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Color-dye injection of monochorionic placentas and correlation with pregnancy complications

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ABSTRACT

Introduction: Vascular anastomoses in monochorionic (MC) twin placenta can be easily identified with color-dye injection. The aim of this study is to analyze the relationship between different type of anastomoses and twin pregnancy complications.
Methods: From January 2011 to October 2014, MC placentas were analyzed with color-dye injection and five group of pregnancies were identified: those that were not complicated (NC), those complicated with selective intrauterine growth restriction (sIUGR), twin–twin transfusion syndrome (TTTS), or twin anemia-polycitemia sequence (TAPS) and those with amniotic fluid discordance (AFD) between twins. Cases of TTTS treated with endoscopic laser coagulation of placenta anastomoses or cases with in utero death of one twin were excluded.
Results: A total of 118 MC placentas were observed, 58 (49%) NC, 35 (30%) sIUGR, 10 (8%) TTTS, 13 (11%) AFD and 2 (2%) TAPS. The median number of anastomoses was 7 (range 1–15), 8 (2–18), 4 (2–11), 7 (2–13) and 1 (1–1), respectively. At least one artero-venous anastomoses was found in the placenta observed, while the prevalence of artero-arterial anastomoses was 95% for NC, 91% for sIUGR, 60% for TTTS, and 77% for AFD; no TAPS placenta had this type of anastomoses. The diameter of arteroarterial anastomoses was greater in the AFD group (3.3 mm), compared to the NC, sIUGR and TTTS groups (2.3, 2.5 and 1.4 respectively, p 0.04).
Discussion: In this large serie of MC placenta analyzed with color-dye injection, a specific distribution of anastomoses emerged for twins with amniotic fluid discordance, which points to a need for intensive surveillance.

1. Introduction

Vascular anastomoses between the circulation of both twins are a characteristic of all monochorionic (MC) placentas [1] and contribute to the complications of these pregnancies, which include twin–twin transfusion syndrome (TTTS), selective intrauterine growth restriction (sIUGR), twin anemia-polycytemia sequence (TAPS). Three types of anastomoses have been described: superficial ones, artero-arterial anastomoses (AAA) and veno-venous anastomoses (VVA), which mediate a bidirectional blood flow, and those that go deep in the cotiledon, artero-venous anastomoses (AVA), which allow unidirectional flow [2].

Color-dye injection has been described as a feasible way for fetal surgeons to analyze MC placentas [3], in order to correlate anatomo-pathological findings with the development of twin complications [4].

Although the sonographic evidence of moderate amniotic fluid discordance between twins has been identified as a risk factor for the development of acute TTTS [5], this finding has not been considered a twin complication, and as a consequence, not much has been described specifically regarding these twin placentas.

The aim of this study was to use color-dye injection to analyze a series of MC placentas with twin complications (including amniotic fluid discordance), and compare them to MC placentas without complications.

2. Methods

All MC placentas examined between January 2011 to October 2014 at Fetal Therapy Unit of V. Buzzi Children's Hospital, Milan,

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were collected. Each placenta was macroscopically evaluated and analysis of amniotic membrane layer was performed to validate chorionicity. Placentas damaged after delivery or those with formalin contamination were excluded from the study. The remaining ones were injected with color dye and subsequently photographed. Color-dye injection was performed according to the protocol previously described by other centers [3]. For each placenta, the amniotic membranes were removed, the cords were cut at five centimeters from their placenta insertion, and the blood clots were pushed out with gentle pressure on the vessels. The umbilical vein and one umbilical artery were incannulated using a 2-mm catheter, and the cords were clamped to prevent accidental removal of the catheters. Four 20 mL syringes, each filled with a different color (blue, green, yellow and red), were used to inject the vessels until the entire ramification of the vascular territories was evident. After the procedure, a picture of the treated placenta along with a measuring tape was taken with a high-resolution digital camera (Fig. 1). In accordance with the aim of the study, cases treated with laser coagulation of the anastomotic vessels or with bipolar cord coagulation of one twin were excluded, as were those complicated with in utero fetal death of one twin or with twin reversed arterial perfusion (TRAP) sequence.

Five groups of MC pregnancies were identified: 1- without complications (NC); 2-complicated with selective intrauterine growth restriction (sIUGR), defined as pregnancies with one estimated fetal weight of one twin below 10th percentile and with Doppler in umbilical artery (UA) according to the classification already described [6]; 3- complicated with twin–twin transfusion syndrome (TTTS), defined as the presence of poly-oligo-hydramnios sequence, with a sonographic evidence of a maximum vertical pocket (MVP) > 8 cm before 20 weeks and >10 cm after 20 weeks in the recipient sac and a MVP $\leq$ 2 cm in the donor sac [7]; 4- complicated with amniotic fluid discordance (AFD), identified by a difference in MVP between twins above 3.1 cm persistent from first observation through the pregnancy, without development of TTTS or sIUGR [5]; 5- complicated with twin anemia polycitemia sequence (TAPS), defined by the presence of prenatal anemia in one twin [peak systolic velocity (PSV) in middle cerebral artery (MCA) value > 1.5 MoM] and polycitemia (PVS in MCA < 1 MoM) in the other twin [8].

