Comparison of the ratio of placental territory discordance to birthweight discordance in placentas of monochorionic diamniotic twins

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Abstract
The ratio of placental territory discordance to birthweight discordance was calculated in monochorionic diamniotic twin placentas. We then determined whether the ratio: (1) correlated with the overall diameter of the placental superficial vascular anastomoses and (2) was different in normal monochorionic diamniotic twin (normal MCDA), twin-to-twin transfusion syndrome (TTTS), and selective intrauterine growth restriction (sIUGR) pregnancies. A prospective study of MCDA placentas was conducted from April 1, 2016, to April 1, 2019, including three groups, normal MCDA (N = 49), TTTS (N = 38), and sIUGR (N = 52). Placentas were studied via dye injection. In the normal MCDA, sIUGR, and TTTS groups, the ratio positively correlated with the overall diameter of the placental superficial vascular anastomoses (Spearman’s = 0.295, 0.619, and 0.530; p = 0.040, < 0.001, and 0.001, respectively) and gradually decreased from normal MCDA to sIUGR and to TTTS (2.88 (0.34, 64.25), 1.70 (0.27, 4.52), and 1.03 (0.12, 15.17); p < 0.001, p = 0.016/0.001/0.005, respectively). The placental territory discordance to birthweight discordance ratio is an effective index reflecting the overall vascular anastomoses in MCDA placentas.

Keywords
Vascular anastomoses, monochorionic twins, placenta, twin to twin transfusion syndrome, selective intrauterine growth restriction
**Introduction**

Twin to twin transfusion syndrome (TTTS) and selective intrauterine growth restriction (sIUGR) are two common complications of monochorionic diamniotic (MCDA) twin pregnancies. Because of the specific placental structure, the two fetuses share different portions of the same placenta and connect with each other through placental superficial vascular anastomoses. Previous studies have shown that the birthweights are determined mainly by the placental territory of each fetus in MCDA twins, although the blood flow exchange between the fetuses through the superficial vascular anastomoses also affects birthweight. Thus, placental territory discordance and birthweight discordance can be combined to assess the influence of the placental superficial vascular anastomoses on birthweight in MCDA twins. The placental territory and birthweight discordance ratios have been used in studies of different varieties of complicated MCDA twin pregnancies. The placental territory discordance ratio is calculated as follows: (larger placental portion – smaller placental portion)/larger placental portion. The birthweight discordance ratio is calculated as follows: (larger twin weight – smaller twin weight)/larger twin weight.

In this study, we calculated the ratio of the placental territory discordance ratio to the birthweight discordance ratio in MCDA twin pregnancies and investigated whether the ratio was correlated with the overall diameter of the placental superficial vascular anastomoses. Furthermore, we compared the ratio of each MCDA twin pregnancy among normal MCDA, sIUGR, and TTTS.

**Methods**

The Fetal Medicine Center in the Department of Obstetrics and Gynecology at Peking University Third Hospital is an emerging referral center in China for the management of complicated MCDA twin pregnancies. Each monochorionic placenta was prenatally diagnosed via ultrasound and pathologically verified after delivery. All monochorionic placentas in our center were evaluated via placental dye injection. This is a prospective case-control study and it was approved by the ethics committee of Peking University Third Hospital. We included all MCDA placentas after the birth of twins at our center from April 1, 2016, to April 1, 2019. Placentas with incomplete dye injection, TTTS post-laser surgery, a fetal anomaly of either twin, and placentas with only twin anemia polythemia sequence (TAPS) were excluded. The surgical indication for TTTS in our center includes TTTS stage II-IV and parts of stage I in accordance with patients’ requirements. We divided the placentas into three groups: normal MCDA, sIUGR, and TTTS.

Maternal and infant characteristics corresponding to the MCDA placentas were also recorded. The birthweight discordance ratio and placental territory discordance ratio are defined above.

