. For AA localization in TAPS placentas: df=1.

TTTS and TAPS placentas. Compared to normal MC placentas, the prevalence of AA anastomoses was significantly lower in the group with TTTS (47%) and TAPS (19%).

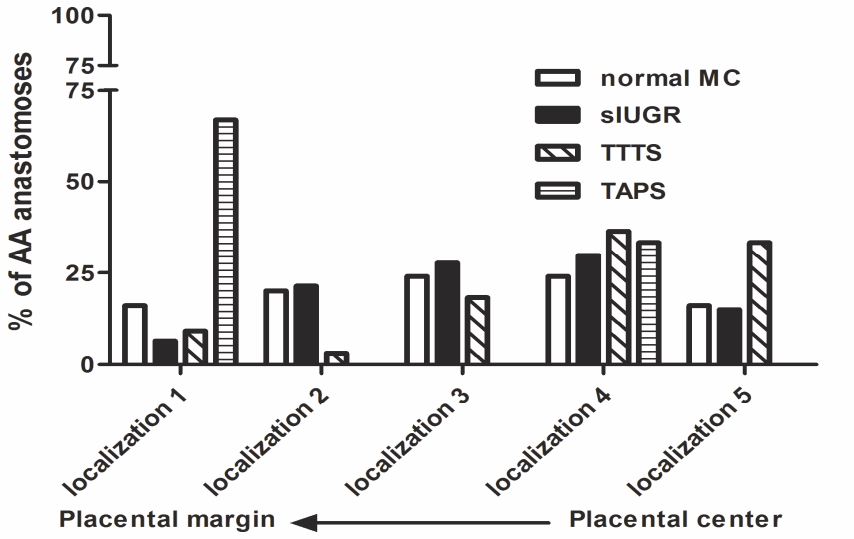

Figure 7 Localization of AA anastomoses in the various subgroups of MC placentas.

As suggested in previous studies,[10, 13, 14] AA anastomoses allow bidirectional flow and equilibration of inter-twin blood volumes, hereby reducing the risk of TTTS and TAPS.
The sizes of these AA anastomoses also vary between the different groups. AA anastomoses are the largest in the sIUGR group (median diameter 2.2mm) and the smallest in diameter in the TAPS group (median diameter 0.3mm). This finding is in accordance with previous studies. [3, 5, 10] Hypothetically, the small diameter of the AA in TAPS placentas does not allow sufficient blood flow for equilibration of hemoglobin levels between the donor and recipient.[14]

The prevalence of AV anastomoses was identical in the 4 groups (almost 100%), but the size and number varied per group. The diameter of AV anastomoses was the largest in the sIUGR group (median diameter 0.5mm) and the smallest in the TAPS group (median diameter 0.1mm). The median number of AV anastomoses in the normal MC, sIUGR and TTTS groups

was double the median number of AV in the TAPS group. The minuscule size and the small number of anastomoses is one of the main characteristics of TAPS placentas.[8]

Our study showed also a higher mean number of anastomoses (mean of 9 to 10 per placenta) compared to other studies (mean range from 1 to 6 per placenta).[13, 15, 16] The cause of this discrepancy is not clear and may be due to improvement of placenta injection technique.

The prevalence and size of VV anastomoses was low and similar in normal MC, sIUGR and TTTS placentas, as reported previously.[1] No VV anastomoses were detected in the TAPS group. The significance and role of VV anastomoses remains to be elucidated.

The most important and novel finding of this study concerns the localization of the anastomoses in the different groups of MC placentas. Our study shows that AV anastomoses are usually evenly distributed along the vascular equator, except in the TAPS group where most anastomoses are localized near the placental margin. In contrast, we found a trend towards an increasing rate of AA anastomoses near the center of the placenta in sIUGR and TTTS. The origin of this variation in distribution of anastomoses is not clear, but could be useful for fetal surgeons performing fetoscopic laser coagulation of vascular anastomoses in MC pregnancies. Several studies reported that the incidence of residual anastomoses after laser surgery was up to 32% and were associated with recurrent TTTS and post-laser TAPS.[12, 17-19] Most residual anastomoses appear to be localized near the placental margin. This could be due to visualization difficulties during fetoscopy because of technical reasons associated with the position and the angle of the fetoscope, or because placental margins may be less well scrutinized during fetoscopic laser surgery. Our study enhances the knowledge that the complete vascular equator must be scrutinized during fetoscopy.

Our data should be interpreted with care due to possible limitations related to the fact that the various subgroups of placentas were not matched for gestational age. In addition, multiple testing was used to compare the various subgroups with the index group of normal MC placentas, which could also have influenced the significance of the data.

In conclusion, understanding the differences in angioarchitecture between the various types of MC placentas may help elucidate the specific role of the various anastomoses in the development of specific complications such as sIUGR, TTTS and TAPS in MC pregnancies. In addition, information on the localization of the various anastomoses may be useful for fetal surgeons involved in fetoscopic laser coagulation of vascular anastomoses.

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Chapter 4

Monochorionic placentas with proximate umbilical cord insertions: definition, prevalence and angio-architecture

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Abstract

Introduction: Not much is known on the occurrence and characteristics of proximate umbilical cord insertions (PCI) in monochorionic (MC) placentas. In addition, a clear definition for PCI is lacking. The purpose of this study was to establish a reference range for the distance between cord insertions and to evaluate the prevalence and angio-architecture of MC placentas with PCI.

Methods: All MC placentas consecutively examined at our center from 2002 to 2014 were included in this study. We excluded MC placentas treated with fetoscopic surgery. The reference range of distance between cord insertions was created using the standard methodology proposed by Royston and Wright. We defined PCI as a cord insertion distance below the 5^{th} centile.

Results and Discussion: A total of 369 MC placentas were analyzed during this study period. The 5^{th} centile was calculated by the equation : 0.027 × gestational age (weeks) +2.91 (cm), and ranged from 3.3 to 4 cm throughout gestation. Accordingly, 18 of the 369 (5%) MC placentas fulfilled the definition criteria for PCI. PCI occurred frequently in MC monoamniotic placentas (53%, 9/17) but were rare in MC diamniotic placentas (3%, 9/352). The prevalence of arterio-arterial (AA) and veno-venous (VV) anastomoses in MC placentas with and without PCI was respectively 100% (18/18) versus 80% (281/351) (P=.12) and 56% (10/18) versus 26% (91/351) (P=.01). The proximity of umbilical cord insertions and its characteristic presence of superficial anastomoses may be a representative of the later splitting of inner cell mass in MC amniotic twins.

Conclusion: The threshold for PCI (5^{th} centile) is approximately 4 cm throughout gestation. PCI are rare in MC diamniotic placentas, but are quite common in MC monoamniotic

placentas. MC placentas with PCI are characterized by higher rates of superficial AA and/or VV anastomoses.

Keywords: Monochorionic twins, proximate cord insertions, twin-twin transfusion syndrome, twin anemia-polycythemia sequence, growth discordance, monoamniotic.

Introduction

Several studies reported a correlation between abnormal cord insertion and adverse perinatal outcome in monochorionic (MC) twin pregnancies [1-4]. Most literature on abnormal cord insertions focuses on the presence and consequences of velamentous or marginal cord insertion. Another special type of abnormal umbilical cord insertion in MC twins, so called proximate cord insertions (PCI), occurs when the cord insertions are very near to each other [5-7]. Recent studies show that in twin-twin transfusion syndrome (TTTS) cases treated with fetoscopic laser coagulation, PCI may lead to difficulty in identifying the inter-twin vascular equator and subsequent treatment failure [8-10]. Not much is known on the occurrence and characteristics of PCI in other subgroups of MC twin pregnancies. The prevalence and characteristics of MC placentas with PCI among various subgroups of MC twins remain to be elucidated. In addition, the current definitions used for PCI are based on arbitrary assumptions and not derived from scientific analysis.

The primary aim of our study was to establish a reference range for the distance between cord insertions based on the analysis of a large cohort of MC placentas. In addition we aimed to evaluate the prevalence of PCI in different subgroups of MC twins and compare the placental angio-architecture in MC placentas with and without PCI.

Materials and methods

All consecutive MC placentas examined at the Leiden University Medical Center from July 2002 to October 2014 were included in this study. MC pregnancies managed with fetoscopic surgery (either laser ablation of vascular anastomoses or selective feticide) were excluded due to iatrogenic destruction of placental angio-architecture. We also excluded placentas due to damage caused by manual placental removal, fixation in formalin or when the

insertion site of umbilical cord was damaged preventing accurate measurements and evaluation of placental angio-architecture. We divided the MC placentas into 5 subgroups including: 1.) normal MC; 2.) TTTS treated conservatively with amnioreduction or expectant management; 3.) spontaneous twin anemia-polycythemia sequence (TAPS); 4.) growth discordance and 5.) monoamniotic (MA). Normal MC twin pregnancies were defined as uneventful MC twin pregnancies. Diagnosis of TTTS was based on the internationally accepted 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 [11]. Diagnosis of spontaneous TAPS was based of prenatal criteria depending on Doppler ultrasound measurements or postnatal criteria using hematological tests as previously reported [12]. Growth discordance was defined as inter-twin birth-weight discordance ≥ 25%.

MC placentas were examined and routinely injected using colored dye according to a protocol described before [13]. Pictures of the injected placenta were then taken using a high-resolution digital camera and a measuring-tape was placed on the placenta to allow various measurements on the digital picture. Examination, classification and injection was performed by 2 of the authors (D.Z., E.L.).

After placental injection, distance between both cord insertions was measured. We defined PCI as a cord insertion distance below the 5^{th} centile. We also measured the ratio between insertion distance and placental diameter by dividing the longest placental diameter by the distance between cord insertions. We recorded the type of umbilical cord insertion as (para-) central, marginal or velamentous. Velamentous cord insertion was defined as a cord directly inserted into the amniotic membrane instead of placental parenchyma and marginal cord insertion was defined as a cord insertion site within 1 cm of the plate edge. We

recorded the number and type of anastomoses. Arterio-arterial (AA) and veno-venous (VV) anastomoses were classified as superficial anastomoses and arterio-venous (AV) anastomoses were classified as deep anastomoses. All measurements were performed using Image J 1.45s (Image J, National Institute of Health, USA). Part of the placental data was included in previous studies to map the localization of vascular anastomoses on placental plate surface and compare the placental characteristics between different forms of TAPS [14, 15].

Information on perinatal outcome was documented for each case in a dedicated database, including gestational age at birth, birth weight and perinatal death (either fetal demise or neonatal death).

The primary aim of our study was to estimate the cut-off value for PCI against gestational age. We also compared the prevalence of PCI in various subgroups of MC twins and studied the characteristics of MC placentas with and without PCI.

Statistics

The gestational age-specific reference range of distance between cord insertions was generated according the standard methodology described by Royston and Wright [16]. Briefly, polynomial least-squares regression was applied to estimate the mean curve of distance between cord insertions in function of gestational age at birth and to calculated scaled absolute residuals. The standard deviation (SD) curve was estimated by the polynomial least-squares regression of the scaled absolute residuals. A centile curve was calculated using the formula: centile = mean + $K \times$ SD (K is the corresponding centile of the normal distribution). Independent-samples t test or Mann-Whitney U test was adapted to compare continuous variables. Chi-square or Fisher's exact test was used to analyze categorical variables, where appropriate. A P value <.05 was considered to show the

statistical significance. SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis.

Results

A total of 405 MC placentas not treated with fetoscopic laser surgery were delivered or shipped to our center for examination during this study period. Thirty-six (9%) cases could not be injected due to damage of cord insertion site (n=16), severely damaged placentas (n=10), placentas fixed in formalin (n=6), severe maceration (n=2) and TRAP (n=2). The remaining 369 (91%) placentas were injected and analyzed, including 197 (53%) normal MC, 48 (13%) TTTS treated with amnioreduction or managed expectantly, 26 (7%) spontaneous TAPS, 81 (22%) growth discordance and 17 (5%) MA. A detailed flowchart to illustrate the inclusion and exclusion of MC placentas is shown in Figure 1.

In the TTTS group, 44% (21/48) were stage 1, 27% (13/48) were stage 2, 23% (11/48) were stage 3 and 6% (3/48) were stage 4. Half of the TTTS cases (n=24) were treated with amnioreduction, while the other half were managed expectantly. Baseline characteristics of these 5 subgroups are summarized in Table 1. The distances between the cord insertions ranged from 0 to 34 cm.

Table 1 Baseline characteristics in the various subgroups of MC placentas

	Normal MC (n=197)	TTTS (n=48)	TAPS (n=26)	sIUGR (n=81)	MA group (n=17)
GA at birth – week[a]	35.3 (32.3-36.4)	29.6 (27.6-33.5)	33.2 (31.0-35.0)	33.9 (29.8-35.8)	32.0 (29.0-33.7)
BW – gram [a]	2250 (1701-2650)	1448 (1128-2021)	1820 (1434-2134)	1561 (1015-2135)	1720 (1088-2070)
BWD - %[a]	9.7 (4.4-15.3)	15.8 (8.1-24.3)	19.5 (9.6-29.6)	31.7 (27.8-38.3)	5.6 (2.0-20.6)
Cesarean - n (%)	54 (28)	22 (46)	11 (42)	49 (61)	14 (82)[b]

Table 1 [a] Denotes median (IQR).[b] Three cases were managed with vaginal delivery due to fetal demise in one twin

Table 2 Characteristics of various types of MC placentas

	Normal MC (n=197)	TTTS (n=48)	TAPS (n=26)	sIUGR (n=81)	MA (n=17)	P_1 Value	P_2 Value	P_3 Value	P_4 Value
PCI– n (%)	7 (4)	0	0	2 (2)	9 (53)	.37	.62	.62	<.01
Distance– (cm) [a]	17 (13-21)	18 (14-21)	18(13-24)	15 (11-19)	3 (1-6)	.40	.23	<.01	<.01
Ratio– % [a]	63 (49-76)	70 (61-80)	69(59-81)	61 (50-71)	22 (9-31)	.02	<.05	.84	<.01
VCI– n (%) [b]	84 (21)	29 (30)	6 (12)	58 (36)	1 (3)	.06	.14	<.01	.01

Table 2 [a] Denotes median (IQR). [b] Refers to the type of cord insertion per fetus. PCI: Proximate cord insertions Distance: Distance between cord insertions.

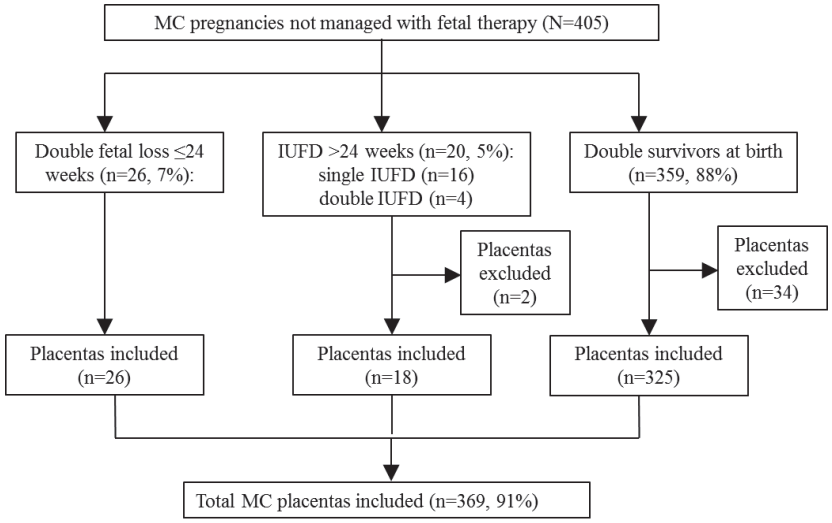

Figure 1 Flow chart showing the derivation of the studied cohort.

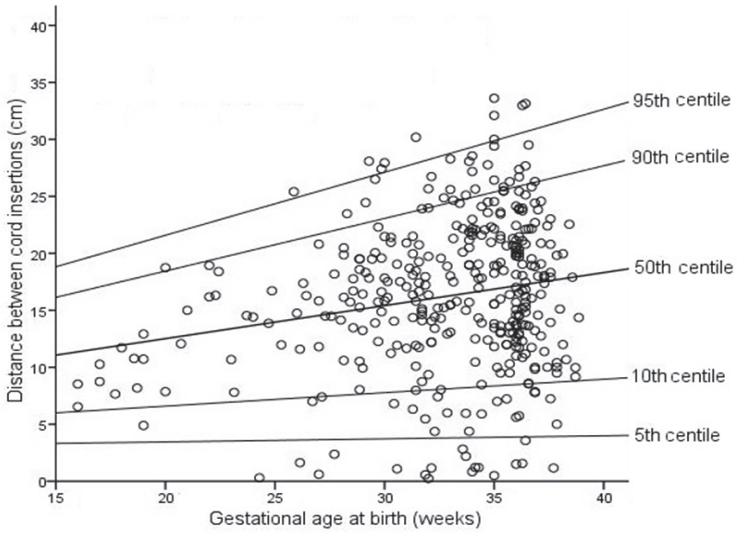

Figure 2 Scatter plot of distance between cord insertions across gestation and estimated reference range with curves of various centiles.

The reference range for distance between cord insertions across gestation between 16^{+0} to 38^{+6} weeks was estimated by the equation: Distance (cm) = 0.290 × gestational age (GA) (weeks) + 6.720 (R^2 =0.04, P = .00). The curve fitting SD was as follows: SD (cm) = 0.134 × GA (weeks) + 1.943 (R^2 =0.04, P = .00). The 5^{th}, 10^{th}, 50^{th}, 90^{th} and 95^{th} centile lines were calculated using the *K* value of -1.96, -1.28, 0, 1.28, and 1.96, respectively and are shown in Figure 2. The 5^{th} centile across gestation between 16^{+0} to 38^{+6} weeks was calculated by the equation: 0.027 × GA (weeks) +2.912 (cm) (yielding a range from 3.3 cm to 4.0 cm). A total of 18 of the 369 MC placentas fulfilled the criteria for PCI. The distances between the cord insertions ranged from 0 to 34 cm. The reference range for distance between cord insertions across gestation between 16^{+0} to 38^{+6} weeks was estimated by the equation: Distance (cm) = 0.290 × gestational age (GA) (weeks) + 6.720 (R^2 =0.04, P = .00). The curve fitting SD was as follows: SD (cm) = 0.134 × GA (weeks) + 1.943 (R^2 =0.04, P = .00). The 5^{th}, 10^{th}, 50^{th}, 90^{th} and 95^{th} centile lines were calculated using the *K* value of -1.96, -1.28, 0, 1.28, and 1.96, respectively and are shown in Figure 2. The 5^{th} centile across gestation between 16^{+0} to 38^{+6} weeks was calculated by the equation: 0.027 × GA (weeks) +2.912 (cm) (yielding a range from 3.3 cm to 4.0 cm). A total of 18 of the 369 MC placentas fulfilled the criteria for PCI. Further analysis showed that PCI occurred frequently in MA placentas (53%, 9/17) but were rare in normal MC (4%, 7/197), TTTS (0%, 0/48), TAPS (0%, 0/26) and growth-discordance (2%, 2/81) placentas, respectively. Additional placental characteristics in the 5 subgroups of MC placentas are reported in Table 2. Two examples of MC placentas with PCI are shown in Figure 3 and 4.

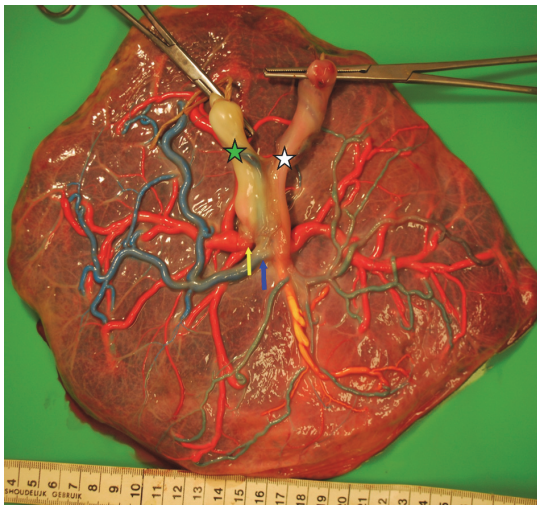

Figure 3 Monochorionic diamniotic placenta with growth discordance (delivery at 35 weeks' gestation, birth weight of 1st and 2nd twin is 3340 grams and 2415 grams, respectively) with proximate cord insertions (≤ 4 cm). Green stars and white stars denote the cord insertions of the first and second twin, respectively. AA and VV anastomoses are indicated with blue and yellow arrows, respectively.

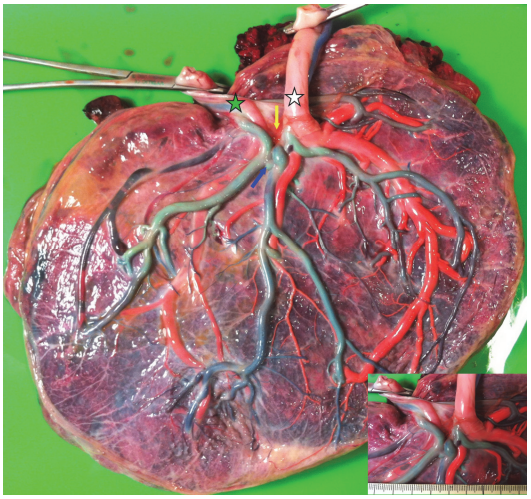

Figure 4: Monoamniotic placenta (delivery at 32 weeks' gestation; birth weight of 1st and 2nd twin is 2210 grams and 2218 grams, respectively) with proximate cord insertions. Green stars and white stars denote the cord insertions of the first and second twin, respectively. AA and VV anastomoses are indicated with blue and yellow arrows, respectively. The inserted picture shows the detailed distance between cord insertions (bottom right).