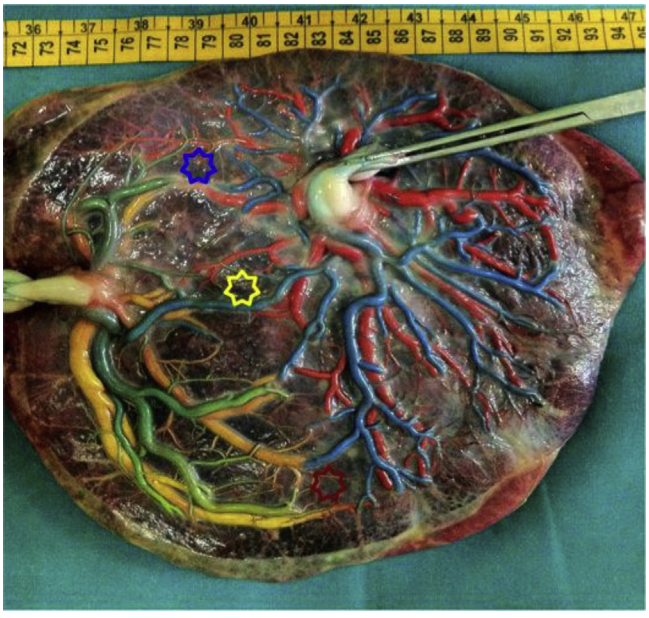

Fig. 1. Monochorionic placenta of not complicated twin pregnancy. In blue and green the arteries, in red and yellow the veins. Blue star: artero-venous anastomoses. Yellow star: artero-arterial anastomoses. Red star: venous-arterial anastomoses.

All the pictures taken were analyzed with Image J 1.49 g (National Institute of Health, USA), in order to identify and measure the caliber of each anastomoses. The caliber of AVA was defined by the artery diameter, while the caliber of AAA or VVA was measured at the level of the vascular equator.

For all placentas, the type of each cord insertion was defined as paracentral, marginal or velamentous, according to previous classifications [9]; the distance between the two cord insertions was measured, and related to placental diameter. In order to evaluate placental sharing, the area of each vascular territory was measured, and a discordance was defined when there was a difference >25% [10].

3. Statistical analysis

For comparison across groups, we used Kruskal–Wallis (continuous variables) and Fisher's exact test (categorical variables). To evaluate correlation between quantitative variables we calculated Spearman's rho. Analyses were performed with Stata 13 (StataCorp. 2013).

4. Results

A total of 191 MC placentas were collected during the study period: 55 of these, which had been treated with laser coagulation of the anastomotic vessels, and 18 which were damaged or contaminated with formalin, were all excluded from the study. The remaining 118 were studied after dye injection: 58 (49%) were NC; 35 (30%) were sIUGR; and 10 (8%) were TTTS that could not be treated with laser coagulation of the anastomotic vessels because of advanced gestational age at diagnosis (5 cases, after 26 weeks g.a., treated with amnioreduction), miscarriage before treatment (1 case at 19 weeks, with II stage Quintero), IUFD of both twins before treatment (2 cases) or lack of patient consent (2 cases, I stage Quintero, treated with amnioreduction); 13 (11%) were AFD while 2 (2%) were TAPS. The characteristics of the 5 groups are shown in Table 1: gestational age at delivery was significantly lower in sIUGR and TTTS group (33 weeks and 28.5 weeks respectively) when compared to NC group (35.5 weeks), while no difference was found with AFD and TAPS groups (35 weeks and 34.5 respectively). The same difference was observed for birthweight, while birthweight discordance was higher in sIUGR (28.2%), TTTS (14.9%) and also in AFD group (18.7%) when compared to NC group (6.3%). The analysis of placentas characteristic is summarized in Table 2.

Anastomoses were found in each placenta injected. The TTTS and TAPS group showed a small number of total anastomoses when compared to the NC group (4 and 1 vs 7 respectively), while in the sIUGR and AFD group, the overall number of anastomoses (8 and 7 respectively) was similar to that of the NC group. The analysis according to type of anastomoses showed no difference in the number and size of AVA, except for the TAPS group where only 1 AVA with a diameter of 0.8 mm was found in both the placentas observed (Fig. 2).

The analysis of placentas with AAA showed a similar number in the sIUGR and NC group (91% vs 95%), significantly higher than in TTTS (60%) and AFD (77%) groups, while no AAA were found in the TAPS group. The size of these vessels was similar in the NC, sIUGR and TTTS groups (2.3 mm, 2.5 mm, 1.4 mm) but significantly larger in the AFD group (3.3 mm) (Fig. 3).

No difference was found as regards VVA, even though these vessels were found in a smaller proportion of placentas (Table 2).

The evaluation of cord insertion showed a significantly higher number of placentas with at least one velamentous cord insertion