Normal MCDA twin pregnancy is defined as uncomplicated monochorionic diamniotic twin pregnancy without fetal anomaly. TTTS is diagnosed based on the combined presence of a maximum vertical pocket (MVP) of ≥8 cm in one sac and
≤2 cm in the other, regardless of the gestational age at diagnosis.5 sIUGR is diagnosed when the ultrasound-estimated weight of either fetus is less than the 10th percentile of the corresponding gestational week.6 TAPS is diagnosed antenatally on the basis of discordant measurements of the middle cerebral artery peak systolic velocity (MCA-PSV; >1.5 multiples of the median [MoM] in donors and <1.0 in recipients), or postnatally based on the large inter-twin hemoglobin (Hb) difference (>8 g/dL) and with at least one of the following parameters: reticulocyte count ratio >1.7 or minuscule placental anastomoses as identified by dye injection.7 In our center, we treat TTTS with fetoscopic laser coagulation of placental vascular anastomoses at 18–26 gestational weeks, the surgical indication for TTTS in our center included TTTS stage II-IV and parts of stage I in accordance with patients’ requirements. Some patients beyond 26 weeks may choose amnioreduction and timely termination of the pregnancy after fetal lung maturation promotion with dexamethasone. sIUGR type I only requires strict observation with biweekly ultrasound, while the treatment of type II and III includes fetal reduction and conservative treatment according to the wishes of the family.

Placenta dye injection was performed as previously described,8 which is also the same as previously published reports from our center.9 Briefly, after delivery, the amniotic membranes were removed, and each umbilical cord was cut 5 cm from its placental insertion site. The placental vessels were gently squeezed to clear out the blood clots. The umbilical vein and one of the umbilical arteries were then cannulated and clamped with an intravenous catheter. The placental vessels were injected with saline until all the branches were visible. The last step was performed using four 20 mL syringes, each filled with a distinctively colored dye (white, green, yellow, and red) to visualize the umbilical cord arteries and veins of the two fetuses. Pictures of the injected placentas placed vertically under a grid harboring a scale were taken with a high-resolution digital camera (Figure 1).
Placental pictures were analyzed using ImageJ, version 1.51j8 for Windows (National Institutes of Health, Bethesda, MD, USA). Superficial artery-to-artery (AA) and vein-to-vein (VV) anastomoses were identified as direct external communications between two homonymous umbilical vessels. For both AA and VV type anastomoses, the caliber was measured as the minimum external diameter along the course of the anastomosis. Deep artery-to-vein (AV) anastomoses were identified where an unpaired artery from one twin was seen penetrating the chorionic plate close to $<1.0\,\text{mm}$ from an unpaired vein from the other twin. The diameter of an AV anastomosis was defined as the diameter of the feeding artery at its narrowest point. The overall diameter refers to the sum of the diameters of all anastomoses in the placenta. Additionally, the placental territory of each fetus was defined by the margins of a specific dye location. The placental territory was measured on photographs using ImageJ software. Individual placental territories were expressed as a percentage of the total area. The vascular equator was first defined according to the locations of all anastomoses, which served as the boundary of the placental territory of the two fetuses. Then, each twin’s placental territory was delineated and measured using the freehand selection feature of the software (Figure 1).

We initially performed Spearman’s correlation analysis between the ratio and the overall diameter of the placental superficial vascular anastomoses in each group and then compared the ratios among the three groups.

**Statistical analysis**

SPSS statistics software, version 24.0 (IBM Corp., Armonk, NY, USA) was used for statistical processing. The measurement data were expressed as mean $\pm$ standard deviation or median (maximum value, minimum value). We first determined whether the measurement data were distributed normally. If both groups had a normal distribution, the data were analyzed using an independent-sample $t$-test and were expressed as mean $\pm$ standard deviation. In the case of non-normally distributed data, the nonparametric Mann-Whitney $U$ test was used, and the data were expressed as medians and ranges. Spearman’s method was used for correlation analysis. The Bonferroni correction was used for multiple comparisons, and their $p$-values were estimated. If the $p$-value was $<0.05$, the difference was considered to be statistically significant. The three groups were compared pairwise, and if the $p$-value after the correction was $<0.0167$ (0.05/3), the difference was considered to be statistically significant.