Table 3 Placental characteristics in MC twins with and without proximate cord insertions

	PCI (n=18)	**No-PCI (n=351)**	**P value**
AV present – n (%)	18 (100)	350 (100)	1.00
AA present – n (%)	18 (100)	264 (80)	.12
VV present – n (%)	10 (56)	91 (26)	.01
VCI – n (%) [a]	1 (3)	177 (25)	.02

Table 3 [a] Denotes the type of cord insertion per fetus.

Characteristics (angioarchitecture and type of cord insertion) of MC placentas with and without PCI are shown in Table 3. The prevalence of AA anastomoses in MC placentas with and without PCI was 100% (18/18) versus 80% (281/351), respectively, P=.12. The prevalence of VV anastomoses in MC placentas with and without PCI was 56% (10/18) and 26% (91/351), respectively, P<.01.

Discussion

This is the first study establishing a reference range for the distance between umbilical cord insertions across gestational age. The reference range and associated equations were based on the evaluation of 369 MC placentas. We defined PCI as a distance between the cords insertions lower than the 5th centile. Accordingly, the cut-off value for PCI ranged from 3.3 to 4.0 cm across gestation. This cut-off value is slightly lower than the 5 cm cut-off used in previous studies [7, 17]. However, the 5 cm cut-off value was based on arbitrary assumptions and not derived from scientific analysis. We propose that a lower cut-off set at 4 cm would be more appropriate. In addition, since the range of the 5th centile varied only slightly throughout gestation (from 3.3 to 4 cm), we suggest that the use of a fixed cut-off set at 4 cm might be easier to use in daily practice instead of a gestational-age-dependent equation.

This study also shows that the prevalence of PCI in various subgroups of MC diamniotic placentas is low (0-4%) whereas in the subgroup of MC monoamniotic placentas PCI are quite common (53%). The significant proximity of umbilical cord insertions may be a representative of the later splitting of inner cell mass in MA twin gestations (around 8-12 days after fertilization) compared to MC diamniotic twin gestations (around 4-8 days after fertilization). The short distance between cord

insertions in MA placentas is reported to be one of the main causal factors leading to the ubiquitous entanglement of umbilical cords [7, 18].

This study also compared the angio-architecture in MC twin placentas with and without PCI. Interestingly, we found that MC placentas with PCI are characterized by a higher incidence of superficial AA (100%) and VV (56%) anastomoses. The cause of the higher rate of superficial anastomoses in MC placentas with PCI is not known and could also be related to the later splitting of inner cell mass. Previous studies showed that the blood flow in superficial AA anastomoses is bidirectional and reduce the inter-twin fluid disequilibrium [19, 20]. The presence of AA anastomoses may thus have a protective effect and be beneficial in cases with PCI, such as reduced risk of TTTS development [19]. The exact role and effect of VV anastomoses in MC placentas is not clear. Although Denbow et al argued that VV anastomoses may increase the perinatal mortality [21], several other studies reported no association between perinatal mortality and VV anastomoses [5-7].

Antenatal detection of umbilical cord insertion site has been steadily achieved with the advance in ultrasound technology [25]. The accuracy of ultrasound examination in the diagnosis of PCI has however not been studied yet. The clinical implication of antenatal detection of PCI also requires further investigation.

The main limitation of our study, besides its retrospective nature, is the relative small number of cases with PCI preventing further analyzing the clinical significance of PCI. Our data should therefore be interpreted with care. Larger studies are required to assess the correlation between clinical outcome and PCI. Ideally, the design of these larger studies should be prospective and start with ultrasound examination in the first trimester of pregnancy. In addition, our data should be also

interpreted with caution due to the inclusion bias of MC twin pregnancies. The majority of TTTS cases examined at our center are treated with fetoscopic laser surgery and these cases were excluded from this study. In a previous study in TTTS cases managed with fetoscopic laser surgery at our center, we reported a 2% (4/252) rate of PCI [9], which is in accordance with the rate of PCI in TTTS cases managed conservatively in this study.

In conclusion, PCI are rare in MC diamniotic placentas, but occur frequently in MC monoamniotic placentas. MC placentas with PCI are characterized by high rates of AA and VV anastomoses. Larger studies are needed to understand the clinical implications of PCI. Based on our analysis, we propose to set "≤4cm" as the fixed cut-off value of PCI and this cut-off should be used in future studies on PCI in MC twins to increase the homogeneity and uniformity between the various studies.

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PART II

Placental characteristics in relation to TTTS and TAPS

Chapter 5

Veno–venous anastomoses in twin–twin transfusion syndrome: a multicenter study

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Abstract

Introduction: The aim of this study is to evaluate the prevalence of veno-venous (VV) anastomoses in a large cohort of monochorionic (MC) twin placentas with twin–twin transfusion syndrome (TTTS) compared to a control group of MC placentas without TTTS.

Methods: All TTTS placentas not treated with fetoscopic laser surgery (TTTS group) and examined at five international fetal therapy centers were included in this study and compared with a control group of MC placentas without TTTS (non-TTTS group). MC placentas were routinely injected with colored dye. We recorded the presence of VV and arterio-arterial (AA) anastomoses.

Results: A total of 414 MC placentas were included in this study (TTTS group, n=106; non-TTTS group, n=308). The prevalence of VV anastomoses was significantly higher in the TTTS group than in the non-TTTS group, 36% (38/106) and 25% (78/308), respectively (p=.04; odds ratio (OR) 1.65; 95% confidence interval (CI): 1.03–2.64). In the subgroup of MC placentas without AA anastomoses, the prevalence of VV anastomoses in the TTTS group and non-TTTS group was 32% (18/57) and 8% (2/25), respectively (p=.03; OR: 5.31; 95% CI: 1.13–24.98).

Discussion: VV anastomoses are detected more frequently in TTTS placentas than in MC placentas without TTTS and may thus play a role in the development of TTTS.

Keywords: Monochorionic placenta, Twin–twin transfusion syndrome, Veno–venous anastomoses

Introduction

Twin–twin transfusion syndrome (TTTS) is a severe complication of monochorionic (MC) twin pregnancies and results from intertwin blood transfusion through placental vascular anastomoses. Almost all MC placentas have vascular anastomoses, but only 9% of MC twins eventually develop TTTS [1]. One of the factors involved in the development of TTTS is the placental angioarchitecture and the type of anastomoses [2]. Three types of anastomoses may be present: arterio-venous (AV) anastomoses, arterio-arterial (AA) anastomoses and veno-venous (VV) anastomoses. AV anastomoses are unidirectional anastomoses, whereas AA and VV anastomoses allow bidirectional blood flow. AA anastomoses are detected more frequently in MC placentas without TTTS (non-TTTS placentas) and may therefore play an important role in preventing the development of TTTS. The protective role of AA anastomoses has been substantiated in *in vitro* and *in vivo* placental studies and mathematical models for TTTS [3-6]. Although the blood flow in VV anastomoses is also bidirectional, a higher prevalence of VV anastomoses in TTTS placentas was reported in a few small studies compared to non-TTTS placentas [6-8]. Data from these small studies suggest that, in contrast to AA anastomoses, VV anastomoses may increase the risk of TTTS [7, 8]. However, other studies reported a comparable or lower prevalence of VV anastomoses in TTTS placentas compared to non-TTTS placentas [4, 9-11]. Discrepancy among these studies can partially be attributed to methodological differences and small sample size of TTTS placentas (range: 10 to 50 TTTS placentas). The aim of this study is to evaluate the prevalence of veno-venous (VV) anastomoses in a large cohort of monochorionic (MC) twin placentas with TTTS compared to a control group of MC placentas without TTTS.

Materials and methods

The majority of TTTS cases in the Netherlands are managed with fetoscopic laser coagulation of vascular anastomoses. Given the paucity of TTTS placentas not treated with fetoscopic laser surgery and the purpose of this study, we contacted four other international tertiary care centers for fetal medicine and experienced in placental injection with color dye, to reach a large sample size. The four other centers were: Women and Infants Hospital (Providence, the USA), University Hospitals Leuven (Leuven, Belgium), Shanghai First Maternity and Infant Hospital (Shanghai, China), Hospital Italiano (Buenos Aires, Argentina). All MC placentas with and without TTTS consecutively examined at Leiden University Medical Center (the Netherlands) from May 2002 to February 2015 were included in the TTTS group and non-TTTS group. All TTTS placentas consecutively examined at Women and Infants Hospital (Providence, the USA) from 2001 to 2014, at University Hospitals Leuven (Belgium) from 2003 to 2014, at Shanghai First Maternity and Infant Hospital (Shanghai, China) from 2009 to 2014 and at Hospital Italiano de Buenos Aires Argentina from 2010 to 2014 were included in the TTTS group. Part of the placental data from the Leiden University Medical center included in this study was reported in two previous publications on the role of VV anastomoses in TTTS [7, 12]. TTTS was diagnosed based on 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 [13]. We excluded all MC twins with TTTS treated with fetoscopic laser coagulation of vascular anastomoses, MC twins with twin anemia polycythemia sequence (TAPS), damaged placentas (due to fixation in formalin, maceration, or other causes) and placentas from triplets or higher order

gestations. Placentas from TTTS cases with stage V or TTTS cases managed with feticide were included in this study if delivery occurred within 1 week after fetal demise or treatment.

Postnatal placental examination and colored-dye injection was routinely performed in these five centers according to protocols described elsewhere [14-16]. Velamentous cord insertion was defined as the insertion of umbilical cord into the amniotic membrane and was recorded during postnatal examination. After injection, the number and type of vascular anastomoses (AV, AA and VV anastomoses) were documented. The type of AV anastomoses was not specified if this was an AV anastomosis in the direction from donor to recipient or in the opposite direction. High-resolution pictures were also taken perpendicularly for post-hoc measurements on computer. Individual placental share was delineated as the venous return area of each twin and was measured on the placental pictures using a computer software Image J 1.45s (Image J, National Institute of Health, USA). Placental sharing difference was calculated by the larger placental share minus the smaller placental share. Placental sharing discordance was defined as a placental sharing difference ≥ 25%. Birth weight discordance was calculated by the following formula: (larger twin birth weight – smaller twin birth weight) / larger twin birth weight x 100.

The following clinical data was also collected, including gestational age at diagnosis of TTTS, Quintero stage, modalities of managing TTTS, gestational age at birth, birth weight and perinatal mortality.

Statistics

A few studies reported the prevalence of VV anastomoses in TTTS placentas and/or non-TTTS placentas [4, 6, 8-11, 17-20]. Accordingly, we calculated that a minimum of 60 TTTS placentas and 250 non-TTTS placentas would be needed to demonstrate a 8% difference in the prevalence of VV anastomoses between groups (33% versus 25%) with a significance of 0.05, a power of 90%, by two-tailed analysis. Student *t* test or Mann-Whitney *U* test was opted for analyzing continuous variable, where appropriate. Chi-square or Fisher's exact test was employed to analyze categorical variables, where appropriate. Significance was considered as a p value < .05. All statistical analysis was processed in SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 490 MC placentas not treated with fetoscopic laser surgery were consecutively examined during the study period. We excluded TAPS placentas (n=29), placentas from triplet pregnancies (n=5), placentas fixed in formalin (n=8) , placentas with severe damage (n=15) and placentas macerated due to fetal demise (> 1 week before delivery) (n=19). Lastly, 414 (84%) MC placentas with complete data on placental angioarchitecture were analyzed in this study, including 106 TTTS placentas and 308 non-TTTS placentas. In the TTTS group, information on gestational age at diagnosis, Quintero staging and treatment was retrieved in 85 (80%) cases. Median gestational age at diagnosis of TTTS was 26.2 weeks (range: 14.0 to 36.6 weeks). Quintero stage at diagnosis was stage I in 22 cases (26%), stage II in 11 (13%), stage III in 36 (42%), stage IV in 5 (6%) and stage V in 11 (13%). In the TTTS group, 32 cases (37%) were managed expectantly, 46 (54%) were treated with amniodrainage, 1 (1%) was treated with cord occlusion, 1 (1%) was treated with

radiofrequency, 3 (4%) were delivered by induced labor and 2 (2%) terminated the pregnancy. Additional clinical characteristics of patients are summarized in Table 1.

Table 1 Patient characteristics.

	Non-TTTS (n=308)	TTTS (n=106)
Gestational age at birth – weeks [a]	33.5 ± 4.2	28.3 ± 4.9
Birth weight of larger twin– grams[a]	2168 ± 717	1309 ± 716
Birth weight of smaller twin– grams[a]	1835 ± 703	1077 ± 623
Birth weight discordance - % [b]	13.7 (5.8-25.4)	17.2 (9.5-28.0)

Birth weight was not available in 13 cases: 4 non-TTTS cases and 9 TTTS cases. [a] Data was shown as mean ± SD. [b] Data was shown as median (IQR).

Median number of overall AV anastomoses in TTTS and non-TTTS placentas was 6 (interquartile range (IQR): 4–8) and 7 (IQR: 4–11), respectively (p=.15). AA anastomoses were observed in 46% (49/106) of the TTTS placentas and in 92% (283/308) of the non-TTTS placentas (p=<.01). The prevalence of VV anastomoses was significantly higher in the TTTS group than in the non-TTTS group, 36% (38/106) and 25% (78/308), respectively (p=.04). In the subgroup of placentas without AA anastomoses, the prevalence of VV anastomoses in the TTTS group and non-TTTS group was 32% (18/57) and 8% (2/25), respectively (p=.03). No significant difference was found in the prevalence of velamentous cord insertion (referred to as umbilical cord insertion per fetus) between TTTS placentas (29%, 62/212) and non-TTTS placentas (25%, 152/616) (p=.19). Further comparison of placental angioarchitecture between TTTS placentas and non-TTTS placentas is displayed in Table 2. An example of a TTTS placenta injected with colored-dye is shown in Figure 1.

Table 2 Placental characteristics

	Non-TTTS (n=308)	TTTS (n=106)	P value	OR (95%CI)
Placental sharing difference - % [a]	20.8 (10.0-35.8)	19.8 (10.0-30.3)	.72	2.20 (.03–164.27)
Placental sharing discordance (>25%) – n (%)	121 (39)	34 (39)	.91	.97 (.60–1.58)
Velamentous cord insertion in smaller twin– n[a] (%)[b]	119 (39)	44 (45)	.12	1.29 (.81–2.05)
Velamentous cord insertion in larger twin– n (%)[b]	29 (9)	13 (13)	.33	1.47 (.73–2.95)
Placentas with AA anastomoses – n (%)	283 (92)	49 (46)	.00	.08 (.04–.13)
Placentas with VV anastomoses – n (%)	78 (25)	38 (36)	.04	1.65 (1.03–2.64)
Placentas with AA and VV anastomoses – n (%)	76 (25)	20 (19)	.22	.71 (.41–1.23)
Placentas with AA and without VV anastomoses – n (%)	207 (67)	29 (27)	.00	.18 (.11–.30)
Placentas with VV anastomoses without AA anastomoses – n (%)	2 (1)	18 (17)	.00	31.3 (7.1–137.5)

[a] Data was shown as median (IQR). [b] Birth weight was not available in 13 pairs of MC twins: 4 non-TTTS cases and 9 TTTS case.

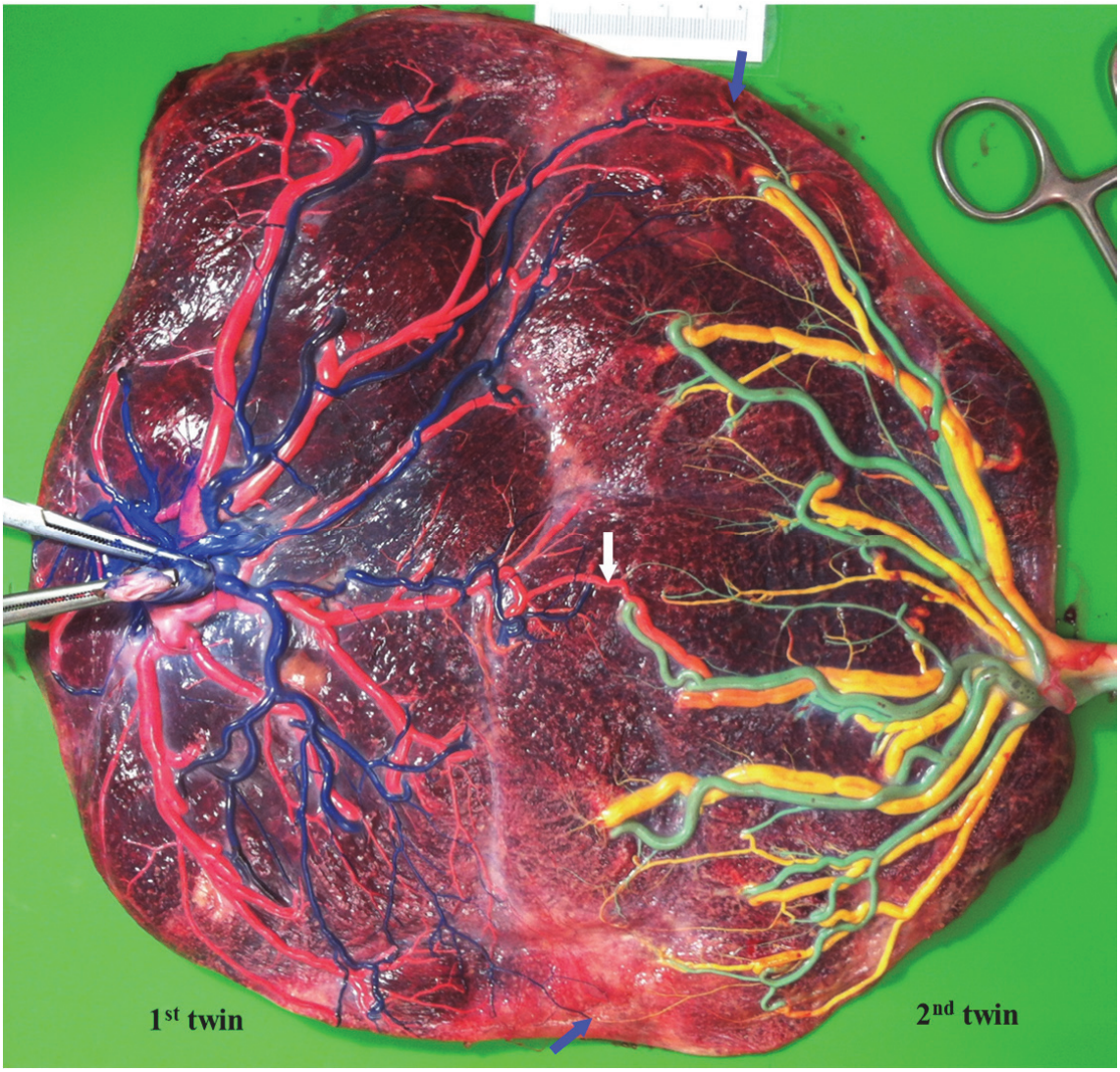

Figure 1 A TTTS (Quintero stage 3) monochorionic placenta managed with amniodrainage and delivered at 34+3 weeks' gestation. The 1st twin is the ex-donor. After delivery, injection with colored dye (blue or green for arteries and pink or yellow for veins) was given to demonstrate the vascular anastomoses. The white and blue arrows denote the VV and AV anastomoses, respectively.

The prevalence of AA and VV anastomoses in TTTS placentas varied from 0 to 57% and from 9 to 50%, respectively, among our 5 centers participating in this study. In the absence of AA anastomoses, the prevalence of VV anastomoses in TTTS placentas from each center tended to be higher (range from 11% to 50%) compared to non-TTTS placentas (8%).

Discussion

The findings reported in this large multicenter study demonstrate that TTTS placentas have a significantly higher prevalence of VV anastomoses compared to non-TTTS placentas. Our results suggest that the VV anastomoses may play a role in the development of TTTS, in particular in the absence of AA anastomoses.

Since most TTTS cases are nowadays managed with fetoscopic laser surgery, only a minority of studies have reported on the angioarchitecture in TTTS placentas not treated with laser [4, 6, 9-11, 21]. However, the reported prevalence of VV anastomoses in TTTS and non-TTTS placentas varied greatly. Bajoria et al. found a lower prevalence of VV anastomoses in TTTS placentas (10%) compared to non-TTTS placentas (100%) [11]. Nevertheless, in this study only 10 MC placentas were included in each group. Diehl et al. also reported a lower incidence of VV anastomoses (11%) in TTTS placentas [21]. Data collection in this study was recorded during fetoscopy prior to laser surgery, which may prevent accurate identification of the number and type of anastomoses [21]. In another three small studies the reported prevalence of VV anastomoses was similar in TTTS placentas (range: 16%-32%) compared to non-TTTS placentas (range: 16%-28%) [4, 9, 10]. In contrast, three recent studies analyzing relatively larger sample size (from 30 to 50 TTTS cases) reported a significantly higher prevalence of VV anastomoses in TTTS placentas (37%-42%) compared to non-TTTS placentas (15%-25%) [6-8]. Based on the power analysis in the present study, a minimum of 60 TTTS placentas were need to compare the prevalence of VV anastomoses between TTTS and non-TTTS placentas. Disparity among these reports may thusbe due to methodological differences, in particular the small number of included TTTS placentas in most previous publications. In the present multicenter study, we succeeded in analyzing the largest cohort

of untreated TTTS placentas (n=106) by using data from 5 international centers with experience in colored-dye injection of MC placentas. We found a significantly higher prevalence of VV anastomoses in TTTS placentas (36%) compared to non-TTTS placentas (25%).

It remains unclear why the presence of VV anastomoses may predispose to the development of TTTS. Unlike the arterial system, the resistance in the venous circulation is low. Inter-twin pressure gradient in the venous circulation is therefore prone to being affected by external impact, such as fetal position. VV anastomoses may then act as AV anastomoses and carry unidirectional blood flow when the inter-twin pressure gradient in venous circulation becomes skewed to one twin. This may, in certain circumstances, lead to the development of TTTS.

