Placenta xxx (2015) 1–4

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

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

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ARTICLE INFO

Article history:
Accepted 19 May 2015

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

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.

1. 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

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http://dx.doi.org/10.1016/j.placenta.2015.05.014
0143-4004/

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.

2. 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 [7,8]. TTTS was diagnosed based on the Eurofetus criteria: polyhydramnios (deepest vertical pocket $\geq$ 8 cm before 20 weeks of gestation or $\geq$ 10 cm after 20 weeks of gestation) in the recipient and oligohydramnios (deepest vertical pocket $\leq$ 2 cm) in the donor [12]. 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 [13–15]. 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 $\geq$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.

2.1. Statistics

A few studies reported the prevalence of VV anastomoses in TTTS placentas and/or non-TTTS placentas [4,6,8–11,16–19]. 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 .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).

3. 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: 14.0–36.6 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.

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 Fig. 1.

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%).

4. 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 [4,6,9–11,20]. 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 [20]. 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 [20]. In another three small studies the reported prevalence of VV

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).

Please cite this article in press as: Zhao DP, et al., Veno–venous anastomoses in twin–twin transfusion syndrome: A multicenter study, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.05.014

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 (%)[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)	(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	31.30 (7.12–137.48)

[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 cases.

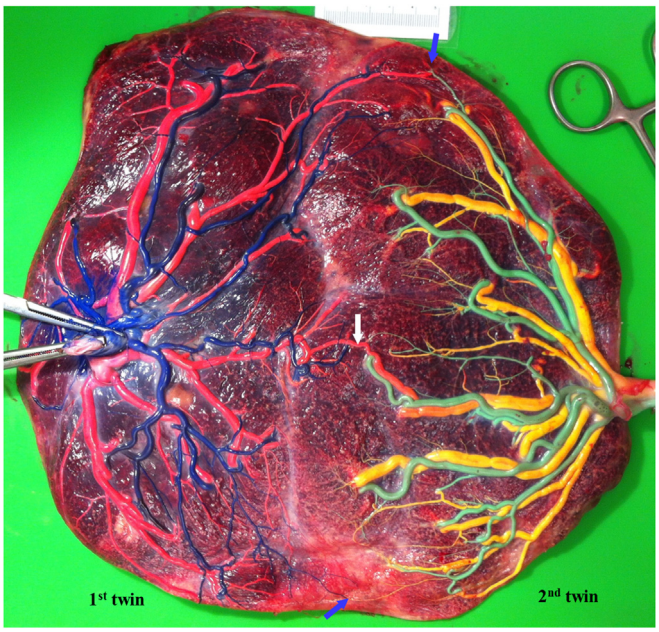

Fig. 1. A TTTS (Quintero stage 3) monochorionic placenta managed with amnio-drainage and delivered at 34 + 3 weeks' gestation. The 1st twin is the ex-donor. After delivery, injection with colored dye (blue or green for arteries and pink or yellow for veins) was given to demonstrate the vascular anastomoses.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

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 thus be 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.

Conflict of interest

We declare that we have no conflict of interest.

Acknowledgment

Luming Sun is the recipient of a grant of Shanghai Committee of Science and Technology grant No. 134119a4400. Tao Duan is the recipient of a grant of National Natural Science Foundation of China (contract grant number: 81270759).

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