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**Title:** Placental characteristics and complications in monochorionic twin pregnancies
**Issue Date:** 2016-11-08
Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas

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Placenta. 2013;34(7):589-93
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,
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>
<thead>
<tr>
<th>Table 1 Baseline characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal MC (n=126)</td>
</tr>
<tr>
<td>GA at birth-wks</td>
</tr>
<tr>
<td>BW - g</td>
</tr>
<tr>
<td>BWD - %*</td>
</tr>
<tr>
<td>Cesarean - n (%)</td>
</tr>
</tbody>
</table>

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. \* 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

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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).
<table>
<thead>
<tr>
<th></th>
<th>Normal MC (n=126)</th>
<th>sIUGR (n=46)</th>
<th>TTTS (n=47)</th>
<th>TAPS (n=16)</th>
<th>P₁ Value</th>
<th>P₂ Value</th>
<th>P₃ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall no. of anastomoses*</td>
<td>8 (4-12)</td>
<td>8 (5-14)</td>
<td>7 (5-11)</td>
<td>4 (3-5)</td>
<td>0.67</td>
<td>0.88</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Overall diameter of all</td>
<td>0.5 (0.3-0.9)</td>
<td>0.6 (0.4-1.2)</td>
<td>0.4 (0.3-0.6)</td>
<td>0.1 (0.1-0.2)</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>anastomoses – mm*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placentas with AV anastomoses -</td>
<td>124 (98)</td>
<td>46 (100)</td>
<td>45 (96)</td>
<td>16 (100)</td>
<td>1.00</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of AV anastomoses per</td>
<td>7 (3-10)</td>
<td>6 (4-13)</td>
<td>6 (4-10)</td>
<td>4 (2-5)</td>
<td>0.74</td>
<td>0.84</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>placenta*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV diameter – mm*</td>
<td>0.4 (0.3-0.7)</td>
<td>0.5 (0.4-0.9)</td>
<td>0.4 (0.3-0.6)</td>
<td>0.1 (0.1-0.2)</td>
<td>&lt;0.01</td>
<td>0.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Placentas with AA anastomoses -</td>
<td>121 (96)</td>
<td>46 (100)</td>
<td>22 (47)</td>
<td>3 (19)</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA diameter – mm*</td>
<td>1.7 (1.0-2.5)</td>
<td>2.2 (1.5-3.1)</td>
<td>0.6 (0.4-1.2)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.04</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Placentas with VV anastomoses -</td>
<td>35 (28)</td>
<td>13 (28)</td>
<td>15 (32)</td>
<td>0 (0)</td>
<td>1.00</td>
<td>0.58</td>
<td>—</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VV diameter – mm*</td>
<td>2.8 (2.0-4.7)</td>
<td>2.4 (1.6-4.5)</td>
<td>1.1 (0.3-3.5)</td>
<td>—</td>
<td>0.85</td>
<td>0.08</td>
<td>—</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Placenta Type</th>
<th>AV Localization (%)</th>
<th>AA Localization (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal MC</td>
<td>AV - %</td>
<td>21, 24, 19, 21, 15</td>
<td>.70</td>
</tr>
<tr>
<td></td>
<td>AA - %</td>
<td>16, 20, 24, 24, 16</td>
<td>.53</td>
</tr>
<tr>
<td>sIUGR</td>
<td>AV - %</td>
<td>20, 21, 19, 21, 19</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>AA - %</td>
<td>6, 21, 28, 30, 15</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>TTTS</td>
<td>AV - %</td>
<td>23, 23, 18, 22, 14</td>
<td>.54</td>
</tr>
<tr>
<td></td>
<td>AA - %</td>
<td>9, 3, 18, 36, 33</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>TAPS</td>
<td>AV - %</td>
<td>39, 26, 12, 14, 9</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>AA - %</td>
<td>67, 0, 0, 33, 0</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

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%).

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.

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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.

References