Our results should be interpreted with care due to several limitations besides the retrospective study design. One important limitation is the exclusion of MC twin pregnancies with fetal demise (and placenta delivery > 1 week after demise). Another important bias was introduced due to exclusion of TTTS cases treated with fetoscopic laser surgery which are not eligible for the purpose of this study. These TTTS placentas had to be excluded since the initial angioarchitecture cannot be evaluated after coagulation of the vascular anastomoses. Since the majority of TTTS cases are treated with laser surgery, the TTTS cohort reported in this study is thus not representative of the general TTTS population. The relative high percentage of Quintero stage 1 and high gestational age at diagnosis reflects the presence of selection bias in this cohort.

Since VV anastomoses cannot be detected accurately during ultrasound assessment in MC twin pregnancies, the direct clinical implication of this study is limited. Nevertheless, this

large study contributes to understand the pathogenesis of TTTS and the associated role of VV anastomoses. The exact mechanisms of VV anastomoses enabling the development of TTTS need further investigation.

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Chapter 6

Placental share and hemoglobin level in relation to birth weight in twin anemia-polycythemia sequence

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Abstract

Introduction: Twin anemia-polycythemia sequence (TAPS) is a newly described form of chronic twin transfusion. Previous observational studies noted a discordance between birth weight and individual placental share in TAPS. The purpose of this study was to investigate if fetal growth in monochorionic (MC) twins with TAPS is determined by placental share or by the net inter-twin blood transfusion.

Methods: All consecutive MC twin placentas of live-born twin pairs with and without TAPS examined at our center between June 2002 and February 2014 were included in this study. Hemoglobin (Hb) levels and individual placental share were evaluated at birth and correlated with birth weight share. We excluded MC twin pregnancies with twin-twin transfusion syndrome.

Results: A total of 270 MC twin pregnancies (TAPS group, n=20; control group without TAPS, n=250) were included in this study. Donors with TAPS had a lower birth weight than recipients in 90% (18/20) of cases, but a larger placental share in 65% (13/20) of cases. In the TAPS group, birth weight share was positively correlated with Hb share at birth ($P<0.01$) but not with placental share ($P=0.54$). In the control group without TAPS, birth weight share was strongly correlated with placental share ($P<0.01$) but not with Hb share ($P=0.14$).

Discussion: A relatively larger placental share may enable survival of anemic twin in TAPS.

Conclusion: In contrast with uncomplicated MC twins, fetal growth in MC twins with TAPS is determined primarily by the net inter-twin blood transfusion instead of placental share.

Keywords: monochorionic twins, twin anemia-polycythemia sequence, placental share, hemoglobin level, fetal growth

Introduction

Twin anemia-polycythemia sequence (TAPS) is a rare condition of monochorionic (MC) twin pregnancies and is characterized by large inter-twin difference in hemoglobin (Hb) level without large differences in amniotic fluid such as in twin-twin transfusion syndrome (TTTS) [1]. The pathogenesis of TAPS is associated with the presence of few tiny arterio-venous (AV) anastomoses, leading to chronic blood loss from the anemic twin (donor) to the polycythemic twin (recipient).[2, 3] TAPS can occur spontaneously or after laser for TTTS (post-laser TAPS)[1].

Fetal growth in MC twins has been demonstrated to be primarily determined by placental share[4-6]. A larger placental share usually leads to a larger birth weight, while a smaller placental share results in a smaller birth weight. In TAPS cases, however, several authors reported contrasting findings. The donor twin in TAPS cases is most often the smaller twin but seems to have a larger placental territory compared to its recipient co-twin[7, 8]. These observations suggest that fetal growth in TAPS twins could be determined by other factors besides placental share. We hypothesized that the net inter-twin blood transfusion in TAPS twins could have a greater contribution to fetal growth than relative placental share.

The aim of this study was to investigate the correlation between birth weight share and placental share and Hb level in MC twin pregnancies with TAPS compared to a control group of MC twin pregnancies without TAPS.

Materials and Methods

All consecutive MC placentas examined at our center, the Leiden University Medical Center, between June 2002 and February 2014 were eligible for this study. We included all MC twin

pregnancies with spontaneous TAPS (study group) and uncomplicated MC twin pregnancies without TAPS (control group). The management protocol for MC twins implies a routine bi-weekly Doppler-ultrasound examination, including middle cerebral artery peak systolic velocity (MCA-PSV) measurements. For the purpose of this study, only MC twin pregnancies resulting in two live-born twins were included. We excluded cases with TTTS, twin reversed arterial perfusion sequence and/or MC pregnancies treated with fetoscopic laser surgery. Fetoscopic laser surgery affects the trajectory of fetal growth in TTTS by iatrogenically destroying the placental angio-architecture, suggesting the inter-twin growth pattern may be different between spontaneous TAPS and post-laser TAPS[9]. Thus, cases with post-laser TAPS were excluded. Cases with incomplete placental injection due to severe damage or fixation in formalin were also excluded. TAPS was diagnosed prenatally or postnatally based on previously published international criteria.[1] Briefly, prenatal diagnosis of TAPS is reached when Doppler ultrasound examinations show an increased MCA-PSV(> 1·5 multiples of the median (MoM)) in one fetus and a decreased MCA-PSV (<1 MoM) in the co-twin; postnatal criteria include an inter-twin Hb difference > 8.0g/dL at birth, inter-twin reticulocyte count ratio > 1.7 and/or placental injection showing only few small anastomoses. Part of the placental data in the present study was reported previously to map the localization of vascular anastomoses on placental plate[10] and to determine the placental characteristics of TAPS placentas[3, 11].

Prenatal and postnatal clinical variables were prospectively recorded for all twins in a dedicated MC twins database, including antenatal and postnatal TAPS stage, antenatal management of TAPS, gestational age at birth, birth weight, gender, mode of delivery, and albumin levels at birth. Hematological investigations including Hb levels and reticulocyte

counts were routinely determined at birth in all MC twins. The inter-twin reticulocyte count ratio was calculated by dividing the reticulocyte count of the twin with lower hemoglobin level by the reticulocyte count of the co-twin. Individual birth weight share was calculated by dividing the birth weight of each infant by the sum of the birth weights of both infants. Individual Hb share was calculated by dividing the Hb level of each neonate by the sum of the Hb levels of both neonates. Individual reticulocyte count share was calculated by dividing the reticulocyte count of each neonate by the sum of the reticulocyte count of both neonates. The inter-twin birth weight discordance was calculated by the following formula: ((birth weight of larger twin – birth weight of smaller twin)/birth weight of larger twin) × 100%.

All MC placentas were routinely examined and injected with colored dye according to a previously reported protocol[12]. High-resolution digital pictures were taken for computer analysis. The number and type of vascular anastomoses and type of cord insertion was recorded after each injection. Umbilical cord insertion site was recorded as central, marginal and velamentous. Velamentous cord insertion was referred as to direct insertion of the umbilical cord into amniotic membrane instead of placental plate. Marginal cord insertion was defined as insertion within 1 cm of the margin Individual placental territory was measured on the digital picture as the area of venous return using Image J 1.45s (Image J, National Institute of Health, USA). Individual placental share was calculated by dividing the placental territory of each infant by the total placental plate surface area. The placental share difference was calculated by subtracting the lower placental share to the larger placental share. We calculated individual birth weight share/placental share (BWS/PS) ratio according to a report from Lewi et al[4]. Briefly, BWS/PS ratio was calculated by dividing the

birth weight share by the corresponding placental share. A BWS/PS ratio close to 1.0 indicates the birth weight share matches the placental share. A BWS/PS ratio of <1.0 means the birth weight is smaller in relation to its placental share. A BWS/PS ratio of >1.0 indicates the birth weight is larger in relation to its placental share.

Statistics

Fisher's exact test and Mann-Whitney U test was applied to analyze categorical variables and continuous variables, respectively. For comparison within twins, the paired *t* test was used for continuous variables and the Mc Nemar test was employed to analyze paired nominal variables. The nonparametric correlation coefficients (Spearman r) was calculated to evaluate the correlation between bi-variables. The straight lines fitting the bivariate diagrams were yielded to determine the slope and intercept using linear regression. Differences with a *P* value <0.05 were regarded as statistical significance. Statistical analysis was performed using GraphPad Prism v6.0 (GraphPad Software Inc. La Jolla, CA 92037 USA).

Results

A total of 704 MC placentas were examined at our center during this study period. We excluded 328 TTTS cases and three spontaneous TAPS managed with fetoscopic laser surgery. Fifty-four TTTS cases managed conservatively or with amniodrainage were also excluded. Forty-nine placentas were excluded due to severe placental damage (n=10), intrauterine fetal demise (n=24), fixation in formalin (n=6) and placenta lost (n=9). Finally, a total of 270 cases with double live-born twins were analyzed in the study, including 20 (7%) MC twin pregnancies with TAPS and 250 (93%) MC twin pregnancies without TAPS.

TAPS was detected antenatally in 40% (8/20) of cases and all TAPS cases met the postnatal diagnostic criteria. Four TAPS cases (20%, 4/20) were managed with intrauterine transfusion and were excluded when relating Hb and reticulocyte count at birth to birth weight. Hb levels and reticulocyte count at birth were measured in all twin pairs with TAPS but were missing in 16% (40/250) of MC twin pairs in the control group without TAPS. The baseline characteristics of MC twins with and without TAPS are displayed in Table 1.

Table 1 Baseline characteristics in MC twin pregnancies with and without TAPS

	TAPS (n=20)	no TAPS (n=250)	P value
Female – n (%)	9 (45)	127 (51)	.40
Caesarean – n (%)	10 (50)	94 (38)	.20
GA at birth – weeks[a]	33 ± 2	34 ± 3	<.01
BW – grams[a]	1771 ± 445	2094 ± 647	<.01
BWD - %[b]	17.8 (8.6-27.4)	13.4 (5.7-25.0)	.29
BWD ≥ 25% - n (%)	6 (30)	67 (27)	.80
HB difference – g/dL[b]	13.8 (12.0-18.1)	1.6 (0.6-3.5)[c]	<.01
Reticulocyte count ratio[d]	3.6 (1.7-10.0)	1.0 (1.0-2.0)[c]	<.01

Table 1 [a] Data displayed as mean ± SD. [b] Data given as median (IQR).[c] The hemoglobin (Hb) values of 40 pairs were not available. GA: gestational age; BW: birth weight; BWD: birth

The placental characteristics of MC twin gestations with and without TAPS are shown in Table 2. The number of vascular anastomoses in TAPS placentas was significantly lower compared to placentas from MC twin pregnancies without TAPS. The majority of donor twins (90%, 18/20) in the TAPS group had a smaller birth weight, but a larger placenta share in 65% (13/20) of cases whereas in the control group twins with smaller birth weight usually had a smaller placental share in 60% cases (151/250) (P=0.03).

In MC twin pairs with TAPS, the anemic twins always had a lower level of albumin and increased reticulocytosis compared to their polycythemic counterparts, 28g/L (IQR:24 g/L-30g/L) vs 35g/L (IQR: 34g/L -36g/L)(P=0.02) and 142‰ (IQR: 114‰-212‰) vs 39‰ (IQR: 36‰-51‰)(P<0.01), respectively. We did not detect these discrepancies between twins with lower Hb and the co-twins with higher Hb in the control group.

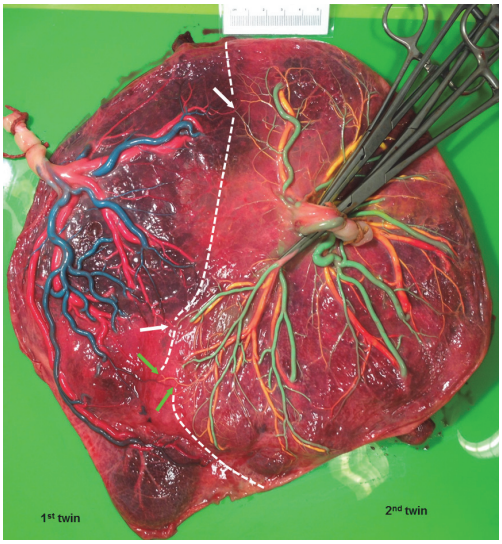

Figure 1 A typical TAPS placenta.

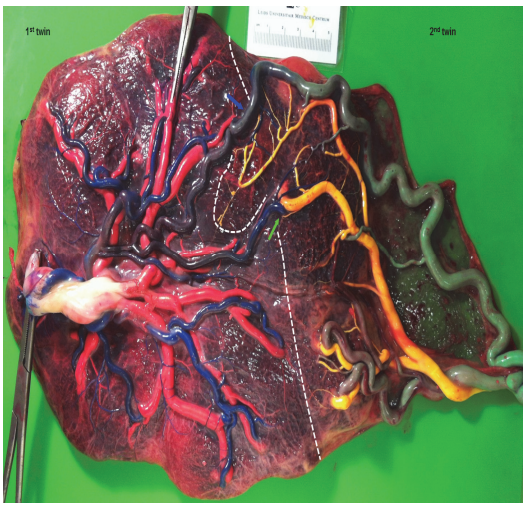

Figure 2 A typical MC placenta with selective intrauterine growth restriction.

Table 2 Placental characteristics in MC twin pregnancies with and without TAPS

	TAPS (n=20)	no TAPS (n=250)	P value
Number of anastomoses – n[a]	4 (3-6)	8 (5-12)	<.01
VCI – n (%)[b]	4 (10)	114 (23)	.06
Placental share difference - %[a]	18.1 (4.0-28.8)	20.1 (9.8-34.4)	.19

Table 2 [a] Data shown as median (IQR). [b] Values given per umbilical cord. VCI: velamentous cord insertion

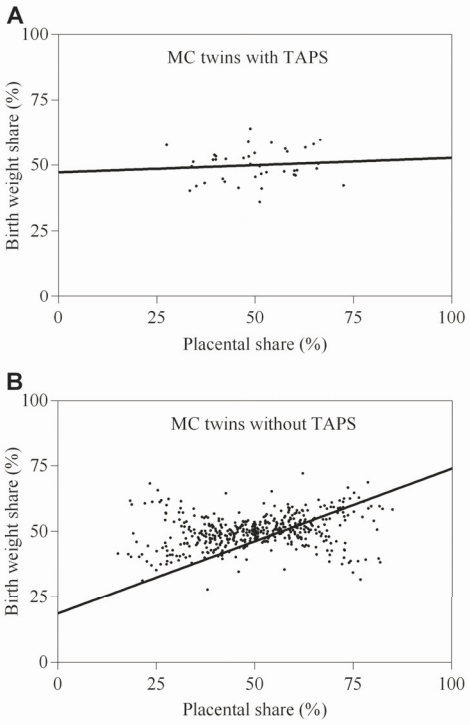

Figure 3 Correlation between placental share and birth weight share in MC twin pregnancies with TAPS (Spearman r=0.14; 95% confidence interval (CI): -0.18 to 0.45; P=0.36; Figure 3A) and without TAPS (Spearman r=0.28; 95% CI: 0.20 to 0.36; P<0.0001; Figure 3B).

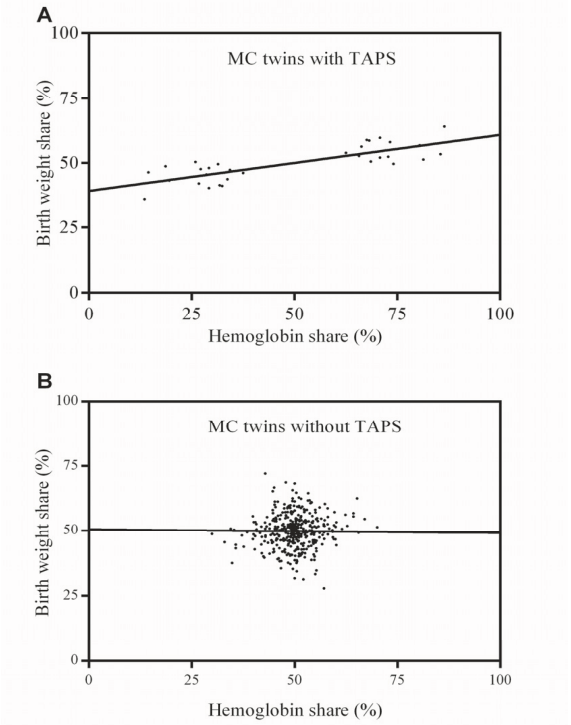

Figure 4 Correlation between Hb share and birth weight share in MC twin pregnancies with TAPS (Spearman r=0.74; 95% CI: 0.51 to 0.87; P<0.0001; Figure 4A) and without TAPS (Spearman r=0.07; 95% CI: -0.03 to 0.17; P=0.14; Figure 4B).

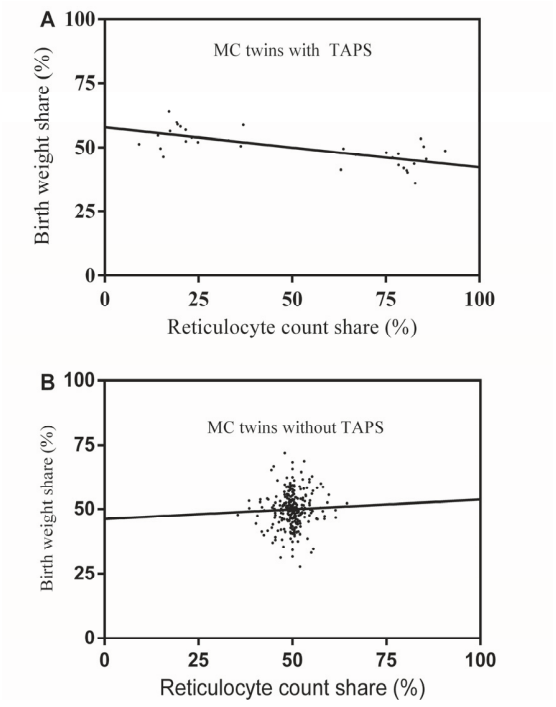

Figure 5 Correlation between reticulocyte count share and birth weight share in MC twin pregnancies with TAPS (Spearman r= -0.62; 95% CI: -0.80 to -0.34; *P*<0.001; Figure 5A) and without TAPS (Spearman r= 0.01; 95% CI: -0.11 to 0.12; *P*=0.92; Figure 5B).

In the TAPS group, the median BWS/PS ratio in the smaller twin and/or anemic twins was < 1.0 and significantly lower than in larger and/or recipient twins (P <0.01), indicating that birth weight share in donors was lower than their corresponding placental share. In contrast, this mismatch between placental share and birth weight share was not detected in the control group of MC twins without TAPS. In the control group the median BWS/PS ratio was 1.0 indicating that birth weight was well reflected in the corresponding placental share. Table 3 shows the comparison within twin pairs with and without TAPS. Examples of placentas originated from MC twins with and without TAPS are shown in Fig 1 and Fig 2, respectively.

Correlation between birth weight share and placental share in MC twins with and without TAPS

Birth weight share was not associated with placental share in MC twins with prenatal TAPS (Spearman r= -0.13; 95% confidence interval (CI): -0.60 to 0.41; *P*=0.63) or TAPS detected prenatally or postnatally (Spearman r=0.10; 95% confidence interval (CI): -0.23 to 0.41; *P*=0.54; Figure 3A). Conversely, in MC twins without TAPS, birth weight share increased linearly with placental share (Spearman r=0.28; 95% CI: 0.20 to 0.36; *P*<0.0001; Figure 3B).

Correlation between birth weight share and Hb share in MC twins with and without TAPS

Birth weight share increased linearly with Hb share in MC twins with TAPS (Spearman r=0.74; 95% CI: 0.51 to 0.87; *P*<0.0001; Figure 4A). In contrast, in MC twins without TAPS, birth weight share was not related to Hb share (Spearman r=0.07; 95% CI: -0.03 to 0.17; *P*=0.14; Figure 4B).

Correlation between birth weight share and reticulocyte count share in MC twins with and without TAPS

Birth weight share was inversely related to the reticulocyte count share in MC twins with TAPS (Spearman r= -0.62; 95% CI: -0.80 to -0.34; *P*<0.001; Figure 5A) whereas this relation between reticulocyte count share and birth weight share was not found in the control group (Spearman r= 0.01; 95% CI: -0.11 to 0.12; *P*=0.92; Figure 5B).

Discussion

This is the first study to investigate the various factors contributing to fetal growth in MC twins with TAPS. Our findings confirm that in uncomplicated MC twins, individual placental share contributes fundamentally to the fetal growth. As shown in this and other studies in uncomplicated MC twins the fetus with the larger placental share has usually also the larger birth weight share[4, 5]. However, this rule does not seem to apply to the category of MC twins with spontaneous TAPS. This study demonstrates that fetal growth in TAPS twins is not determined by the placental share. Donors in TAPS twins have mostly (90% of cases) a smaller birth weight share but the majority (65%) have a larger placental share. Conversely, the recipient twins have a larger birth weight but a smaller placental share. This inverse correlation between placental share and birth weight was recently noted by several authors in recent reports[7, 8]. Our results suggest that the placental share is not the primary contributor to fetal growth in MC twins with TAPS regardless of the timing at diagnosis (prenatal or postnatal). Instead, fetal growth in TAPS twins seems to be predominantly determined by the net inter-twin blood flow. The chronic blood loss from the donor twin through the vascular anastomoses into the circulation of the recipient twin causes anemia and a decreased red cell mass in the donor, whereas the recipient presents polycythemia and an increased red cell mass. These differences in red cell mass may have an important effect on fetal growth and birth weight. In addition, as shown in a previous study

Table 3 Comparison within MC twin pairs with and without TAPS

	MC twin pregnancies with TAPS (n=20)		P value	MC twin pregnancies without TAPS (n=250)		P value
	Smaller twin (n=20)	Larger twin (n=20)		Smaller twin (n=250)	Larger twin (n=250)	
Birth weight - grams[a]	1592 ± 399	1949 ± 424	<0.01	1916 ± 623	2273 ± 624	<0.01
Placental share - %[b]	51.4 (41.9-60.0)	48.6 (40.0-58.1)	0.77	45.1 (37.6-54.6)	54.9 (45.5-62.4)	<0.01
BWS/PS ratio[c]	0.90 (0.78-1.03)	1.11 (0.98-1.31)	0.02	1.00 (0.98-1.07)	1.00 (0.95-1.02)	0.15
	Anemic twin (n=20)	Polycythemic twin (n=20)	P value	Twin with lower Hb level (n=210)[d]	Twin with higher Hb level (n=210)[d]	P value
Birth weight - grams[a]	1705 ± 401	1980 ± 424	<0.01	2055 ± 614	2133 ± 674	0.62
Placental share - %[b]	51.7 (43.2-60.2)	48.3 (39.8-56.8)	0.37	47.4 (38.8-58.9)	52.6 (41.1-61.2)	0.18
BWS/PS ratio[c]	0.86 (0.77-1.03)	1.17 (0.98-1.32)	<0.01	1.02 (0.98-1.08)	0.98 (0.94-1.02)	0.15

Table 3 BWS/PS ratio: birth weight share/placental share ratio. a Data displayed as mean ± SD. b Data given as median (IQR). c Data given as median (95%CI). d The values of 40 pairs were not available.

by Verbeek et al., donor twins in TAPS have not only a significantly lower Hb level but also lower albumin and total protein levels[8]. Furthermore, blood loss-causing chronic hypoxia represented by increased reticulocyte count may also affect the growth of the anemic donor twin. We speculate that chronic loss of blood, nutritional elements and associated chronic hypoxia may contribute to the impaired fetal growth and lower birth weight in donor twins with TAPS.