**Results**

From April 1, 2016, to April 1, 2019, a total of 191 monochorionic twin pregnancies were delivered at our center. Following delivery, we recorded the birthweights of each baby. The placentas were obtained with informed consent from the patients after the approval of the ethics committee of the hospital. Eight placentas with
incomplete dye injections, 17 placentas with laser coagulation, 19 placentas with fetal abnormalities, and 8 placentas with TAPS were excluded from the study. The remaining 139 placentas with complete dye injections were divided into three groups: 49 normal MCDA, 52 sIUGR, and 38 TTTS. The TTTS group included 15 stage I cases, 5 stage II, 6 stage III, 9 stage IV, and 3 stage V; the sIUGR group included 18 type I cases, 23 type II, and 11 type III.

The comparisons of maternal and infant characteristics among the three groups are presented in Table 1. The three groups were similar regarding maternal age, utilization of assisted reproductive technology (ART), early fetal reduction, incidence of pregnancy-induced hypertension, and gestational diabetes mellitus. The gestational age at delivery of the TTTS group was lower than that of the normal MCDA.

|                                | Normal MCDA (n = 49) | sIUGR (n = 52) | TTTS (n = 38) | p-Value |
|--------------------------------|----------------------|----------------|---------------|---------|
| Maternal age (years)           | 29 (23, 40)          | 30 (23, 38)    | 30 (22, 37)   | 0.108   |
| ART—n/N (%)                    | 2/49 (4.1)           | 2/52 (3.8)     | 1/38 (2.6)    | 1.000   |
| Early fetal reduction—n/N (%)  | 1/49 (2.0)           | 1/52 (0)       | 1/38 (2.6)    | 0.528   |
| Pregnancy-induced hypertension—n/N (%) | 4/49 (8.1) | 5/52 (9.6)      | 3/38 (7.9)    | 1.000   |
| Gestational diabetes mellitus—n/N (%) | 5/49 (10.2) | 5/52 (9.6)      | 3/38 (7.9)    | 1.000   |
| Gestational age at diagnosis (weeks) | –                  | 25.9 (17.4, 32.7) | 22.0 (16.9, 33.6) | 0.007   |
| Gestational age at delivery (weeks) | 36.1 (19.4, 38.6) | 33.3 (24.1, 38.9) | 29.7 (17.6, 36.0) | <0.001* |
| Fetal birthweight (g)          | 2440 (720, 3210)    | 1700 (610, 3110) | 1170 (630, 3000) | <0.001/ <0.001/ <0.001* |
| Fetal birthweight discordance ratio | 0.10 ± 0.08         | 0.34 ± 0.14     | 0.33 ± 0.19   | <0.001  |
| Placental territory discordance ratio | 0.25 (0.05, 0.75) | 0.59 (0.10, 0.80) | 0.33 (0.05, 0.86) | <0.001  |
| Overall diameters of the placental superficial vascular anastomoses (mm) | 6.7 (0.6, 20.1) | 5.2 (0.8, 24.6) | 7.3 (0.5, 22.4) | 0.050   |
| The ratio of placental territory discordance ratio to fetal birthweight discordance ratio | 2.88 (0.34, 64.25) | 1.70 (0.27, 4.52) | 1.03 (0.12, 15.17) | <0.001* |

*p-value was compared pairwise between normal MCDA with sIUGR, normal MCDA with TTTS and sIUGR with TTTS.
and sIUGR groups. The birthweight decreased from normal MCDA to sIUGR to TTTS group. The birthweight discordance ratio in normal MCDA was lower than that in the sIUGR and TTTS groups. The placental territory discordance ratio of the sIUGR group was higher than those of the normal MCDA and TTTS groups.

In the normal MCDA, sIUGR, and TTTS groups, the ratio was positively correlated with the overall diameter of the placental superficial vascular anastomoses ($r = 0.295, 0.619, \text{ and } 0.530; p = 0.040, <0.001, \text{ and } 0.001$, respectively) (Figure 2). The ratio gradually decreased from normal in the MCDA group to below normal in the sIUGR and TTTS groups.