It is not clear, however, why donor twins with TAPS often have larger placental shares than the recipient twins. Chronic blood loss may lead to chronic hypoxia and depletion of nutrition in the placental share of the donor twin with TAPS. This could theoretically stimulate compensatory placental expansion to transport more oxygen and nutrition to the fetus, consequently leading to a relative larger placental share in relation to the fetal size[13-15]. The increased reticulocyte count in anemic twin with TAPS indicates its exposure to intrauterine environment of chronic hypoxia. Another, most plausible explanation, could be that the larger placental share found in donor twins is a result of selection bias in this study due to the inclusion of only TAPS cases with two live-born infants. We speculate that donor twins with TAPS with a significantly smaller placental share may be at increased risk of fetal demise due to the accumulation of potential risks (chronic blood loss and smaller placental share). As shown by several authors, MC twin pregnancies are at increased risk of unexplained fetal demise, which could in part be related to severe placental share discordances[16, 17]. Underreporting of donor twins with smaller placental shares may have occurred due to our study design.

The main limitation of this study, besides its retrospective nature, is related to the introduction of a selection bias attributed to the exclusion of cases with fetal demise. Since

fetal demise consequently leads to placental maceration, these cases had to be excluded from the study due to the impossibility of measuring the individual placental share or Hb levels. Our date should therefore be interpreted with care as they only relate MC pregnancies resulting in double survivals.

In conclusion, our study shows that fetal growth in MC twins with TAPS is determined primarily by the net inter-twin transfusion rather than the placental share. Our data may help elucidate the various factors determining fetal growth in MC twins pregnancies with and without TAPS.

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Chapter 7

Laser surgery in twin-twin transfusion syndrome with proximate cord insertions

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Abstract

Objective: To estimate the prevalence of proximate cord insertions in twin-twin transfusion syndrome (TTTS) and evaluate the outcome after fetoscopic laser coagulation surgery.

Methods: We included all TTTS cases treated with laser at our center between 2002 and 2013. Placentas were examined after birth and injected with colored dye. TTTS cases without complete placental injection study were excluded. We recorded the presence of proximate cord insertions (distance < 5cm) after birth and the presence and types of residual anastomoses. We compared the clinical outcome and placental findings in cases with and without proximate cord insertions.

Results: The prevalence of proximate cord insertions in TTTS placentas was 2% (4/252). Perinatal mortality in the TTTS group with and without proximate cord insertions was 13% (1/8) and 12% (61/496), respectively (P=1.0). Residual anastomoses were detected in all placentas with proximate cord insertions (100%, 4/4) compared to 27% (66/248)(P<.01) in TTTS placentas without proximate cord insertions.

Conclusion: Fetoscopic laser coagulation in TTTS cases with proximate cord insertions is challenging due to technical difficulties in visualizing the vascular equator and results in an increased risk of incomplete laser treatment.

Keywords: proximate umbilical cord insertions, twin-twin transfusion syndrome, fetoscopic laser coagulation surgery

Introduction

The omnipresent vascular anastomoses are the anatomic prerequisite for twin-twin transfusion syndrome (TTTS).[1] Fetoscopic laser coagulation of the vascular anastomoses is considered to be the most effective treatment for TTTS.[2] On pre-operative ultrasound, and again at the start of the fetoscopy, the cord insertion sites and the location of the vascular equator between both cord insertions are evaluated. When the distance between cord insertions is very close, so-called proximate cord insertions, technical difficulties in identifying the vascular equator may occur. In addition, the anastomoses in these cases may have a larger diameter than usual. Fetoscopic laser coagulation surgery may then either not be considered as the first treatment of choice or may fail due to technical limitations in identifying anastomoses.[3]

To date, the prevalence and clinical consequences of proximate cord insertions in TTTS placentas have not been studied. The aim of the present study was to compare the outcome after laser surgery in TTTS cases with and without proximate cord insertions.

Materials and methods

All TTTS pregnancies treated with fetoscopic laser coagulation surgery at Leiden University Medical Center between April 2002 and March 2013 were eligible. The technique used for fetoscopic laser coagulation was either the standard selective laser technique or, more recently, the Solomon technique.[4] According to our guideline, laser surgery is performed in TTTS stage II, III or IV and in cases with stage I with clinical symptoms of polyhydramnios.[5] Diagnosis of TTTS was based on the

internationally accepted criteria: polyhydramnios (deepest vertical pocket ≥ 8cm before 20 weeks or 10cm after 20 weeks of gestation) in the recipient sac and oligohydramnios (deepest vertical pocket ≤ 2cm) in the donor sac.[2] We included in the present study only TTTS cases with complete placenta examination after birth including colored dye injection to identify residual anastomoses. Injection with colored dye was performed according to our previously published protocol.[6] Severely damaged placentas were excluded from the postnatal placenta injection study. We also excluded placentas in case of single or double intrauterine fetal demise (IUFD) when delivery occurred more than a week later after demise and the corresponding placenta-sharing was macerated. Pictures of the injected placenta were taken using a high-resolution digital camera. A measuring-tape was placed on the placenta to allow post-hoc measurements on the digital picture. We recorded the presence of proximate cord insertions, defined as a distance between both cords insertion sites < 5 cm, prenatally detected by ultrasound and measured at postnatal placental examination. We recorded the presence, number and types of residual anastomoses. All measurements were performed using Image J 1.45s (Image J, National Institute of Health, USA). Part of placental data reported in this study was reported in previous publications.[7-9]

The following perinatal data were recorded: TTTS stage, gestational age at laser, recurrence/reversal of TTTS, post-laser twin anemia-polycythemia sequence (TAPS), gestational age at birth and perinatal death (either fetal demise or neonatal death). Treatment failure in fetoscopic laser coagulation surgery was defined as the presence of residual anastomoses after color dye injection.

The primary aim of this study was to estimate the prevalence of proximate cord insertions and compare the clinical outcome and placental characteristics in TTTS cases with and without proximate cord insertions.

Statistics

Mann-Whitney U test was used to compare continuous variables and Fisher's exact test was used to analyze categorical variables. Statistical significance was considered when a P-value <.05. SPSS Statistics v20.0 (SPSS Inc., Chicago, IL, USA) was applied to perform statistical analysis.

Results

During the study period, 432 TTTS twin pregnancies were consecutively managed with fetoscopic laser coagulation surgery at our center. A total of 148 (35%) placentas were not delivered at our center or not shipped back to our center. Of the remaining 284 placentas, we excluded 11 placentas due to remote (> 1week) single or double fetal demise and 21 cases due to severe damage to the cord insertions or placenta, or fixation in formalin. Complete placental data, including colored dye injection studies, were available for 252 placentas and included in the present study. Proximate cord insertions were detected in 4 TTTS cases, yielding overall prevalence of 2% (4/252). No TTTS cases with proximate cord insertions were treated with other intervention (such as umbilical cord clamping) during the study period. Examples of placentas with and without proximate cord insertions after color dye injection were shown in Figure 1 and 2, respectively. Baseline characteristics of TTTS cases with and without proximate cord insertions are summarized in Table 1.

Table 1 Baseline characteristics

	PCI (n=4)	No PCI (n=248)
GA at laser - weeks[a]	19 (16-23)	20 (18-23)
Quintero stage - n (%)		
Stage 1	0	21 (8)
Stage 2	2 (50)	86 (35)
Stage 3	2 (50)	126 (51)
Stage 4	0	15 (6)
Anterior placenta- n (%)	1 (25)	78 (31)

[a]Denotes median (IQR). PCI: proximate cord insertions.

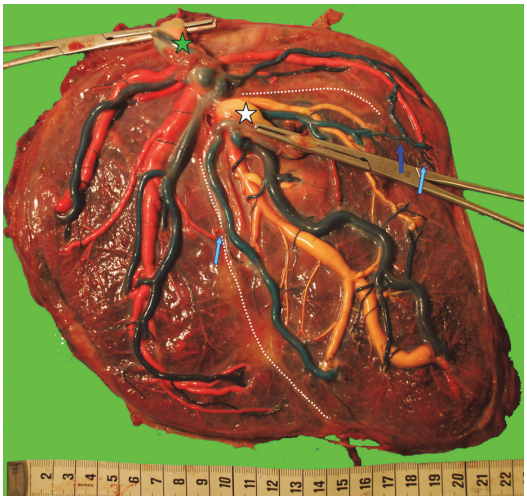

Figure 2 TTTS placenta with proximate cord insertion (delivery at 30 weeks) after colored dye injection: AA and VV residual anastomoses are indicated with blue stars and yellow arrows, respectively. Light-blue arrows indicate several residual AV anastomoses The white dotted lines show the laser coagulation demarcation line.

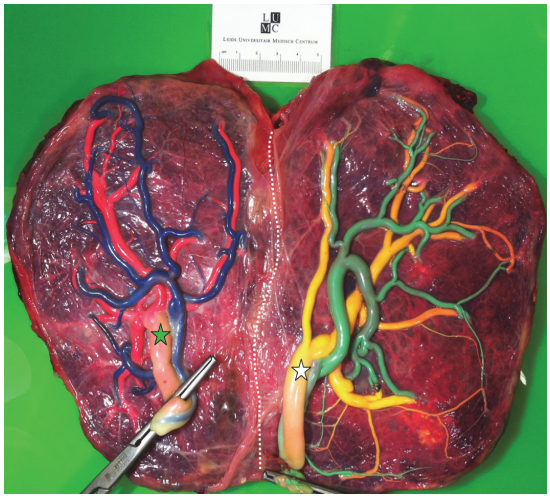

Figure 1 TTTS placenta without proximate cord insertion (delivery at 32 weeks) after colored dye injection: Solomon technique was used to coagulate the whole vascular equator (white dotted line). No residual anastomoses are found.

Clinical outcome data: Post-laser TAPS in the group with and without proximate cord insertions was 25% (1/4) and 14% (35/248), (P=.46). No cases of recurrent TTTS were detected in the proximate cord insertions group, compared to 2% (5/248)(P=1.00) in the group without proximate cord insertions. In the case of post-laser TAPS in the group with proximate cord insertions, laser surgery was performed at 25 weeks' gestation but failed because of difficulty in identifying the vascular equator and severe bleeding. At 27 weeks' gestation, evidence of severe fetal anemia in the

donor and polycythemia in the recipient were detected on Doppler ultrasound examination, confirming the diagnosis of TAPS. An emergency caesarean section was performed at 27 weeks' gestation due to signs of fetal distress in the donor. Hemoglobin level at birth in the donor was 2.6 g/dL (reticulocyte count 172‰) versus hemoglobin level of 18.7 g/dL in the recipient (reticulocyte count 72 ‰), confirming the diagnosis of TAPS. The anemic donor died after 4 weeks of intensive care treatment due to severe cerebral injury (intraventricular hemorrhage grade 4) and severe respiratory distress syndrome. Overall perinatal survival in the TTTS group with and without proximate cord insertions was 87% (7/8) and 88% (435/496), respectively (P=1.00). Further details on the clinical outcome of all TTTS pregnancies are shown in Table 2.

Table 2 Pregnancy outcome in TTTS twins with and without proximate cord insertion

	PCI (n=4)	No PCI (n=248)	P value
Recurrent TTTS – n (%)	0 (0)	5 (2)	1.00
Post-laser TAPS– n (%)	1 (25)	35 (14)	.46
GA at birth – weeks[a]	32 (27-34)	32 (29-35)	.89
Perinatal mortality– n (%)[b]	1 (13)	61 (12)	1.00

[a] Denotes median (IQR). [b] Means the number of fetus.

Placental outcome data: Residual anastomoses were detected after colored dye injection in each TTTS placentas with proximate cord insertions (100%, 4/4) compared to 27% (66/248)(P<.01) in TTTS placentas without proximate cord insertions. The median number of residual anastomoses in TTTS placentas with and without proximate cord insertions was 13 (interquartile range (IQR): 7-24) and 0

(IQR: 0-1), respectively (P<.01) The overall prevalence of residual arterio-arterial (AA) and veno-venous (VV) anastomoses in TTTS placentas with proximate cord insertions was significantly higher compared to placentas without proximate cord insertions, 100% (4/4) versus 5% (13/248)(P<.01) and 50% (2/4) versus 4% (9/248)(P<.01), respectively. In the case with proximate cord insertions and post-laser TAPS, the diameter of the residual AA anastomosis was very small (< 1 mm). Further details on the injected placentas with and without proximate cord insertions are shown in Table 3.

Table 3 Characteristics of placenta with and without proximate cord insertions

	PCI (n=4)	No PCI (n=248)	P value
Presence of RA – n (%)	4 (100)	66 (27)	.01
No. of overall RA [a]	13 (7-24)	0 (0-1)	<.01
Residual AV -n (%)	13 (7-23)	0 (0-0)	<.01
Residual AA -n (%)	4 (100)	13 (5)	<.01
Residual VV -n (%)	2 (50)	9 (4)	.01

RA: residual anastomoses a Denotes median (IQR).

Discussion

In this study, we evaluated the prevalence and clinical consequences of proximate umbilical cord insertions in TTTS twins treated with fetoscopic laser coagulation surgery. We found that proximate cord insertions are rare in TTTS cases (2%, 4/252). Residual anastomoses were detected in each case with proximate cord insertions

and TAPS developed in one of these cases, emphasizing the difficulty in achieving dichorionization in these rare cases. Our study suggests that TTTS pregnancies with proximate cord insertions may pose a technical challenge for fetal surgeons due to problems related to the identification of the vascular equator, impeding complete laser photocoagulation of all potential anastomoses.

This important technical limitation of laser surgery in these specific TTTS cases was first reported in another small case series (n=6) by Gandhi et al in 2011.[3] The authors reported an overall perinatal mortality of 42% (5/12) in TTTS cases with proximate cord insertions, and in one case treated with laser surgery, both fetuses died due to residual anastomoses located between the close cord insertions. Based on their experience, Gandhi et al suggest that other fetal interventions (such as selective feticide or amnioreduction) should be considered in these cases.[3] Interestingly, we found that the rate of perinatal survival in the 4 cases with proximate cord insertions was similar to the control group. One of the possible explanation for the lack of association between proximate cord insertions, residual anastomoses and adverse outcome could be related to the nature of these anastomoses. As shown after placental injection, each case with proximate cord insertions had an AA anastomosis. As previously shown, AA anastomoses are known to prevent the development of TTTS or TAPS (provided the AA anastomosis is sufficiently large).[8-10] In one case, however, post-laser TAPS occurred despite the presence of an AA anastomosis, but the size of the anastomosis was very small. This is in agreement with one of our case reports.[10]

This peculiar placental angio-architecture in TTTS placentas with proximate cord insertions appears to be similar to monoamniotic placentas. Monoamniotic

placentas are also characterized with proximate cord insertions and a high prevalence of AA and VV anastomoses.[11] Concomitantly, monoamniotic twin pregnancies have also a reduced risk of developing TTTS due to the presence of AA anastomoses.[11, 12]

Several studies have investigated the relation between cord insertion distance and perinatal outcome in monochorionic twin pregnancies.[3, 13, 14] Importantly, international consensus agreement on the definition of proximate cord insertions is lacking. The optimal method to determine the clinically most useful cord distance, by using cord distance as continuous variable, and asses its correlation with outcome parameters, would require a dataset too large to be practical. We therefore propose the empirically chosen 5 cm cut-off for use in future studies at least until better evidence for another cut-off becomes available. In one study by Nikkels et al, a higher perinatal mortality rate was found in placentas with short cord insertion distance.[13] However, a wider cut-off value (<14 cm) for proximate cord insertions was chosen.[13] In another study by Hack et al, using a lower cut-off value(<5cm), no clear relationship between clinical outcome and distance between cord insertions in monochorionic and monoamniotic pregnancies was found.[11, 14] In accordance with Hack et al, we also found no difference in perinatal outcome between TTTS pregnancies with and without proximate cord insertions in this study adopting <5cm as the cut-off value as well.

Our data should be interpreted with care due to the retrospective nature of the study and the small number of cases with proximate cord insertions, preventing accurate statistical analysis. Larger (multicenter studies) are required to evaluate the perinatal morbidity and mortality in TTTS cases with proximate cord insertions and

determine if fetoscopic laser surgery is the most effective treatment in such cases. Another potential limitation of our study lies in the fact that we excluded cases if placental injection was not performed, including macerated placentas with TTTS. Excluding cases with IUFD may have introduced a potential, but unavoidable selection bias.

In conclusion, although proximate cord insertions are rare in TTTS placentas, they create a great technical challenge for fetal surgeons due to the difficulty in determining the vascular equator. More studies are required to confirm these findings and determine the best treatment in TTTS cases with proximate cord insertions. In addition, determination of a uniform cut-off value to define "proximate cord insertions" would be useful to allow accurate comparisons between different cohorts in the near-future. For future studies, we propose the uniform use of "5 cm" as cut-off to define proximate cord insertions, either detected antenatally (during pre-operative ultrasound scan) or measured postnatally at placental examination.

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PART III

Chorioamnionitis and early onset sepsis in TTTS after laser surgery

Chapter 8

Histological Chorioamnionitis and Funisitis After Laser Surgery for Twin–Twin Transfusion Syndrome

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Abstract

Objective: To estimate the incidence of histological chorioamnionitis and funisitis after fetoscopic laser surgery for the management of twin–twin transfusion syndrome.

Methods: A case–control study was performed at the Leiden University Medical Center from 2013 to 2014. All twin–twin transfusion syndrome managed with laser surgery during the study period were included and compared to a control group of all monochorionic twins not treated with laser surgery. We excluded cases with fetal demise or higher-order pregnancies. Placentas were reviewed for the presence and degree of chorioamnionitis and presence or absence of fetal inflammatory response. The primary outcome was the incidence of histological chorioamnionitis and funisitis after laser surgery. Odds ratios (OR) and 95% confidence intervals (CI) for primary outcomes were calculated. A p value of <.05 was considered as statistical significance.

Results: Sixty-two patients managed with laser surgery were included in the study group and compared to 64 patients in the control group. The incidence of histological chorioamnionitis was 13% (8/62) in the laser group versus 5% (3/64) in controls (OR 3.0, 95% CI .8-11.9, *P*=.12). Funisitis occurred in 8% (10/124) in the laser group versus 0% in controls (OR 11.1, 95% CI 1.3-96.9, *P*=.03). Histological chorioamnionitis with or without funisitis after laser surgery was associated with shorter laser-to-delivery interval (median 6.6 (range 3.4-14.1) versus 13.6 (4.4-20.1) weeks, *P*<.01) and lower gestational age at birth (median 28.1 (range 23.1-32.6) versus 32.7 (24.4-37.0) weeks, *P*<.01).

Conclusion: These findings suggest twin–twin transfusion syndrome managed with laser surgery are at increased risk of funisitis.

Introduction

Twin–twin transfusion syndrome affects around 10% of monochorionic twin pregnancies, resulting in 80% to 100% perinatal mortality if left untreated.[1] Twin–twin transfusion syndrome results from an unbalanced exchange of blood between the donor twin and recipient twin through placental vascular anastomoses, which are present in almost all monochorionic twins. Fetoscopic laser surgery is the optimal treatment for severe twin–twin transfusion syndrome and leads to an improved perinatal outcome.[2] In laser surgery the surgeon introduces a fetoscope into the amniotic cavity of the recipient twin and coagulates the connecting vessels with a laser beam. Postoperative complications include recurrent twin–twin transfusion syndrome, twin anemia–polycythemia sequence, premature rupture of membranes (PROM) and preterm birth.[3-8] The risk of intrauterine infection may be increased due to the invasive nature of laser surgery. A possible association between laser surgery and chorioamnionitis has been suggested.[3-13] Chorioamnionitis, especially concomitant with funisitis, is the main factor of preterm birth and cerebral injuries in neonates.[14] Chorioamnionitis represents the maternal inflammatory response while funisitis is the fetal inflammatory response and is secondary to severe chorioamnionitis. The occurrence of intrauterine infection may thus compromise the treatment efficacy of laser surgery. However, good-quality studies with histological evaluation are not available. The exact incidence of histological chorioamnionitis and funisitis after laser surgery remains unclear.

The primary purpose of this study was to investigate the incidence of histological chorioamnionitis and funisitis after fetoscopic laser surgery for the treatment of twin–twin transfusion syndrome.

Materials and Methods

A case–control study was conducted at the Leiden University Medical Center from March 2013 to December 2014. All consecutive twin–twin transfusion syndrome cases treated with laser surgery at our center were included in the study group. Monochorionic twins delivered at our center but not treated with laser surgery during the same study period were included in the control group. We excluded triplet or higher order pregnancies and monochorionic twin pregnancies with single or double fetal demise. Diagnosis of twin–twin transfusion syndrome was based on the Eurofoetus criteria: presence of a deepest vertical pocket of amniotic fluid in the donor ≤ 2 cm and ≥ 8 cm in the recipient of before 20 weeks of gestation or ≥ 10 cm after 20 weeks.[10] Severity of twin–twin transfusion syndrome was assessed according to Quintero's staging system.[15] If twin–twin transfusion syndrome were diagnosed, the patients were counseled regarding the following management alternatives in details: expectant management, amnioreduction and fetoscopic laser surgery. Fetoscopic laser surgery was performed in TTTS with Quintero stage 1 with clinical symptoms of polyhydramnios, or Quintero stage 2-4.

Amnioreduction was performed using an 18-gauge needle under continuous ultrasound visualization. Amniotic fluid in the sac of the recipient was reduced to a deepest pool of 5 cm. No prophylactic antibiotics were administered prior to amnioreduction given the low risk (<1%) of intrauterine infection after intervention.[16]

Solomon technique was used in all the twin–twin transfusion syndrome cases managed with laser surgery. Detailed information on the operative procedure was described previously.[17] In brief, prophylactic tocolysis using indomethacin (100 mg, one dose, rectal administration, 2 hours before operation) and antibiotics using cefuroxime (1.5 g, intravenous

administration prior to fetoscopic laser surgery) was administered periopreatively. Under continuous ultrasound guidance, a fetoscope was introduce into the amniotic sac of the recipient twin. After visualization of the vascular equator, all anastomoses were coagulated using a diode or Nd:YAG laser device (Dornier MedTech, Wessling, Germany) with power setting from 20 to 70 W. After ablation of vascular anastomoses one by one, a laser line was drawn from one edge of the placenta to the other. After each laser procedure, amnioreduction was performed to drain the excessive amniotic fluid to a deepest vertical pocket of 6 cm. The number of coagulated anastomoses, total laser energy and duration of operation were recorded. Repeated fetoscopic laser surgery was considered if recurrent or reversed twin–twin transfusion syndrome occurred at gestational age of ≤ 26 weeks. After fetoscopic laser surgery, patients were monitored at our center in combination the monitoring at the referring hospital. All lasered placentas were shipped to our center after delivery.

Diagnosis of twin anemia–polycythemia sequence was based on prenatal or postnatal criteria as described previously. Prenatal criteria includes Doppler ultrasound detecting an increase in middle cerebral artery peak systolic velocity > 1.5 multiples of the median in the anemic fetus that coincided with a decrease in middle cerebral artery peak systolic velocity < 1 multiples of the median in the polycythemic fetus. Postnatal criteria consists of hematologic tests (inter-twin Hemoglobin difference > 8.0g/dL and inter-twin reticulocyte count ratio > 1.7) and placental injection showing only few small anastomoses present.[18] If twin anemia–polycythemia sequence developed, intrauterine blood transfusion with or without partial exchange transfusion was considered depending on clinical evaluation. Prophylactic antibiotics were not routinely administered prior to intrauterine blood

transfusion due to the low risk (form 0 to 1%) of intrauterine inflammation after procedure.[19]

Written informed consent was obtained from each patient included in the study. The study was approved by the institutional ethics committee (the Leiden University Medical Centre Medical Ethics Committee (MEC P07.261).

All monochorionic twin placentas were evaluated according to a fixed protocol. Placental tissues were collected using a minor adaptation of the method described by Burton et al.[20] In brief, tissue-samples were collected from the umbilical cord, placenta and membrane area of each twin. Tissue-samples were collected from three sites of each umbilical cord; one close to the fetus, one at the middle of the cord and one near the placental insertion site. A membrane roll of each side was taken from the rupture site to placental margin. Macroscopically normal placental parenchyma from the placental share of each twin was sampled and processed for histological evaluation. Histological chorioamnionitis was diagnosed as the presence neutrophilic granulocytes in the chorionic plate or the extraplacental membranes. Histological chorioamnionitis was defined as one or more of the following categories: acute subchorionitis or chorionitis, acute chorioamnionitis or necrotizing chorioamnionitis. Diagnosis of funisitis is based on the presence of neutrophilic granulocytes in the wall of the umbilical vessel(s) and Wharton's jelly. Funisitis was defined as one or more of the following items: chorionic vasculitis, umbilical phlebitis, umbilical vasculitis (inflammation in one or two umbilical arteries ± umbilical vein) and umbilical panvasculitis (inflammation in 3 vessels).[21] Histological chorioamnionitis is recognized as the maternal host response, while funisitis suggests fetal inflammatory response secondary

to severe histological chorioamnionitis.[22] The grading and staging of maternal and fetal inflammatory response was according to the criteria proposed by Redline et al. (see Table 1, which illustrates the grading and staging of histological chorioamnionitis and funisitis).[23]

Perinatal outcomes were documented prospectively in a dedicated database. The following variables were recorded, including gestational age at laser surgery, premature rupture of membranes (PROM), gestational age at PROM, maternal fever (≥38 °C), gestational age at birth, delivery mode, birth weight, full neonatal blood count and C-reactive protein levels (maximum value within first 72 hours after birth), severe cerebral injury, early–onset neonatal sepsis and neonatal mortality. Severe cerebral injury was defined and recorded in accordance with a standard protocol at our center[24]. Early–onset neonatal sepsis was defined as the onset of sepsis within 72 hours after birth[25] and recorded as proven or suspected sepsis. Proven early–onset sepsis was diagnosed if the blood culture was positive. Suspected early–onset neonatal sepsis was defined if antibiotic treatment was administrated for 5-7 days in an infant with signs of infection though without positive blood culture. All preterm neonates born at a gestational age below 35 weeks from mothers with PROM are routinely placed on antibiotics at birth. Neonates delivered after 35 weeks are only placed on antibiotics depending on other clinical findings or risk factors for suspected perinatal infection. Neonatal mortality was defined as death within 28 days after birth.

Statistics

In general population of preterm birth, the incidence of histological chorioamnionitis ranges from 30% to 70%.[26-28] We thus expected histological chorioamnionitis present in 30% of the control group of MC twins not managed with laser surgery (because we thought that this group had a low risk of histological chorioamnionitis) and in 55% of the study group of

twin–twin transfusion syndrome managed with laser surgery (based on the expected gestational age at birth in the laser group of 31-32 weeks corresponding to a incidence of 52% of histological chorioamnionitis according to the data on the gestational age-dependent frequency of chorioamnionitis reported by Mueller-Heubach et al.).[27] The clinical outcome including gestational age at birth in TTTS cases was significantly improved after the introduction of fetoscopic laser surgery. We calculated that 61 placentas in each group were required to demonstrate a 25% absolute difference in histological chorioamnionitis (i.e. 30% vs 55%), with a significance of 0.05 and a power of 80%, by two-tailed analysis. The normality of continuous variables was assessed using Shapiro-Wilk test. Unpaired t test or Mann-Whitney U test was adopted to compare continuous variables, as appropriate. Generalized estimating equation with exchangeable structure was employed to evaluate the odds ratio (OR) of laser surgery in relation to funisitis. Results of categorical variables are compared using Fisher's exact test or Chi-square test, as appropriate. A p-value < 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 20 (SPSS, inc., Chicago, Illinois, USA).

Results

During the study period, 71 patients managed with fetoscopic laser surgery and 75 patients not managed with fetoscopic laser surgery were eligible for this study. Nine (13%) twin–twin transfusion syndrome cases managed with fetoscopic laser surgery and 8 (11%) monochorionic twin pregnancies not treated with laser surgery were excluded due to single or double fetal demise. Three triplet pregnancies not undergoing laser surgery were also excluded. The remaining 62 twin–twin transfusion syndrome cases managed with laser surgery were included in the study group and 64 monochorionic twins without laser surgery

were included in the control group. In the study group of twin–twin transfusion syndrome treated with laser surgery, 19% (12/62) cases were stage 1, 39% (24/62) stage 2, 42% (26/62) stage 3. Post–laser twin anemia–polycythemia developed in 4 cases and was treated with intrauterine transfusion and/or repeated laser surgery. In the control group, two monochorionic twin pregnancies with spontaneous twin anemia–polycythemia sequence were managed with intrauterine blood transfusions and 6 late twin–twin transfusion syndrome cases were treated with amniodrainage. Clinical baseline characteristics of two groups were displayed in Table 2.

Table 2. Baseline characteristics

Variables	TTTS treated with laser (n=62)	Control group (n=64)	*P*
Gestational age at birth – wk	31.8 ± 3.5	33.3 ± 3.1	<.01
Birth weight – gr	1646 ± 607	1906 ± 667	<.01
PROM – n (%)	21 (34)	4 (6)	<.01
Maternal fever (≥38 ºC) – n (%)	11 (16)	5 (8)	.16
Antenatal corticosteroid treatment – n (%)	42 (68)	20 (31)	<.01
Maternal antibiotic treatment – n (%)*	21 (24)	6 (9)	<.01
Cesarean delivery – n (%)	27 (44)	31 (48)	.58

Data was shown as mean ± SD or number (%). TTTS: twin–twin transfusion syndrome; PROM: premature rupture of membrane. * Antibiotics were administered due to clinical indications such as PROM or maternal fever, but not for prophylaxis prior to laser surgery.

Table 1. Grading and staging of histological chorioamnionitis and funisitis

Diagnostic categories		Histological chorioamnionitis	Funisitis
Grading	1	≥5 clusters, ≥2 neutrophilic granulocytes each cluster per high power field	≥5 clusters, ≥2 neutrophils each cluster per high power field
	2	Presence of confluent neutrophilic granulocytes, ≥ 3 isolated foci of neutrophilic granulocytes, continuous band of neutrophilic granulocytes, or microabscesses	Presence of near confluent intramural neutrophilic granulocytes in chorionic or umbilical vessels
Staging	1	Infiltration of neutrophilic granulocytes limited in chorion	Infiltration of neutrophilic granulocytes limited in chorionic vessels and/or umbilical vein
	2	Infiltration of neutrophilic granulocytes into chorioamniotic membrane	Infiltration of neutrophilic granulocytes into umbilical artery
	3	Necrotizing (severe) chorioamnionitis, karyorrhexis of neutrophilic granulocytes, necrosis in amnion	Presence of neutrophilic granulocytes and associated debris in concentric bands-rings-halos around umbilical vessels (severe fetal inflammatory response)

We found that the incidence of histological chorioamnionitis in the study group and the control group was 13% (8/62) versus 5% (3/64), respectively (OR 3.0, 95% confidence interval (CI) .8-11.9, *P*=.12). The incidence of funisitis in the study group was significantly higher compared to the control group, 8% (10/124) versus 0% (0/128), respectively (OR 11.1, 95% CI 1.3-96.9, *P*=.03). In the control group, no histological chorioamnionitis nor funisitis occurred in monochorionic twins managed with amniodrainage or intrauterine transfusion. In the study group, histological chorioamnionitis without funisitis occurred in one of the four cases with post-laser twin anemia–polycythemia sequence. In this case, repeated laser surgery was planned but the intervention was cancelled due to signs of intrauterine inflammation (premature contractions and maternal fever, and delivery at 23^{+1} weeks). In the study group, histological chorioamnionitis was detected in the placental share of both the ex–donor and ex–recipient in 50% (4/8) cases, in the placental share of the ex–recipient alone in 38% (3/8) cases and in the placental share of the ex–donor alone in 12% (1/8) cases. The ten cases with funisitis in the study group occurred in five twin pairs (both in the ex-donors and ex-recipients). All cases with funisitis also had histological chorioamnionitis. Analysis of histological findings between two groups was summarized in Table 3. An example of histological chorioamnionitis and funisitis is shown in Figure 1 and 2, respectively.

Analysis of the association of several perinatal variables with the occurrence of histological chorioamnionitis in the study group of twin–twin transfusion syndrome treated with laser surgery is shown in Table 4. Median gestational age at PROM in twin–twin transfusion syndrome cases with and without histological chorioamnionitis was 25.2 (range 25.1-28.0) and 30.6 weeks (15.9-35.1) weeks, respectively (p=.04).

Table 3. Incidence of histological chorioamnionitis and funisitis in TTTS after fetoscopic laser surgery and control group

Variables	TTTS treated with laser (n=62 pregnancies, 124 fetuses)	Control group (n=64 pregnancies, 128 fetuses)	OR (95% CI)[+]	*P* value
Histological chorioamnionitis – n (%)	8/62 (13%)	3/64 (5%)	3.0 (.8-11.9)	.10
Grade 1 and stage 1	1/8 (13%)	1/3 (33%)		
Grade 2 and stage 1	3/8 (38%)	2/3 (67%)		
Grade 2 and stage 2	1/8 (13%)	NA	NA	NA
Grade 2 and stage 3	3/8 (38%)	N/A		
Funisitis – n (%)[*]	10/124 (8%)	0	11.1 (1.3-96.9)	.03
Grade 1 and stage 1	4/10 (40%)	NA		
Grade 2 and stage 1	1/10 (10%)	NA	NA	NA
Grade 1 and stage 2	5/10 (50%)	NA		

Data was displayed as number (%).TTTS: twin–twin transfusion syndrome; OR: odds ratio; CI: confidence interval. NA: not applicable.
*Denotes funisitis present in each infant instead of pregnancies. To be able to compute odds ratios, we added one positive outcome to the control group. As a result, the estimates of the odds ratios are conservative, and their significance is underestimated.

Table 4. Comparison of risk factors between TTTS after fetoscopic laser surgery with and without histological chorioamnionitis

Risk factors	TTTS with acute chorioamnionitis (n=8 pregnancies, 16 fetuses)	TTTS without acute chorioamnionitis (n=54 pregnancies, 110 fetuses)	P value
Gestational age at operation – wk	21.7 (16.2-25.0)	19.6 (15.4-27.4)	.15
Quintero stage – n (%)			
1	3	9	
2	2	22	1.0
3	3	23	
4	0	0	
Anterior placenta – n (%)	3 (38)	18 (35)	.67
PROM – n (%)	3 (38)	18 (33)	.60
Gestational age at PROM – wk	25.2 (25.1-28.0)	30.6 (15.9-35.1)	.04
PROM-to-delivery interval – day	9 (2-15)	4 (1-126)	.49
Intraprocedural bleeding – n (%)	1 (13)	3 (6)	.43
Total amount of laser energy – J	5460 (1928-14924)	4120 (600-10100)	.06
Number of anastomoses coagulated – n	6 (3-13)	6 (3-23)	.79
Duration of operation – min	34 (24-40)	30 (12-65)	.12

Data was displayed as median (range) or number (%).TTTS: twin–twin transfusion syndrome; PROM: premature rupture of membranes

Table 5. Histological chorioamnionitis in TTTS treated with fetoscopic laser surgery in relation to perinatal outcome

Variables	TTTS with acute chorioamnionitis (n=8 pregnancies, 16 fetuses)	TTTS without acute chorioamnionitis (n=54 pregnancies, 108 fetuses)	P value
Laser-to-delivery interval – wk	6.6 (3.4-14.1)	13.6 (4.4-20.1)	<.01
Gestational age at birth – wk	28.1 (23.1-32.6)	32.7 (24.4-37.0)	<.01
C-reactive protein – mg/L	7.5 (1.0-64.0)	1.0 (1.0-54.1)	<.01
Leukocyte count – 10^9/L	13.2 (2.6-23.8)	9.2 (2.0-23.9)	.07
Early-onset neonatal sepsis – n /N (%) *	2/14 (14%) [b]	4/100 (4%) [c]	.13
Cerebral injury – n/N (%) *	4/14(29%)	13/106 (12%)	.83
Neonatal mortality – n/N (%) *	2 (13%)	6 (6%)	.30

Data was displayed as median (range) or number (%).TTTS: twin–twin transfusion syndrome; * Refers to per infant rather pregnancy. Three infants died soon after birth and in 11 neonates were lost to follow up therefore no any test or bacterial culture was performed.

Comparison of perinatal outcome between twin–twin transfusion syndrome cases with and without histological chorioamnionitis after fetoscopic laser surgery was presented in Table 5. Median of laser-to-delivery interval in twin–twin transfusion syndrome with and without histological chorioamnionitis after fetoscopic laser surgery was 6.6 (range 3.4-14.1) versus 13.6 (range 4.4-20.1) weeks, respectively (p<.01).

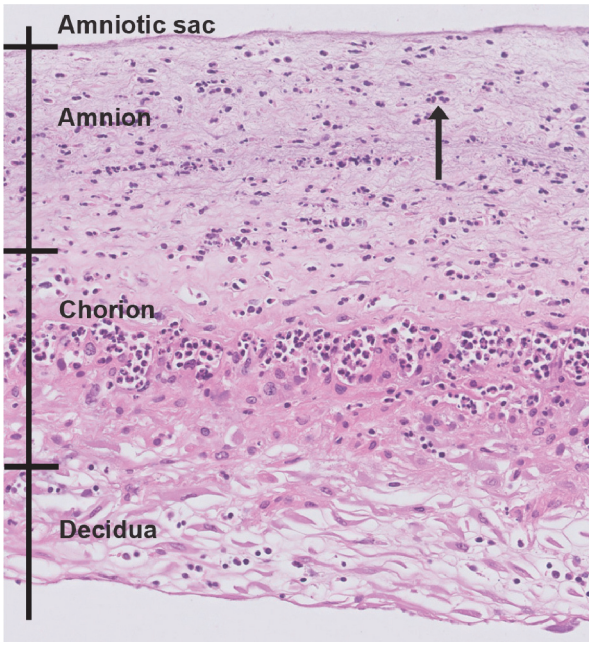

Figure 2 An example of histological chorioamnionitis in a placenta from twin–twin transfusion syndrome treated with fetoscopic laser surgery. Histological chorioamnionitis (stage 2 and grade 2) occurred with diffuse distribution of neutrophils (blue arrow).

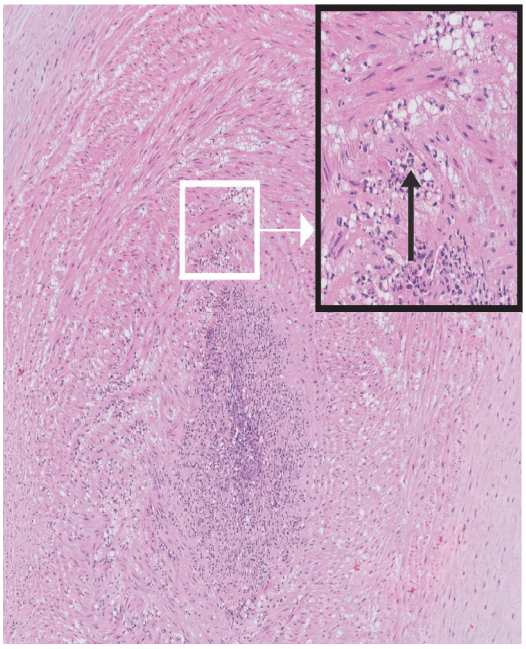

Figure 1 An example of funisitis following fetoscopic laser surgery. Umbilical arteritis (stage 2 and grade 2) occurred. The infiltrated neutrophils were nearly confluent in the transverse section of arterial wall. A close view of umbilical arteritis (white square) was shown in the enlarged picture (black square). Neutrophils were indicated by the blue arrow.

Discussion

This study presents the histological evaluation of the maternal and fetal inflammatory response after fetoscopic laser surgery for twin–twin transfusion syndrome. We found that the risk of funisitis (8%) was significantly increased after laser. The incidence of histological chorioamnionitis was also higher in the study group compared to the control group (13%

versus 5%), but no statistical significance was found. Our data suggest that fetoscopic intervention is not only associated with an increased risk of PROM, but also with an increased risk of funisitis.

The occurrence of intrauterine inflammation after laser surgery in the literature varies from 0 to 4%.[3-13] In these studies, the diagnosis of intrauterine inflammation was defined as clinical chorioamnionitis, based on clinical indicators such as maternal fever and fetal tachycardia. However, clinical chorioamnionitis does not correspond well to histological chorioamnionitis.[29, 30] Importantly, histological chorioamnionitis is a more reliable indicator for intrauterine inflammation compared to clinical chorioamnionitis.[31] Intrauterine inflammation in severe chorioamnionitis may extend to the umbilical cord and the fetus, resulting in funisitis (inflammation in the cord) and fetal inflammatory response. Therefore, the higher incidence of histological chorioamnionitis and funisitis detected in this study may be a more reliable indicator of the true risk and the severity of intrauterine inflammation after laser.

The cause of increased risk of funisitis after laser is not well known. The increased risk may be related to the nature of the fetal surgical intervention. The use of a relatively large fetoscope (diameter typically 3 to 4 mm), creating a membrane defect (and high risk of PROM)[32] and the use of laser energy creating iatrogenic placental necrosis may induce maternal inflammatory response ('sterile inflammation'), even with fetal involvement.[22, 33] Given that the risk factors for funisitis and histological chorioamnionitis are nearly identical[34] and that the sample size of cases with funisitis in this study is small, we evaluated the role of several perinatal risk factors only for histological chorioamnionitis. We found that lower gestational age at PROM was associated with the occurrence of

histological chorioamnionitis after laser. However, the limited number of pregnancies with histological chorioamnionitis prevented further examination of any independent association between gestational age at PROM and histological chorioamnionitis after laser.

Previous studies reported that both histological chorioamnionitis and funisitis contribute to the increased risk of short-term adversity.[14, 35, 36] In our study, we found that histological chorioamnionitis was related to shorter laser-to-delivery interval and lower gestational age at birth. Noticeably, the longer laser-to-delivery interval in cases without intrauterine inflammation may be partially iatrogenic since active management may be considered in cases with PROM at early gestational age. In a recent study, the risk of impaired long-term neurodevelopmental outcome was higher in preterm infants with funisitis than histological chorioamnionitis,[37] emphasizing the clinical consequences of funisitis. A larger study with long–term neurodevelopmental follow up is needed to compare the risk of histological chorioamnionitis with or without funisitis in infants with twin–twin transfusion syndrome treated with laser.