**Discussion**

In this study, we found that the ratio of the placental territory discordance ratio to birthweight discordance ratio positively correlated with the overall diameters of the placental superficial vascular anastomoses for each of the MCDA, sIUGR, and TTTS groups. Moreover, the ratio gradually decreased from the normal MCDA group to the sIUGR group and to the TTTS group. This result suggests the presence of different compensatory mechanisms in the placental superficial vascular anastomoses among normal MCDA, sIUGR, and TTTS pregnancies.

Placental territory and superficial vascular anastomosis are two major factors of the MCDA twin placenta that influence the prognosis of the monochorionic diamniotic twin pregnancy. Lewi et al.\(^2\) found that, in unequally shared placentas, the two fetal circulations were more tightly linked than in equally shared placentas, which could reduce the birthweight discordance for a given placental territory discordance. Therefore, the current view is that birthweight is determined by the allocated placental share while vascular anastomoses can compensate for the difference in birthweight to some extent in MCDA pregnancies.\(^2,10\) Thus, it is possible that the newborn birthweight could reflect the interaction of placental territory and vascular anastomoses in the placenta of MCDA twins. Therefore, theoretically, it is possible to evaluate the influence of vascular anastomoses in MCDA twin pregnancies by determining the placental territory and newborn weight.

Newborn birthweight can be used as firstly, an indicator of pregnancy outcome and secondly, as a prognosis of complicated twin complications. Based on the aforementioned roles of the placental territory and vascular anastomoses, we calculated the ratio of the placental territory discordance ratio to the birthweight discordance ratio. The positive correlation between the ratio and the overall diameter of the vascular anastomoses in the three groups suggested that the ratio could reflect the influence of vascular anastomoses in MCDA twin pregnancies.

Interestingly, by further comparing the ratios among the three groups, we found that it gradually decreased from normal MCDA to sIUGR and then to TTTS, which indicates that the influence of the vascular anastomoses on MCDA twins is different. Our result is consistent with Zhao et al.\(^11\) study that found that the prevalence, size, number, and localization of the various types of anastomoses differed among normal MCDA, sIUGR, and TTTS placentas, concluding that the superficial vascular anastomoses affect normal MCDA, sIUGR, and TTTS differently.
Figure 2. Correlation between the ratio and the overall diameter of the anastomoses in the three groups: (a) the correlation in normal MCDA group, (b) the correlation in sIUGR group, (c) the correlation in TTTS group.
Furthermore, the ratios in sIUGR and TTTS were lower than that of the normal MCDA. Our findings appear consistent with Yinon’s study, where a longitudinal cohort study of pregnant women with normal MCDA twins analyzing the maternal plasma levels of soluble vascular endothelial growth factor receptor-1 (EGF1), placental growth factor (PGF), and soluble glycoprotein endoglin found that TTTS and sIUGR were characterized by decreased angiogenic activity which may affect the vascular anastomosis formation. The ratios obtained in our study appear to be equivalent to the angiogenic activity in Yinon’s study, with both decreasing from normal MCDA to sIUGR and TTTS.

The limitations of this study include the overlap of part of the sIUGR and TTTS groups which may introduce bias in the comparison between the two groups; however, even though the final TTTS and sIUGR diagnoses were made before delivery, significant differences still existed in the post hoc comparison of the two groups. The lower gestational age of the TTTS placentas may have influenced their overall vascular diameter and the absence of data on the directions of the AV anastomoses may also have influenced the evaluation of the effects of vascular anastomoses on birthweight.

Conclusion

In conclusion, despite certain limitations, the current placental morphological study suggested that the ratio of placental territory discordance to birth weight discordance might represent the regulatory weight of placental superficial anastomoses in the birthweights of twin babies. This ratio could be used as an index reflecting the overall vascular anastomoses in MCDA placentas. In future studies, we hope to develop an index that can reflect the vascular anastomoses of the placenta in the first trimester using CRL and placental area measurement to facilitate early pregnancy predictions.

Author contributions

WXJ conceived the study concept and wrote the manuscript. LLY, SHF, and YPB collected the data and contributed to data analysis. ZYY contributed to data acquisition. WY contributed to manuscript revision.

Declaration of conflicting interests

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