The main limitation of our study is the selection bias due to the exclusion of cases with fetal demise. Severe intrauterine inflammation may lead to fetal demise. Exclusion of these cases may thus lead to an underestimation of the true risk of intrauterine inflammation after laser. However, fetal death itself may also stimulate intrauterine inflammation. The cause–effect relationship between fetal death and intrauterine inflammation is difficult to ascertain. In addition, TTTS itself may be a risk factor for intrauterine inflammation. The ideal control group would have been twin–twin transfusion syndrome managed without laser. However, this is considered unethical since the optimal treatment for this condition is laser surgery. Interestingly, the incidence of histological chorioamnionitis in this study (13%) is quite low

compared to the general incidence in preterm births (30% - 70%).[26, 38] This discrepancy may be explained by the lack of consistent diagnostic criteria and methods for histological chorioamnionitis.[39] The high rate of cesarean section, preoperative use of indomethacin and prophylactic antibiotic, antibiotic treatment for PROM and corticosteroid treatment in the study group may also contribute to the lower rate of histological chorioamnionitis.[14, 22, 40] As discussed above, placental necrosis after laser may be associated with intrauterine inflammation. The occurrence of intrauterine inflammation after laser may thus be confounded by the amount of laser energy and duration of operation. Furthermore, the incidence of histological chorioamnionitis in this study (13%) is higher than that after other invasive procedures such as amniodrainage and intrauterine transfusion (less than 1%).[16, 19] Papanna et al. reported the size of operative cannula for laser surgery contributes to the occurrence of post–laser PROM, which is closely related to intrauterine inflammation.[32] Further studies are warranted to investigate whether the improvement of operation instruments (smaller diameter) could reduce the risk of intrauterine inflammation.

In conclusion, fetoscopic laser surgery appears to increase the risk of histologically determined intrauterine inflammation.

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Chapter 9

Increased Risk Of Early-Onset Neonatal Sepsis After Laser Surgery For Twin-Twin Transfusion Syndrome

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Abstract

Objective: To investigate the occurrence of early-onset neonatal sepsis (EOS) in twin-twin transfusion syndrome (TTTS) managed with laser surgery.

Study design: We performed a prospective cohort study of all consecutive TTTS cases treated with laser surgery (TTTS group) delivered at the Leiden University Medical Center. We recorded the occurrence of EOS, defined as a positive blood culture ≤72 hours postpartum (proven sepsis) or administration of a full course of antibiotics due to risk factors or signs of sepsis, in the absence of a positive blood culture (suspected sepsis). Perinatal variables in the TTTS group were compared with uncomplicated monochorionic twins (no-TTTS group). A multivariate model was generated, examining the association between EOS and gestational age at birth, interval between laser surgery and birth, anterior placenta, laser period (first study period: 2002-2008; second study period: 2009-2015) and preterm premature rupture of membranes (PPROM).

Results: The rates of combined suspected and proven EOS in the TTTS group and no-TTTS group were 16% (68/208) and 10% (55/271), respectively (relative ratio (RR) 1.74, 95% confidence interval (CI) 1.19-2.55). Multivariate analysis showed that EOS in the TTTS group was independently associated with lower gestational age at birth (odds ratio (OR) 0.75, 95% CI 0.63-0.88), first study period (OR 2.25, 95% CI 1.08-4.67) and PPROM (OR 2.47, 95% CI 1.28-4.75

Conclusion: The rate of EOS in the TTTS group is low, but increased compared to the no-TTTS group. EOS in TTTS is independently associated with premature delivery, earlier laser period and PPROM.

Keywords: twin-twin transfusion syndrome, laser surgery, early-onset neonatal sepsis

Twin-twin transfusion syndrome (TTTS) is a severe condition complicating about 10% of monochorionic diamniotic twin pregnancies (Lewi et al., 2008). Laser surgery is the optimal treatment for severe TTTS. Although the neonatal outcome has significantly improved after introduction of laser surgery, several complications are reported, such as preterm premature rupture of membranes (PPROM), clinical or histologic chorioamnionitis, recurrent or reversed TTTS and post-laser twin anemia-polycythemia sequence (TAPS). PPROM is partly due to the specific nature of the fetoscopic intervention itself, in which a fetoscope is introduced into the amniotic cavity causing iatrogenic PPROM [1-5]. Previous studies also demonstrated that the risk of chorioamnionitis and early–onset neonatal sepsis (EOS) is increased after invasive intrauterine procedures, such as amniocentesis or fetal blood sampling, in both singletons and twins [6-12]. EOS is usually caused by bacterial pathogens, most commonly group B streptococcus or Escherichia coli, transmitted vertically from mother to infant before or during delivery and is one of the leading causes of neonatal mortality and morbidity [13-15]. The risk of EOS following laser surgery for TTTS has however not yet been studied.

The purpose of this study was to investigate the occurrence and risk factors of EOS after laser surgery for the treatment of TTTS.

Materials and Methods

Design and study population

All consecutive monochorionic twins delivered at the Leiden University Medical Center between March 2002 and April 2015 were eligible for this study. We included all monochorionic twins with TTTS treated with laser surgery (TTTS group) and compared them with a control group of uncomplicated monochorionic twins (no-TTTS group).

Monochorionic twins with TTTS not managed with laser surgery and TAPS cases were excluded. We also excluded monochorionic twins with single or double fetal demise. These twins had to be excluded since sepsis workup was not performed, preventing the diagnosis of EOS. TTTS was diagnosed according to the Eurofetus criteria, defined as a maximal vertical pocket (MVP) of <2 cm in the donor combined with a MVP of >8 cm in the recipient before 20 weeks gestation and >10 cm thereafter [1]. Staging of TTTS was defined according to Quintero's system [16]. The procedure of laser surgery for TTTS was published previously [17]. Since 2008, the Solomon laser technique was introduced to reduce the risk of residual anastomoses and associated post–operation complications [18]. We thus divided the study period into two groups, that is the first group from 2002 to 2008 (first study period) and the second group from 2009 to 2015 (second study period). Diagnosis of TAPS was based on previously published international criteria [17].

Outcome variables

The following perinatal variables were recorded, including gestational age at laser surgery, study period (first study period: 2002-2008; second study period: 2009-2015), placental localization, gestational age at birth, birth weight and birth weight discordance, mode of delivery, maternal fever (≥38 ºC), use of epidural anesthetics during labor and PPROM (<37 weeks gestational age for >24 hours). Data from maternal vaginal or urinary cultures were mostly not available and therefore not recorded. After birth, routine blood tests were performed in all preterm neonates that were born before the gestational age of 37 weeks. C–reactive protein (CRP) levels and immature/total neutrophil ratios (I/T ratio) were documented within the first 72 hours after birth. Elevated CRP was defined as a level >20 mg/L. An I/T ratio >0.22 was suggestive of infection [19]. Blood cultures were performed

within 72 hours after birth in all children with suspected perinatal infection. Diagnosis of EOS was based on a positive blood culture within the first 72 hours postpartum (proven EOS) or the necessity for administration of a full course (5-7 days) of antibiotics due to perinatal risk factors and/or clinical signs of neonatal infection, though without a positive blood culture (suspected EOS). Blood cultures were evaluated for the chance of contamination. When a Coagulase Negative Staphylococcus was found, these were only considered proven EOS if there were clinical and/or laboratory signs (CRP >20 mg/L or I/T ratio >0.22) of infection. Risk factors for perinatal infection included one or more of the following items: maternal group B streptococcal colonization, maternal bacteriuria or infection, preterm birth (gestational age <37 weeks) following spontaneous labor, ruptured membranes >24 hours in preterm birth, maternal fever (≥38°C) or parenteral antibiotics given to mother for (suspected) invasive bacterial infection. The primary outcome was the occurrence of proven and/or suspected EOS. Secondary outcomes were CRP and I/T ratio in the first 72 hours after birth. Potential risk factors for EOS that were studied included gestational age at laser surgery, gestational age at birth, interval between laser surgery and birth, anterior placenta, study period (first study period: 2002-2008; second study period: 2009-2015) and PPROM.

Statistical analysis

Kolmogorov-Smirnov test was used to assess the normality of continuous variables. Student *t* test or Mann-Whitney test were applied to analyze continuous variables, where appropriate. Chi square test or Fisher exact test was used to compare dichotomous data. Paired variables were compared using McNemar or Wilcoxon signed rank test, if applicable. The results were displayed as median and interquartile range (IQR), mean and range or number and percentage, where appropriate. The potential risk factors for EOS were analyzed in a univariable regression model (generalized estimating equation). The factors

significantly associated with EOS in the univariate analysis were further used to build a multivariable regression model (generalized estimating equation) to assess their independency in relation to EOS, because pair wised comparison in twins is possible in this binominal model. The results of correlation and risk factor analysis were displayed as odds ratio (OR) and 95% confidence interval (CI). Statistical significance was assumed if $p<0.05$ in all analyses, besides the univariate analysis ($p<0.10$). Statistical analysis was performed using *IBM SPSS Statistics 22.0*® (IBM Corporation, Armonk, New York, USA).

Results

During the study period 479 eligible twin pairs with two live born neonates were delivered at our hospital and included in the study, of which 208 (43%) in the TTTS group after laser surgery and 271 (57%) in the no-TTTS group. The baseline characteristics of the two study groups are summarized in table 1. In the TTTS group, 14% (28/208) of the twin pairs were Quintero stage 1, 32% (67/208) stage 2, 51% (105/208) stage 3 and 4% (8/208) stage 4. Median gestational age at laser surgery was 20 weeks (IQR 18-23 weeks). In the no-TTTS group, selective fetal growth restriction occurred in 65/271 (24%) twin pairs and 14/271 (5%) twin pairs were monoamniotic.

Comparison of primary and secondary outcomes between the TTTS group and no-TTTS group is shown in Table 2. The rate of proven EOS was 2% (10/208) in the TTTS group and 0.4% (2/271) in the no-TTTS group (relative ratio (RR) 6.67, 95% CI 1.45-30.59 $p=0.02$). The rate of suspected EOS was 14% (58/208) in the TTTS group and 10% (53/271) in the no-TTTS group (RR 1.50, 95% CI 1.01-2.24, $p=0.04$). The overall rate of EOS (proven or suspected) was significantly higher in the TTTS group compared to the no-TTTS group, 16% versus 10% respectively (RR 1.74, 95%CI 1.19-2.55, $p<0.01$). In the TTTS group after laser surgery,

Table 1 Clinical baseline

	TTTS group (n=208)	Missing	No-TTTS group (n=271)	Missing	p value
Epidural during labor	43 (21%)	44 (21%)	57 (21%)	72 (27%)	.44
Maternal fever (≥38 ºC)	29 (14%)	30 (14%)	17 (6%)	25 (9%)	<.01
Caesarian section	83 (40%)	2 (1%)	116 (43%)	1 (.4%)	.41
Gestational age at birth – weeks[a]	32.7 (30.1-35.6)		34.9 (31.7-36.3)	1 (.4%)	<.01
Birth weight – gram [b]	1765 (1271-2139)		2049 (1520-2580)		<.01
Birth weight discordance – %	11.0 (4.7-21.1)		11.3 (5.5-24.0)		.84
PPROM (> 24 hours) [b, c]	116 (28%)		28 (5%)		<.01
Interval between PPROM and birth – hours [b, c]	1.7 (.0-54.4)		.3 (.0-6.4)		<.01

Data is displayed as median (IQR) or number (%). [a] In the TTTS group the gestational age at birth was known in all neonates. [b] Denotes the number of fetus instead of twin pairs. [c] For these variables the data were complete.

Table 2 Primary and secondary outcomes in the TTTS group and control group.

		TTTS group (n=416)[a]	Missing	No-TTS group (n=542)[a]	Missing	p value	OR (95% CI)[b]
Early onset sepsis	Overall	68 (16%)	2 (1%)	55 (10%)	0 (0%)	<0.01	1.13 (0.74-1.71)
	Suspected	58 (14%)	–	53 (10%)	–	0.04	0.95 (0.62-1.46)
	Proven	10 (2%)	–	2 (0.4%)	–	<0.01	5.03 (1.08-23.54)
C-reactive protein – mg/L		7.0 (1.0-104.0)	95 (23%)	4.1 (1.0-68.0)	177 (33%)	<0.01	–
Elevated C-reactive protein (> 20 mg/L)		28 (7%)	95 (23%)	21 (4%)	177 (33%)	0.13	–
Immature/total neutrophil ratio		0.10 (0.05-0.18)	334 (80%)	0.06 (0.05-0.09)	450 (83%)	<0.01	–

Data is displayed as median (IQR) or number (%).C-reactive protein is displayed as mean (range). [a] Denotes the number of fetus instead of twin pairs. [b] Çorrected for gestational age at birth.

Table 3 Comparison between the ex-donor and the ex-recipient twins in TTTS managed with laser surgery.

		Ex– Donor (n= 206) [a]	Missing	Ex–Recipient (n=206) [a]	Missing	p value
Early onset sepsis	Overall	35 (17%)	1 (1%)	33 (16%)	1 (1%)	0.69
	Suspected	31 (15%)	–	27 (13%)	–	0.41
	Proven	4 (2%)	–	6 (3%)	–	0.75
C-reactive protein – mg/L		8.2 (1.0-104.0)	48 (23%)	5.9 (1.0-75.0)	45 (22%)	0.34
Elevated C-reactive protein (> 20 mg/L)		15 (7%)	48 (23%)	13 (6%)	45 (22%)	1.00
Immature/total neutrophil ratio		0.09 (.05-.15)	164 (80%)	0.10 (0.05-0.20)	166 (81%)	0.10
PPROM (> 24 hours)[b]		46 (22%)		69 (34%)		<0.01
Interval between PPROM and birth – hours[b]		1.3 (0.0-15.5)		2.6 (0.0-95.3)		0.02

Data is displayed as median (IQR) or number (%). C-reactive protein is displayed as mean (range). [a] For two twin pairs the donor and recipient were unknown. [b] For these variables the data were complete

Table 4 Analysis of risk factors for neonatal early–onset sepsis in TTTS group treated with laser surgery.

	Neonates with proven or suspected EOS (n=68)	Neonates without proven or suspected EOS (n=346)	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Gestational age at laser – weeks	20.0 (16.3-23.0)	20.0 (18.0-23.0)	0.97 (0.87-1.08)	0.58	–	–
Gestational age at birth – weeks	30.1 (28.0-31.9)	33.4 (30.4-35.7)	0.75 (0.67-0.83)	<0.01	0.75 (0.63-0.88)	<0.01
Time interval between laser and birth – weeks	10.4 (7.0-13.8)	13.3 (10.0-16.0)	0.89 (0.84-0.95)	<0.01	1.04 (0.93-1.15)	0.50
Anterior placenta localization, yes Anterior placenta localization, no	36 (53%) 32 (47%)	129 (37%) 217 (63%)	1.89 (0.99-3.63)	0.06	1.54 (0.75-3.14)	0.24
First study period: 2002-2008 Second study period: 2009-2015	39 (57%) 29 (43%)	127 (37%) 219 (63%)	2.32 (1.20-4.47)	0.01	2.25 (1.08-4.67)	0.03
PPROM (>24 hours), yes PPROM (>24 hours), no	36 (53%) 32 (47%)	80 (23%) 266 (77%)	3.74 (2.06-6.81)	<0.01	2.47 (1.28-4.75)	<0.01

Data is displayed as median (IQR) or number (%).

CRP and I/T ratio were also significantly increased compared to the no-TTTS group (both $p<0.01$). After controlling for gestational age at birth no difference in suspected EOS was detected in the TTTS group compared to the no-TTTS group (OR 0.95, 95% CI 0.62-1.46, $p=0.82$), but the odds ratio of proven EOS was still increased (OR 5.03, 95% CI 1.08-23.54, $p=0.04$).

Analysis between ex-donors and ex-recipients in the TTTS group showed no difference in suspected or proven EOS (Table 3). However the occurrence of PPROM was significantly higher in ex-recipients than in ex-donors, 69/206 (34%) versus 46/206 (22%) respectively (RR 1.75, 95% CI 1.13-2.71 $p=0.01$). Overall EOS occurred in 62% (42/68) of cases in both neonates within the same twin pair and in only one neonate of the twin pair in the remaining 38% (26/68) of EOS cases.

Univariate analysis of potential risk factors for the occurrence of EOS in TTTS managed with laser surgery showed that EOS was associated with lower gestational age at birth (OR 0.75, 95% CI 0.67-0.83, $p<0.01$), shorter interval between laser surgery and birth (OR 0.89, 95% CI 0.84-0.95, $p<0.01$), anterior placenta (OR 1.89, 95% CI 0.99-3.63, $p=0.06$), first study period (OR 2.32, 95% CI 1.20-4.47, $p=0.01$) and PPROM (OR 3.74, 95% CI 2.06-6.81, $p<0.01$). After multivariate analysis only lower gestational age at birth (OR 0.75, 95% CI 0.63-0.88, $p<0.01$), first study period (OR 2.25, 95% CI 1.08-4.67, $p=0.03$) and PPROM (OR 2.47, 95% CI 1.28-4.75, $p<0.01$) were independently associated with the occurrence of EOS (Table 4). In the group of neonates with proven EOS in the TTTS group, 60% (6/10) of infections was due to infection with gram positive bacteria (Coagulase Negative Staphylococcus, n = 4 with elevated CRP 13, 101 68, 75 mg/L, respectively; Staphylococcus Aureus, n=1, Enterococcus Faecalis, n=1) and 40% (4/10) of infections was due to gram negative bacteria (Escherichia coli, n=2, Klebsiella Pneumoniae, n=2). In the two cases with proven EOS in the control group,

infection was due in both cases to Coagulase Negative Staphylococcus, with CRP 33 and 67 mg/L, respectively.

Discussion

This is the first study to investigate the occurrence of EOS following laser surgery for the treatment of TTTS. Our findings show that the rate of EOS in TTTS treated with laser surgery is slightly increased compared to monochorionic twins without invasive fetoscopic intervention. Risk factor analysis shows that EOS after laser surgery for TTTS is independently associated with lower gestational age at birth, first study period and PPROM. Small studies have linked invasive intrauterine procedures, such as chorionic villus sampling, amniocentesis and fetal blood sampling, to the occurrence of EOS [6-12]. Reports on the frequency of EOS after laser surgery are however lacking. EOS is usually caused by bacterial pathogens transmitted vertically from mother to the fetus perinatally, resulting in intrauterine infection and ensuing EOS [13]. Previous studies have suggested a possible association between laser surgery and increased risk of chorioamnionitis [5, 20-25]. In a cohort of 266 TTTS cases treated with laser surgery, Rossi et al reported that the risk of iatrogenic PPROM and clinical chorioamnionitis was 38% and 0%, respectively [23]. However, the diagnosis of chorioamnionitis was not based on histopathological evaluation and the risk of neonatal EOS was not reported. In another larger cohort of 602 TTTS cases treated with laser surgery, Stirnemann et al reported a 4% incidence of clinical chorioamnionitis, but again data on neonatal EOS were not described [25]. In a prospective study, Lewi et al. reported a rate of neonatal sepsis of 4% in monochorionic twin pregnancies [26]. However, the frequency of neonatal sepsis in the subgroup of TTTS managed with laser surgery was not specified and the definition of neonatal sepsis was not

clearly described. In our study we found that the rate of proven EOS was 2% in monochorionic twins managed with laser surgery and 0.4% in monochorionic twins not managed with laser surgery. Laboratory investigations also showed higher CRP levels and I/T ratio in the TTTS group. Whether these result from bacterial infection or post-operative inflammation after laser surgery is not clear.

This study further analyzed the association between several perinatal variables with the occurrence of EOS in the subgroup of TTTS treated with laser surgery. We found that lower gestational age at birth, first study period and PPROM were independently associated with EOS after laser surgery. Iatrogenic PPROM is a known risk associated with invasive intrauterine procedures. In accordance with previous studies, we also found an increased risk of PPROM (28%) after laser surgery (Yamamoto et al., 2005; Rossi et al., 2008). PPROM may facilitate the invasion of microorganisms into the amniotic cavity, leading to histopathological and/or clinical chorioamnionitis and resulting in fetuses exposed to high risk of infection [10, 27]. PPROM may also lead to premature delivery, which is a known risk factor for both EOS as well as late onset sepsis [28-30]. In addition, in neonates with lower gestational age at birth, the innate and adaptive immunity may be more immature, increasing the risk of infection [31]. Lastly, we also found an increased risk of EOS in the first study period. The decrease in risk of infection in the second, most recent study period, might be a direct effect of the learning curve related to laser surgery. Increased experience and improved laser technique within the last years may result in a reduction of procedure related complications [32].

Univariate analysis in this study also showed that shorter interval between laser surgery and birth and anterior placenta are associated with EOS. However, after correction in multivariate analysis they were no longer associated with the occurrence of EOS. A shorter

interval between laser surgery and birth might be a result of PPROM and chorioamnionitis and result in premature delivery. Anterior placenta increases the complexity of the fetal surgical intervention and may thus increase the risk of perinatal infection due to a longer operation time and a higher risk of complications such as iatrogenic PPROM [5]. This hypothesis should be further explored in other cohorts.

An important asset of this study is that it is a large prospective cohort study, including monochorionic twins with and without TTTS. Furthermore, it is the first study to investigate the occurrence of EOS after laser surgery for TTTS, an important evaluation of an invasive procedure, which is increasingly being used throughout the world. However, our study has several limitations for which the results should be carefully interpreted. An important limitation is that histopathological evaluation of the placenta was not routinely performed. In addition, by excluding monochorionic twin pregnancies with fetal demise from this study, we may have introduced a selection bias. However, these cases had to be excluded since sepsis workup was not performed in these neonates, preventing diagnosis of EOS. If demise in these cases was due to perinatal infection, the rate of EOS in the TTTS group would have been higher and our data may underestimate the true occurrence of EOS. On the other hand, neonatal infection in some cases in the TTTS group could be the result of prematurity due to immature immune system and not be a direct result of the laser surgery. Therefore our data may also overestimate the true risk of infection after laser. However, studying this aspect is not considered ethical, since the majority of TTTS is currently managed with laser surgery [1, 3].

Conclusions

The occurrence of EOS in TTTS managed with laser surgery is low, but increased compared to

uncomplicated monochorionic twins. Future studies and developments focusing on improvement of laser technique and instruments may reduce the risk of (iatrogenic) PPROM, chorioamnionitis and perinatal sepsis. Neonatologists should be aware of the risk of EOS when caring for newborns treated with laser surgery for TTTS during pregnancy.

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Part IV

Summary, General Discussion and Future Perspectives

Summary

Monochorionic (MC) pregnancies account for around one third of all twin gestations, but contribute disproportionately to the occurrence of adverse perinatal outcomes. Increasing body of evidence shows that perinatal outcomes in MC twins are strongly associated with the complications resulting from the unique angioarchitecture in MC placentas, in particular the placental vascular anastomoses. Due to the extensive application of prenatal ultrasound examination, an increasing number and types of complication dedicated for MC twins are being diagnosed. Delineation of the placental characteristics classified by specific complications may shed light on the pathophysiology of various complications in MC twins. One of the great successes in fetal therapy is the introduction of fetoscopic laser coagulation of vascular anastomoses for the treatment of twin–twin transfusion syndrome (TTTS). The investigation on postoperative complications in TTTS placentas is crucial for the further improvement of fetoscopic laser surgery and improvement of perinatal outcome. Since 2002, all MC placentas delivered at the Leiden University Medical Center (LUMC) are consecutively being examined and injected with colored dye. This large database of MC placentas (n=940 in 2016) allows detailed investigation of the pathogenesis and clinical outcome of these rare diseases.

Monochorionic Placentas: analysis and characteristics

In *Chapter 2* we compared the prevalence of vascular anastomoses and evaluated the sharing discordance in 134 MC placentas and 124 dichorionic (DC) placentas. This study demonstrates that vascular anastomoses are present in 99% (133/134) MC placentas and 0% DC placentas ($p < .01$). Placental share discordance between MC twins is significantly larger compared to DC twins, 19.8 (interquartile range (IQR) 8.1-33.3) and 10.8 (IQR 6.2-

19.0), respectively (p<.01). Vascular anastomoses–associated complications occurred in 16% (22/134) MC twins. Our findings show that vascular anastomoses are almost ubiquitous in MC placentas, but non-existent in DC placentas. In addition, unequal placental sharing appears to be more common in MC than in DC placentas. However, our data should be interpreted with care due to the referral nature of our center. Since twin pregnancies referred to our centers usually underwent complications related to the vascular anastomoses, the findings on MC twins in this study may be overestimated.

The aim of the study described in *Chapter 3* was to estimate the prevalence, number, size and localization of the anastomoses using color dye injection in MC placentas with complications (selective intrauterine growth restriction (sIUGR), n = 46; TTTS placentas, n = 47; spontaneous twin anemia–polycythemia (TAPS), n=16) compared to 126 uncomplicated MC placentas. The prevalence of arterio-arterial (AA) anastomoses in uncomplicated MC, sIUGR, TTTS and TAPS placentas was 96%, 98%, 47% and 19%, respectively. Median number of anastomoses in uncomplicated MC, sIUGR, TTTS and TAPS placentas was 8 (IQR: 4-12), 8 (IQR: 5-14), 7 (IQR: 5-11) and 4 (IQR: 3-5), respectively. We concluded that the prevalence, size, number and localization of the various types of vascular anastomoses differ between uncomplicated MC, sIUGR, TTTS and TAPS placentas. However, the ex vivo nature of this study should be taken into account when extrapolating our in vitro findings to the in vivo situation such as visualization of vascular anastomoses during fetoscopy.

In *Chapter 4* based on the measurements in 369 MC placentas, a reference range for the distance between cord insertions was generated to determine the cut–off value of proximate cord insertions (PCI). Additionally, the prevalence and angio-architecture of MC placentas with PCI was further evaluated. The 5th centile of the established reference range

was adopted to define PCI and was calculated by the equation : 0.027 × gestational age (weeks) +2.91 (cm), yielding a range from 3.3 to 4 cm throughout gestation. Accordingly, 18 of the 369 (5%) MC placentas fulfilled the definition criteria for PCI. The prevalence of arterio-arterial (AA) and veno-venous (VV) anastomoses in MC placentas with and without PCI was respectively 100% (18/18) versus 80% (281/351) (P=.12) and 56% (10/18) versus 26% (91/351) (P=.01). Based on our analysis, we propose that the use of a fixed cut-off set at 4 cm might be easier to use in daily practice instead of a gestational-age-dependent equation.

Placental characteristics in relation to specific complications

The objective of the multicenter study described in *Chapter 5* was to evaluate the prevalence of veno-venous (VV) anastomoses in a large cohort of 106 monochorionic (MC) twin placentas with twin–twin transfusion syndrome (TTTS) compared to a control group of 308 MC placentas without TTTS. The prevalence of VV anastomoses was significantly higher in the TTTS group than in the non-TTTS group, 36% (38/106) and 25% (78/308), respectively (p=.04; odds ratio (OR) 1.65; 95% confidence interval (CI): 1.03–2.64). In the subgroup of MC placentas without AA anastomoses, the prevalence of VV anastomoses in the TTTS group and non-TTTS group was 32% (18/57) and 8% (2/25), respectively (p=.03; OR: 5.31; 95% CI: 1.13–24.98). Our results suggest that the VV anastomoses may play a role in the development of TTTS, in particular in the absence of AA anastomoses.

Previous observational studies noted a discordance between birth weight and individual placental share in TAPS. The purpose of the study in *Chapter 6* was to investigate if fetal growth in monochorionic (MC) twins with TAPS is determined by placental share or by the net inter-twin blood transfusion. We analyzed 20 TAPS cases and 250 uncomplicated MC

twins and found that birth weight share in the TAPS group was positively correlated with hemoglobin (Hb) levels share at birth (P<0.01) but not with placental share (P=0.54). In contrast, in the group of uncomplicated MC twins, birth weight share was strongly correlated with placental share (P<0.01) but not with Hb levels share (P=0.14). Our findings suggest that fetal growth in MC twins with TAPS is determined primarily by the net inter-twin blood transfusion instead of placental share and a relatively larger placental share may enable the survival of the anemic twin in TAPS.

In *Chapter 7* we report the prevalence of proximate cord insertions and associated clinical consequences in TTTS treated with fetoscopic laser surgery (FLS). The prevalence of proximate cord insertions in TTTS placentas was 2% (4/252). Residual anastomoses were detected in all placentas with proximate cord insertions (100%, 4/4) compared to 27% (66/248)(P<.01) in TTTS placentas without proximate cord insertions. Our findings suggest that fetoscopic laser coagulation in TTTS cases with proximate cord insertions is challenging due to technical difficulties in visualizing the vascular equator and results in an increased risk of incomplete laser treatment.

Histologic chorioamnionitis and early–onset sepsis in TTTS after laser surgery

The frequency of intrauterine inflammation and associated consequences in TTTS managed with fetoscopic laser surgery is presented in *Chapter 8*. In this case–control study performed at our center from 2013 to 2014, we included all TTTS pregnancies managed with fetoscopic laser surgery (n=62) and compared to a control group of monochorionic (MC) twin pregnancies not treated with fetoscopic laser surgery (n=64). The incidence of histologic chorioamnionitis was 13% (8/62) in the laser group versus 5% (3/64) in controls (odds ratios (OR) 3.0, 95% confidence interval (CI) .8-11.9, *P*=.12). Funisitis occurred in 8% (10/124) in

the laser group versus 0% in controls (OR 11.1, 95% CI 1.3-96.9, *P*=.03). Histologic chorioamnionitis following fetoscopic laser surgery was associated with shorter laser-to-delivery interval (*P*<.01) and lower gestational age at birth (*P*<.01). These findings suggest TTTS cases managed with FLS are at increased risk of funisitis.

In *Chapter 9* we compared the rate of early–onset neonatal sepsis (EOS) in a cohort of all consecutive TTTS cases treated with laser surgery compared to a cohort of uncomplicated monochorionic twins delivered at our center. The rate of proven or suspected EOS in the TTTS group and no-TTTS group was 16% (68/208) and 10% (55/271), respectively (relative ratio (RR) 1.74, 95% confidence interval (CI) 1.19-2.55). Multivariate analysis showed that EOS in the TTTS group was independently associated with lower gestational age at birth (odds ratio (OR) 0.75, 95% CI 0.63-0.88), earlier study period (OR 2.25, 95% CI 1.08-4.67) and PPROM (OR 2.47, 95% CI 1.28-4.75). In conclusion, The rate of EOS in the TTTS group is low, but increased compared to the no-TTTS group. EOS in TTTS is independently associated with premature delivery, earlier laser period and PPROM. Our findings suggest that neonates with TTTS after laser surgery are at increased risk of EOS.

Nederlandse Samenvatting

Ongeveer een derde van alle tweelingzwangerschappen is een Monochoriale (MC) zwangerschap en deze zwangerschappen gaan gepaard met een verhoogde kans op nadelige perinatale uitkomsten. Er is toenemend bewijs waaruit blijkt dat de perinatale uitkomsten bij MC tweelingen met complicaties sterk zijn geassocieerd met de unieke angioarchitectuur in MC placenta's, met name de vasculaire anastomoses in de placenta. Als gevolg van de uitgebreide toepassing van prenataal echo-onderzoek, kan een toenemend aantal en typen van complicaties specifiek voor MC tweelingen uiteindelijk worden gediagnosticeerd. Afbakening van de placentakenmerken geclassificeerd voor specifieke complicaties kan inzicht geven in de pathofysiologie van verschillende complicaties bij MC tweelingen. Een van de grote successen in de foetale therapie is de introductie van foetoscopische lasercoagulatie van vasculaire anastomoses als behandeling van tweeling-tweeling transfusie syndroom (TTS). Het onderzoek naar postoperatieve complicaties van TTS placenta's is cruciaal voor de verdere verbetering van foetoscopische laserchirurgie en verbetering van de perinatale uitkomst. Sinds 2002, worden alle MC placenta's, die aan het Leids Universitair Medisch Centrum (LUMC) zijn geleverd, onderzocht en met kleurstof geïnjecteerd. Deze grote database van MC placenta's (n = 940 in 2016) maakt het mogelijk om gedetailleerd onderzoek te doen naar de pathogenese en klinische uitkomst van deze zeldzame ziekten.

Monochoriale placenta’s: analyse en karakteristieken

In *Hoofdstuk 2* vergeleken we de prevalentie van vasculaire anastomosen en evalueerden we de aandeeldiscordantie in 134 MC placenta’s en 124 dichoriale (DC) placenta’s. Deze studie toont aan dat vasculaire anastomoses aanwezig zijn in 99% (133/134) van de MC

placenta’s en 0% van de DC placenta's (p <0.01). Het verschil in placenta-aandeel bij MC tweelingen is aanzienlijk groter in vergelijking met DC tweelingen, 19.8 (inter quartile range (IQR) 8.1-33.3) en 10.8 (IQR 6.2-19.0) respectievelijk (p <0.01). Complicaties gerelateerd aan vasculaire anastomosen kwamen voor bij 16% (22/134) van de MC tweelingen. Onze resultaten tonen aan dat vasculaire anastomosen bijna altijd aanwezig zijn in MC placenta’s, maar afwezig in DC placenta. Bovendien lijken ongelijke placentadelen vaker voor te komen in MC dan in DC placenta’s. Aangezien tweelingzwangerschappen die verwezen werden naar ons centrum in verband met complicaties meestal vasculaire anastomosen hadden, kunnen de bevindingen over MC tweelingen in dit onderzoek overschat zijn.

Het doel van de studie beschreven in *Hoofdstuk 3* was om de prevalentie, het aantal, de grootte en de lokalisatie van de anastomosen te schatten met behulp van kleurstofinjectie in MC placenta’s met complicaties (selectieve dysmaturiteit (sIUGR), n = 46; TTS placenta's, n=47; spontane Twin-Anemia-Polycythemia Syndrome (TAPS), n=16) in vergelijking met 126 ongecompliceerde MC placenta's. De prevalentie van arterio-arteriële (AA) anastomosen in ongecompliceerde MC, sIUGR, TTS en TAPS placenta’s was 96%, 98%, 47% en 19%, respectievelijk. Het mediane aantal anastomoses in ongecompliceerde MC, sIUGR, TTS en TAPS placenta was 8 (IQR: 4-12), 8 (IQR: 5-14), 7 (IQR: 5-11) en 4 (IQR: 3-5) respectievelijk. Wij concludeerden dat de prevalentie, grootte, aantal en lokalisatie van de verschillende vasculaire anastomosen tussen de ongecompliceerd MC, sIUGR, TTS en TAPS placenta verschillen. Echter, moet rekening gehouden worden met het ex vivo karakter van de studie bij de extrapolatie van onze in vitro bevindingen naar de in vivo visualisatie van vasculaire anastomosen tijdens fetoscopy.

In *Hoofdstuk 4* werd op basis van de metingen in 369 MC placenta's een referentiewaarde voor de afstand tussen navelstrenginserties gegenereerd om de afkap waarde te bepalen van nabije navelstreng insertie [proximate cord insertion, (PCI)] te bepalen. Daarnaast werd de prevalentie en angio-architectuur van MC placenta's met PCI verder geëvalueerd. De 5^{e} percentiel van de vastgestelde referentiewaarde werd aangenomen om PCI te definiëren en werd berekend aan de hand van de volgende formule: 0.027 x zwangerschapsduur (weken) +2.91 (cm), leidend tot een bereik van 3.3-4 cm tijdens de gestatie. Hiermee voldeden 18 van de 369 (5%) MC placenta's aan de criteria voor de definitie van PCI. De prevalentie van arterio-arteriële (AA) en veno-veneuze (VV) anastomosen in MC placenta's met en zonder PCI was respectievelijk 100% (18/18) versus 80% (281/351) (p = 0.12) en 56% (10/18) versus 26% (91/351) (p = 0.01). Op basis van onze analyse stellen we het gebruik van een vaste afkapwaarde van 4 cm voor, wat in de praktijk gemakkelijker te gebruiken zou kunnen zijn dan een zwangerschapsduurafhankelijke vergelijking.

Placentakarakteristieken met betrekking tot specifieke complicaties

Het doel van de multicenter studie beschreven in *Hoofdstuk 5* was om de prevalentie van veno-veneuze (VV) anastomoses in een groot cohort van 106 MC tweelingplacenta's met TTS te vergelijken met een controlegroep van 308 MC placenta's zonder TTS. De prevalentie van VV anastomosen was aanzienlijk hoger in de TTS groep dan in de niet-TTS groep, 36% (38/106) en 25% (78/308) respectievelijk (p = 0.04; odds ratio (OR) 1.65; 95% betrouwbaarheidsinterval (BI): 1.03-2.64). In de subgroep van MC placenta's zonder AA anastomoses was de prevalentie van VV anastomosen in de TTS groep en niet-TTS groep 32% (18/57) en 8% (2/25), respectievelijk (p = 0.03; OR: 5.31; 95% BI: 1.13-24.98). Onze

resultaten suggereren dat de VV anastomosen een rol kunnen spelen bij de ontwikkeling van TTS, met name in afwezigheid van AA anastomoses.

Eerdere observationele studies toonden een discrepantie tussen geboortegewicht en individueel placentaratio in TAPS. Het doel van het onderzoek in *Hoofdstuk 6* was om te onderzoeken of foetale groei in MC tweelingen met TAPS wordt bepaald door het ratio van het aandeel van de placenta of door de netto inter-tweeling bloedtransfusie. We analyseerden 20 TAPS tweelingen en 250 ongecompliceerd MC tweelingen met als uitkomst een positieve correlatie tussen geboortegewichtratio in de TAPS en hemoglobine (Hb) ratio bij de geboorte (P <0,01), maar niet met het placenta-aaandeelratio (p = 0.54). Daarentegen was in de groep van ongecompliceerde MC tweelingen het geboortegewichtratio sterk gecorreleerd met het placenta-aandeelratio (p <0.01), maar niet met het Hb-ratio (p = 0.14). Onze bevindingen suggereren dat de groei van de foetus in MC tweelingen met TAPS vooral wordt bepaald door de netto inter-tweeling bloedtransfusie in plaats van het placenta-aandeelratio en dat een relatief groter placenta-aandeel ratio de overleving van de anemische tweeling in TAPS mogelijk maken.

In *Hoofdstuk 7* beschrijven we de prevalentie van PCI en geassocieerde klinische gevolgen in TTS behandeld met foetoscopische laserchirurgie (FLC). De prevalentie van PCI in TTS placenta's was 2% (4/252). Restanastomosen werden gedetecteerd in alle placenta's met PCI (100%, 4/4) in vergelijking met 27% (66/248) (P <0,01) in TTS placenta's zonder PCI. Onze bevindingen suggereren dat FLC bij TTS gevallen met PCI uitdagend is door technische moeilijkheden bij het visualiseren van de vasculaire evenaar en resulteert in een verhoogd risico op onvolledige laserbehandeling.

Histologische chorioamnionitis en vroege sepsis in TTS na laserbehandeling

De frequentie van intra-uteriene inflammatie en de bijbehorende gevolgen in TTS behandeld met FLC wordt gepresenteerd in *Hoofdstuk 8*. In deze case-control studie uitgevoerd in ons centrum in 2013-2014, includeerden we alle TTS zwangerschappen behandeld met FLC (n = 62) en vergeleken we deze met een controlegroep van MC tweelingzwangerschappen die niet behandeld waren met FLC (n = 64). De incidentie van histologische chorioamnionitis was 13% (8/62) in de lasergroep versus 5% (3/64) in de controlegroep (odds ratio (OR) 3.0, 95% betrouwbaarheidsinterval (BI) 0.8-11.9, p =0. 12). Funisitis was aanwezig in 8% (10/124) in de laser groep versus 0% in de controlegroep (OR 11.1, 95% BI 13-96.9, p = 0.03). Histologische chorioamnionitis na FLC was geassocieerd met kortere laser-tot-bevalling interval (p <0,01) en kortere zwangerschapsduur bij de geboorte (p <0,01). Deze bevindingen suggereren dat TTS gevallen behandeld met FLC een verhoogd risico op funisitis hebben.

In *Hoofdstuk 9* vergeleken we het percentage vroege neonatale sepsis in een cohort van alle opeenvolgende TTS gevallen behandeld met laserchirurgie en vergeleken dit met een cohort van ongecompliceerde MC tweeling die geboren zijn in ons centrum. Het percentage van bewezen of vermoedelijke vroege neonatale sepsis in de TTS groep en non-TTS groep was 16% (68/208) en 10% (55/271) respectievelijk (relatieve ratio (RR) 1.74, 95% betrouwbaarheidsinterval (BI) 1.19 -2,55). Multivariate analyse toonde aan dat vroege neonatale sepsis in de TTS groep onafhankelijk geassocieerd was met een kortere zwangerschapsduur bij de geboorte (odds ratio (OR) 0.75, 95% BI 0.63-0.88), eerdere studieperiode (OR 2.25, 95% CI 1.08-4.67) en PPROM (OR 2.47, 95% CI 1.28-4.75). Concluderend is het percentage vroege neonatale sepsis in de TTS groep laag, maar

toegenomen ten opzichte van de non-TTS groep. Vroege neonatale sepsis in TTS is onafhankelijk geassocieerd met vroeggeboorte, eerdere laser periode en PPROM. Onze bevindingen suggereren dat pasgeborenen met TTS na laserbehandeling een verhoogd risico op vroege neonatale sepsis hebben.

General Discussion

A. Monochorionic Placentas: analysis and characteristics

Although all twin pregnancies are at increased risk of abnormal placentation, several types of placental anomalies are exclusive to monochorionic (MC) pregnancies such as vascular anastomoses, abnormal umbilical cord insertion and unequal placental sharing.

1. Vascular anastomoses

Vascular anastomoses are extremely rare in dichorionic (DC) twins but ubiquitous in MC twins. These anastomoses play a crucial role in the well-being of MC twins and have therefore been the subject of great interest by perinatologists in the past decades. Several injection methods have been proposed to demonstrate the presence and types of vascular anastomoses using air, milk, barium and colored dye. In our recent cohorts using colored dye, we found on average 8 to 11 vascular anastomoses per placenta (Chapter 2 and 3) whereas other studies reported a smaller number of anastomoses, on average 1 to 6 vascular anastomoses per placenta.[1-3] This disparity is likely attributable to differences in injection techniques. In particular the use of air or milk may not be appropriate to detect small size anastomoses and to distinguish between the various types of anastomoses. The difference between the three types of vascular anastomoses (arterio-arterial (AA), veno-venous (VV) and arterio-venous (AV) anastomoses) can easily be detected when using color dye injection. Small size anastomoses can only be detected through accurate and thorough injection with colored dye. Since detection of these minuscule anastomoses is one of the crucial diagnostic criteria for twin anemia-polycythemia sequence (TAPS),[4] high quality injection technique is of paramount importance for the diagnosis of TAPS (see section B.2 on TAPS).

2. Abnormal umbilical cord insertion

Another placental characteristic that is typical to MC twins is abnormal placental cord insertion, namely velamentous cord insertion (VCI). The rate of VCI per fetus is significantly higher in MC twins (35%) compared to DC twins (8%).[5] The higher frequency of VCI in MC twins may be associated with the unequal allocation of blastocyst to each twin during the twining process, leading to lower growth potential of placental mass to protect the chorionic vasculature. VCI is associated with adverse perinatal outcome in MC twins, which may be in part due to the smaller corresponding placental share.[6, 7] As a consequence of the higher prevalence of VCI, MC twins also carry an increased risk of vasa previa and concomitant increased risk of rupture of the vessels during delivery. We recently reported two cases of MC twin pregnancies with rupture of vasa previa which led to acute blood loss in one twin followed by acute exsanguination from its co-twin through the vascular anastomoses.[8, 9] Acute double exsanguination led to double fetal demise in one case and double severe perinatal asphyxia in the other case. Antenatal detection of vasa previa in MC twins is thus of paramount importance.

Another abnormal form of cord insertion which appears to be unique to MC twins is the presence of proximate cord insertions (PCIs). Interestingly, PCIs are around 18 times more likely to occur in monoamniotic (MA) placentas (53%) compared to diamniotic twins (3%) (Chapter 4), suggesting a possible association of PCIs with twining process. In addition, we found that arterio-arterial (AA) and veno-venous (VV) anastomoses were more prevalent in MC placentas with PCIs (100% and 56%, respectively) compared to MC placentas without PCIs (80% and 26%, respectively). Our findings support the theory proposed by De Paepe et al. that the interaction between both cord insertions may play a role in the formation of

angio-architecture in MC placentas.[10] A crucial step of fetoscopic laser surgery is the visualization of vascular equator.[11] However, the presence of PCIs results in difficulty in identifying the vascular equator and high risk of residual anastomoses and treatment failure of fetoscopic laser surgery (Chapter 7). Interestingly, in a recent study the authors reported successful performance of fetoscopic laser surgery in TTTS with PCIs except when the two umbilical cords have a joint part near the insertion site.[12] Noticeably, in case of PCIs fetal surgeons may consider not to coagulate the part of vascular equator between the side-by-side cord insertions to avoid damage to quite large anastomoses or umbilical cords. However, the large vascular anastomose may be obscured by the PCIs, leading to failed detection by preoperational sonography. Therefore, we agree with proposal by the authors that evaluation of the feasibility of fetoscopic laser surgery for TTTS with PCIs should be done during fetoscopy, rather than ultrasound examination.

3. Unequal placental share

In most studies, unequal placental share is empirically defined as a placental share difference of 20% or greater, yielding a rate of around 50% in MC twins. The unequal placental share in MC twins is theorized to result from the poor implantation site represented by the presence of VCI and disequilibrated cleavage of inner cell mass at twinning. MC twins with birth weight discordance of ≥20% have a 2 to 5 times likelihood of unequal placental share compared to MC twins with concordant growth, suggesting that unequal placental share is the main contributor to growth discordance, and may lead to selective intrauterine growth restriction (sIUGR).[13] In MC twins, a larger placental share usually leads to a larger birth weight, while a smaller placental share results in a smaller birth weight. However, the placental share usually does not well correspond to the birth

weight share, i.e. birth weight share/placental share ratio is significantly higher in the smaller twin compared to the larger twin in sIUGR.[14, 15] This may be explained by the more shared circulation as a result of more efficient network of vascular anastomoses with larger anastomotic size, more superficial anastomoses and shorter distance between cord insertions.[14, 16]

B. Placental characteristics in relation to specific complications: twin-twin transfusion syndrome and twin anemia-polycythemia sequence

Vascular anastomoses are the anatomical prerequisite for the net intertwin transfusion enabling the development of twin-twin transfusion syndrome (TTTS) or TAPS. Although the placental angioarchitecture in TTTS and TAPS placentas is different from uncomplicated MC twins (Chapter 5 and 6), the role of placental angioarchitecture in the pathophysiology of TTTS and TAPS is not fully understood yet. One recent hypothesis is that all chronic intertwin transfusion first results in intertwin hemoglobin discordance leading to intertwin osmotic gradient.[17] Subsequently, extraction of fluid from the donor to the recipient may ensue when large anastomoses are present (TTTS placenta) or may be limited when only minuscule anastomoses are present (TAPS placenta). Another theory is that TTTS results from fetal circulatory imbalance and its secondary effect of vasoactive hormones (renin-angiotensin system and endothelin-1), leading to large differences in amniotic fluid production and the development of oligo-polyhydramnion sequence.[18] In contrast, TAPS results mainly from slow chronic intertwin blood transfusion through the minuscule vascular anastomoses leading to differences in Hb levels without discordant amniotic fluid levels.[19]

1. TTTS placentas

Although AV anastomoses carrying unidirectional blood flow are essential for the pathogenesis of TTTS, the number and size of AV anastomoses in TTTS placentas is not different from that in uncomplicated MC placentas.[20] The main difference in the angioarchitecture of TTTS placentas is the lower prevalence of AA anastomoses (around 35%) compared to uncomplicated MC twins placentas (above 90%), suggesting a protective role of AA anastomoses against TTTS.[3, 21] In contrast to AA anastomoses, VV anastomoses occurs more frequently in TTTS placentas (around 35%) than uncomplicated placentas (about 25%).[3] In particular in the absence of AA anastomoses, the presence of VV anastomoses seems to predispose MC twins to the development of TTTS. Unlike the arterial system, the resistance in the venous circulation is low. Inter-twin pressure gradient in the venous circulation is therefore prone to being affected by external impact, such as fetal position. Theoretically, VV anastomoses may then act as AV anastomoses and carry unidirectional blood flow when the inter-twin pressure gradient in venous circulation becomes skewed to one twin. This may, in certain circumstances, lead to the development of TTTS. Given the more recent theory on the pathogenesis of TTTS as discussed above, VV anastomoses with blood flow of low resistance may facilitate the extraction of fluid from donor to recipient when the intertwin Hb difference is present.

The prevalence of VCI is similar between TTTS and uncomplicated MC twins, suggesting that there is no causal relation between development of TTTS and velamentous insertion of the umbilical cord. However, if TTTS occurs, VCI is most often detected in the donor twin.[7] The presence of VCI in TTTS may thus be related to the donor versus recipient status, but not the pathogenesis of TTTS. Velamentous insertion of the cord is associated with the magistral

pattern of chronic vasculature.[10] The few and lager chorionic arteries in the placental territory of the donor twin may tend to be affected by the heart beat and have a higher blood pressure, facilitating the blood loss from the donor to the recipient.

2. TAPS placentas

The typical angioarchitecture of TAPS placentas demonstrates few and miniscule anastomoses (diameter < 1mm), on average 4 in spontaneous TAPS placentas and 2 in post-laser TAPS placentas.[22] AA anastomoses occur strikingly less frequently in TAPS placentas (<20%) compared to uncomplicated MC placentas (above 90%),[23] allowing the accumulation of Hb difference without adequate compensation. Interestingly, most anastomoses in post-laser TAPS placentas are localized near the edge of placenta. These small anastomoses on the margin of the placenta are probably more difficult to detect and can be missed during fetoscopic laser surgery.

Another feature of TAPS placentas is the larger ratio between cord insertion distance and placental diameter compared to uncomplicated MC placentas (69% vs 63%, respectively, *P*<.05).[24] This may result in the diminution of vascular caliber along the vascular equator and smaller vascular anastomoses.

The placental share discordance in TAPS placentas is comparable to uncomplicated MC placentas.[25] However, unlike uncomplicated MC twins, the smaller twin in TAPS who is often the anemic twin usually has a larger placental share, suggesting that fetal growth in TAPS is determined primarily by the net inter-twin blood transfusion instead of placental share. Importantly, the larger placental share found in donor twins could be a result of selection bias in our study due to the inclusion of only TAPS cases with two live-born infants. We speculate that in TAPS donor twins with a significantly smaller placental share may be at

increased risk of fetal demise due to the accumulation of potential risks (chronic blood loss and smaller placental share).

C. Placental complications and associated neonatal outcomes after fetoscopic laser surgery for TTTS: histologic chorioamnionitis, funisitis and early–onset neonatal sepsis

An important aspect that had not been evaluated in detail is related to the impact of intrauterine inflammation after laser surgery for TTTS. Recent studies have shown that intrauterine inflammation are related to adverse fetal and neonatal outcomes and associated with adverse long-term neurodevelopmental outcome.[26-28]

1. **Chorioamnionitis and funisitis**

In our case-control study with histologic evaluation of placenta and umbilical cord (Chapter 8), we found an higher risk of intrauterine inflammation (13% of histologic chorioamnionitis (CA) and 8% of funisitis) in TTTS cases treated with fetoscopic laser surgery compared to previous reports based on clinical CA (0-4%).[29-39]This disparity may be attributable in part to the different definitions of intrauterine inflammation. Noticeably, around half of histologic CA occurs without clinical signs and symptoms including maternal fever, the essential criterion to diagnose clinical CA. Given that funisitis is associated with higher risk of adverse short and long-term outcome than histological CA only,[27] the more important finding in our study is the increased risk of funisitis after fetoscopic laser surgery, suggesting the necessity of postpartum histologic evaluation of the placenta and umbilical cord in TTTS cases treated with laser surgery. Although both the operational and perinatal variables were recorded and analyzed, this study however failed in identifying the risk factors for the

occurrence of histological CA after fetoscopic laser surgery. Nevertheless, our study was primarily designed and powered to display a difference in the risk of histologic CA between the study group of TTTS managed with fetoscopic laser surgery and a control group of mostly uncomplicated MC twins without fetoscopic laser surgery. Analysis of risk factor for histologic CA after fetoscopic laser surgery requires a larger study.

2. Early-onset neonatal sepsis

The clinical implication of histologic CA is the direct association with early-onset neonatal sepsis (EOS). EOS is one of the leading causes of neonatal morbidity and mortality. In our large prospective cohort study (Chapter 9), we found that the incidence of EOS after fetoscopic laser surgery was 16% (2% proven EOS and 14% suspected EOS). The rate of EOS in our cohort is substantially higher compared to the general population of MC twins reported by Lewis et al (4%).[40] This higher incidence of EOS found in our TTTS cohort may be explained by the TTTS disorder itself and the invasive nature of fetoscopic laser surgery leading to increased risk of prematurity and preterm previable rupture of membranes (PPROM). Although fetoscopic laser surgery significantly improves the clinical outcome in TTTS, perinatologists should be aware of the potential risk of intrauterine inflammation and EOS. Future studies and developments focusing on improvement of laser technique and instruments may reduce the risk of (iatrogenic) PPROM, intrauterine inflammation (infection) and EOS.

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Future Perspectives

Although postnatal examination of monochorionic (MC) placentas using color dye injection have shed more light on the pathophysiology of various disorders in MC twins, the examination is usually performed by visual inspection, and is time-consuming and prone to interobserver variation. In addition, the *in vitro* nature of postnatal examination of MC placentas limits the extrapolation of the findings into evaluation of *in vivo* scenarios.

This chapter focuses on various proposals for research directions to further improve our understanding of the pathophysiology of disorders in MC twins. Improved knowledge on the various aspects and characteristics of MC placentas may also help optimize the prenatal management and intrauterine therapy.

A. Monochorionic placenta: analysis and characteristics

1. Development of computerized methods for placental analysis

To minimize interobserver variation and diminish the time used for placental analysis, a dedicated software program may be useful to perform various measurements of placental morphology. For example, the distribution of chorionic vasculature is categorized grossly as disperse, magistral or mixed by visual inspection and is related to the donor and recipient status in TTTS.[1] In singletons, chorionic vasculature patterns are associated with placental insufficiency and fetal growth.[2] **Computerized analysis** would be useful to define the type of chorionic vasculature patterns based on objective parameters such as density of chorionic vessels and number of vascular branches, enabling subsequent investigations on the clinical consequences of chorionic vasculature patterns and differences between donor and recipient placental angioarchitecture.

2. Prenatal evaluation of placental angioarchitecture

Perinatal risk of MC twins is strongly related to the placental anomalies such as vascular anastomoses, velamentous cord insertion (VCI) and unequal placental share.[3] As a result, prenatal detection of placental anomalies could be beneficial to the risk evaluation in MC twins. Prenatal ultrasound is already a reliable tool to detect the presence of VCI or other abnormalities of cord insertion.[4] VCI is the suggestive of smaller placental share and is an independent risk factor for growth discordance and fetal demise.[5] In addition, distance between cord insertions can be measured during ultrasound examination, allowing the detection of proximate cord insertions (PCIs). This may enable the investigation of **correlation between PCIs and clinical outcomes, including fetal demise**.

Detection of the different types of anastomoses and evaluation individual placental share with prenatal ultrasound examination is more difficult if not extremely challenging and not yet reliable. Werner et al. recently reported a preliminary method to identify the vascular equator using ultrasound and resonance magnetic imaging (MRI).[6] Individual placental share may be evaluated based on the combination of **identifying vascular equator with contouring the placental edge**. Although arterio-venous (AV) anastomoses and veno-venous (VV) anastomoses cannot yet be detected with prenatal Doppler ultrasound, reliable detection of arterio-arterial (AA) anastomoses during ultrasound examination has been reported.[7] AA anastomoses carry bidirectional blood flow and can compensate for a wide range of net cross-sectional area of AV anastomoses, preventing the development of TTTS and TAPS.[8] A prospective study is needed to evaluate the predictive value of the combination of **prenatal detection of VCI and AA anastomoses** in relation to the perinatal outcome of MC twins.

B. Placental characteristics in relation to specific complications: Twin–twin transfusion syndrome and Twin anemia–polycythemia sequence

1. Twin-twin transfusion syndrome

Since the majority of TTTS cases are now treated with fetoscopic laser ablation of vascular anastomoses, evaluation of the various types of anastomotic patterns on the placenta after delivery is not possible. However, color dye injection to detect residual anastomoses remains a crucial tool for **quality control**, for the evaluation of the **learning curve** of fetal surgeons and serves as an important **teaching tool** for all perinatologists involved in the care of MC twins.

Although the rate of residual anastomoses and associated post-laser TAPS or recurrent TTTS is reduced due to the application of Solomon technique for laser surgery, residual anastomoses are reported to be detected in up to 20% lasered TTTS placentas in the Solomon trial.[9, 10] With increased familiarity and experience with the Solomon technique, , we expect a decrease in the rate of residual anastomoses in the future. Large, preferably multicenter studies are required to evaluate the inter-individual, intra-individual and inter-center differences in prevalence of residual anastomoses after laser surgery using Solomon technique. Importantly, factors such as localization of the placenta (anterior or posterior), TTTS stage at operation and experience level of the fetal surgeons must be taken into account. Evidently, comparisons of the rate of residual anastomoses between the various fetal centers and between operators can only reliably be performed if placental examination is done adequately and accurately using color dye injection. Evaluation of post-laser placentas using only air injection should not be considered as a valid tool for the detection of residual anastomoses (in particular for the detection of minuscule

anastomoses).[11] Similarly, examination of post-laser placentas performed by inexperienced injectors will also lead to a higher rate of false-negative results.

Fetoscopy also provides the possibility to record the placental angioarchitecture *in vivo*. In a study from Eschbach et al., the authors found that the presence of AA anastomoses detected during fetoscopy was associated with the death in donor twin while the absence of AV anastomoses directed from recipient to donor twin was related to death in recipient twin after laser surgery.[12] In addition, the presence of VV anastomoses is related to the development of TTTS.[13-15] However, the **predictive value of VV anastomoses** in relation to clinical outcome after laser surgery is unclear. This may be due to the limited reliability of detection of vascular anastomoses by fetoscopy.

In the majority of TTTS cases managed with fetoscopic laser surgery, functional dichorionicity can be reached,[10] and post-laser fetal growth is therefore mainly dependent on the individual placental territory. However, the current fetoscopy operation instrument does not include a measurement component to objectively quantify the individual placental territory. Quintero et al. recently reported the feasibility of visible light spectroscopy to identify individual anastomoses and differentiate the individual placental territory of recipient and donor before laser ablation based on the measurement of oxygenation.[16] Further studies are required to evaluate the feasibility and accuracy of this method to define the types of vascular anastomoses and individual placental territory, and subsequently to assess their predictive value for clinical outcome after laser surgery.

2. Twin anemia polycythemia sequence

TAPS is a newly-described form of chronic intertwin transfusion occurring in 5% of MC twins.[17, 18] Due to the rarity of this disorder, previous studies on the characteristics of

TAPS placenta were often limited by the small sample size. The correlation between the **pathogenesis of TAPS and placental angioarchitecture** (including types of cord insertion, unequal placental share) remains to be elucidate, ideally, in prospective multicenter studies with large sample size. In addition, prenatal *in vivo* evaluation of placental characteristics may be also useful for the prediction of clinical outcome in TAPS. Given the miniscule nature of vascular anastomoses in typical TAPS placentas, future studies should probably focus on prenatal evaluation of the difference in color of each individual placental territory rather than on the type of anastomoses. Interestingly, ultrasound examination is already useful in detecting a difference in echodensity and thickness between the placental territory of the anemic and polycythemic twin in a part of TAPS.[18] Further investigation is warranted to show the average **difference in echodensity** between the placental territory of the anemic and polycythemic in general population of TAPS and to assess the feasibility of this difference to define individual placental share.

Recently, we proposed the color difference of the maternal side as an additional criterion for the postnatal diagnosis of TAPS and differential diagnosis between TAPS and acute peripartum TTTS.[19] However, the color difference on maternal side was measured in only 19 TAPS placentas without classification of spontaneous TAPS or post-laser TAPS. The applicability of **color difference on maternal side** to diagnose TAPS should be assessed in larger studies and in studies including cases with acute peripartum TTTS.

3. Selective intrauterine growth restriction

Selective intrauterine growth restriction (sIUGR) is a severe disorder occurring in 10% of MC twins and results from unequal placental sharing.[20, 21] In the majority of previous studies, unequal placental share is empirically defined as a placental share discordance of ≥ 20%.

However, a scientific definition of unequal placental share has not yet been reported and is urgently needed. The optimal method to determine the threshold of unequal placental share that significantly increase adversity of perinatal outcome is to use placental share difference as continuous variable and asses its correlation with outcome parameters.

In addition to unequal placental sharing, sIUGR placentas are also characterized by an increased incidence of VCI and large AA anastomoses.[22, 23] Prenatal detection of the combination of these three factors may help distinguish sIUGR twins from other MC twins and **classify the various types of sIUGR**, in relation to the Gratacos staging.[24]

C. Fetal inflammatory response syndrome after fetal interventions

With the improvement of survival rate after fetoscopic laser surgery for TTTS, attention is shifting towards survival without long-term morbidity. Unfortunately, post-laser complications such as preterm previable rupture of membranes (PPROM) and intrauterine inflammation, remain a challenge in management of TTTS.[10, 25, 26] Both PPROM and intrauterine inflammation (including clinical or histological chorioamnionitis) are associated with premature delivery and adverse short- and long-term outcome.[27, 28] Recent studies show that the risk of adverse outcome is higher in chorioamnionitis with fetal involvement, termed as fetal inflammatory response syndrome (FIRS).[29] Although funisitis is the histologic indication of FIRS, an elevated concentration of interleukin-6 (IL-6, >11 pg/mL) in fetal plasma is the crucial diagnostic criterion for FIRS.[29] The invasive nature of fetal interventions for TTTS or feticide predisposes the fetus to intrauterine inflection and secondary inflammatory response. In addition, iatrogenic necrosis after fetal interventions may result in ‘sterile inflammation’ without the presence of microbe in amniotic sac.[30] Importantly, postmortem involution due to fetal demise after fetal interventions may

prevent the diagnosis of intrauterine inflammation based on the histologic evaluation of placenta. The **measurement of IL-6 in fetal plasma** enables the detection of severe intrauterine inflammation in all liveborns. Interestingly, Kunze et al. recently reported the feasibility of measurement of IL-6 in amniotic fluid collected noninvasively after PPROM.[31] Since PPROM occurs in around 30% after fetoscopic laser surgery or 20% after feticide,[10, 25, 32] this method may be promising for **prenatal detection of FIRS after fetal intervention**.

In conclusion, advances in prenatal ultrasound examination and invasive fetal therapy not only provide an opportunity to improve perinatal survival rate in complicated MC twins, but also to understand the pathophysiology of the various fetal disorders and to build prediction models based on *in vivo* variables obtained prenatally.

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PART V: Appendices

Publications

1. Spruijt MS, Tameeris E, **Zhao DP**, Middeldorp JM, Haak MC, Oepkes D, Lopriore E. Incidence and causes of intentional fetal or neonatal demise in twin-twin transfusion syndrome. *Submitted.*
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Curriculum Vitae

De-Peng Zhao was born on 20th August, 1984 in Zan Huang county, China. He graduated from Shijiazhuang Sili No.1 Middle School in 2004. After his graduation, he was admitted to Jilin Medical University and studied medicine there from 2004 to 2009. After obtaining his bachelor degree, he continued his medical studies at the Shanghai First Maternity and Infant Hospital, Tongji University from 2009 to 2012. In 2012, he contacted Prof. Dr. Enrico Lopriore to apply for a PhD position in Leiden. Afterwards, He submitted the admission letter from Prof. Enrico Lopriore to the China Scholarship council and was awarded a 4-year scholarship for his PhD training in Leiden. Here, Prof. Enrico Lopriore and Prof. Dick Oepkes led him into the field of fetal medicine. His PhD project is mainly focused on the characteristics and complications of monochorionic placentas, successfully yielding this thesis.

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List of Abbreviations

AA	arterioarterial
AV	arteriovenous
CA	chorioamnionitis
CI	confidence interval
DC	Dichorionic
EONS	early-onset neonatal sepsis
FIRS	fetal inflammatory response syndrome
FLS	fetoscopic laser surgery
IL-6	interleukin-6
IQR	interquartile range
MC	Monochorionic
OR	odds ratios
PPROM	preterm previable rupture of membranes
PCI	proximate cord insertions
sIUGR	selective intrauterine growth discordance
TAPS	Twin anemia-polycythemia sequence
TTTS	Twin–twin transfusion syndrome
VCI	velamentous cord insertion
VV	Venovenous