Placental characteristics in twin-to-twin transfusion syndrome and twin anemia-polycythemia sequence

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Chapter 8

General discussion and future perspectives

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Great strides have been made in recent years to improve our understanding of the intertwin transfusion syndromes associated with monochorionic (MC) pregnancies. Placental studies with colored dye injection have proved essential to the understanding of the pathophysiology of the various complications and the detection of new disorders in MC twin pregnancies. Both twin-to-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) develop from a net imbalance of blood flow mainly over arterio-venous (AV) anastomoses. However, there are striking differences in the placental angioarchitecture and therefore in the amount of blood flow which results in the different clinical presentations.

This thesis presents a number of studies on the placental angioarchitecture of both TTTS and TAPS to investigate the pathogenesis of these diseases.

In *Chapter 2* we give a detailed description of the technique of placental dye-colored injection. This technique is of paramount importance in studying the placental angioarchitecture of MC placentas. Further a review of literature is given regarding the differences in placental architecture between various types of MC placentas, including normal MC placentas, TTTS placentas (with and without laser treatment), TAPS placentas, selective intrauterine growth restriction (sIUGR) placentas, monoamniotic placentas, twin reversed arterial perfusion sequence (TRAP) placentas and bipartite placentas.

Twin-to-twin transfusion syndrome (TTTS)

Extensive placental research during the last two decades has led to a clearer understanding of the pathogenesis of TTTS. The ubiquitous presence of deep, unidirectional arterio-venous (AV) anastomoses is the main cause of unbalanced blood flow and the development of TTTS. TTTS cannot develop without these anastomoses. Another crucial finding is related to the role of arterio-arterial (AA) anastomoses. Various studies have shown that AA anastomoses occur less frequently in TTTS placentas compared to uncomplicated MC placentas and imply that AA anastomoses play a protective role in preventing the development of TTTS [1,2]. AA anastomoses are bidirectional and have a low resistance. It is believed that they can compensate for hemodynamic imbalances by creating an equilibrating shift. Another important aspect of MC placentas, besides the type of vascular anastomoses, is related to the type of umbilical cord insertions. Controversy persists as to whether velamentous cord insertions (VCI) play a role in the development of TTTS. Several authors showed an increased rate of VCI in TTTS and suggested that VCI may lead to TTTS because of utero-placental insufficiency caused by VCI [3-6]. However, other studies contradicted those findings [7-9]. The main limitations of these previous studies was the small sample size. We therefore set up a large multicenter study, with enough power to detect significant differences and determine the role of VCI in TTTS compared to normal MC placentas (*Chapter 3*). In this study of 304 TTTS placentas and 326 normal MC placentas, VCI was detected in 36.8% of placentas with TTTS versus

35.9% without TTTS (p = 0.886). The presence of VCI in one twin was associated with small for gestational age twin and severe birth weight discordance, as is to be expected. In the normal MC group an increase in intrauterine fetal demise was seen from 4.6% to 14.1% (p = 0.027), as well as a decrease in gestational age at birth in the presence of VCI. Our study suggests that VCI is not associated with the development of TTTS, invalidating previous hypotheses. Both VCI and TTTS are independent risk factors for intrauterine fetal demise and lower gestational age at birth.

Although the protective role of AA anastomoses has largely been demonstrated, it is still not clear why TTTS may still develop, in a minority of cases, in the presence of an AA anastomosis. We hypothesized that the protective role of AA anastomoses could be related to the diameter of this vessel: a smaller AA anastomosis could, hypothetically, be less likely to equilibrate the intertwin difference of blood than a larger anastomosis due to the higher resistance. We studied the incidence and diameter of AA anastomoses in TTTS placentas in *Chapter 4*. In accordance with previous studies, we found a lower incidence of AA anastomoses in TTTS placentas than in normal MC placentas (37% and 91%, respectively, p < 0.001). However, we found no significant difference in the diameter of AA anastomoses in the TTTS group and the group without TTTS. A potential limitation of our study was the small sample size and possible lack of significant power. In a recent study we repeated the evaluation described here above but this time with a larger set of placentas. In this second larger study the diameter of the AA anastomoses was found to be smaller than in the control group of uncomplicated MC placentas, contesting our previous findings [10]. A larger diameter of the AA anastomosis allows a greater volume of blood to pass through because of a decreased vascular resistance (Poiseuille's law) and therefore could allow for equilibration of the hemodynamic imbalances. Further studies, preferably also by other research groups, are necessary to verify these findings.

Although the role of AA and AV anastomoses in the development of TTTS has been widely investigated, the role of VV anastomoses remains unclear and has received very little attention. VV anastomoses, in analogy to AA anastomoses, are superficial anastomoses and allow bidirectional flow. However, venous blood pressure is lower than arterial blood pressure. This could, hypothetically, lead to blood transfusion as a result of the influence of external factors such as the position of the fetuses on the venous blood pressure. In an effort to unravel the role of veno-venous (VV) anastomoses in MC placentas and specifically in TTTS we performed a single center study focusing on these VV anastomoses (*Chapter 5)*. The prevalence of VV anastomoses is significantly higher in TTTS placentas than in normal MC placentas, 37% and 7% respectively (p < 0.01). This suggests that VV anastomoses may support the development of TTTS [11]. In MC placentas with VV anastomoses the overall perinatal mortality was 16% versus 10% in the group without VV anastomoses (p = 0.02). The presence of VV anastomoses is associated with perinatal mortality, but is not an independent risk factor. However, the

presence of VV anastomoses was associated with and was an independent risk factor for TTTS ($p < 0.01$, OR: 3.59; 95% CI: 1.72-7.47). VV anastomoses-related perinatal mortality may be due to the high rate of TTTS (and its complications) in MC twins with VV anastomoses. This study is limited by its relatively small sample size. At present we are conducting a multicenter study with 5 other centers to confirm our conclusions. As shown in these and other studies, the type and size of anastomoses may play an important role in the development of TTTS. However, other placental factors remain to be elucidated such as the importance of placental share. Differences in placental share are known to be related to the development of sIUGR but could also play a role, in combination with additional factors, in the development of TTTS. In addition, most studies reported here above, analyzed MC placentas on a macroscopic level, but very few attempts have been made at studying the characteristics of TTTS placentas at a microscopic level. In one study looking at possible microscopic differences, deep-hidden anastomoses were found using casting techniques to evaluate chorionic-plate anastomoses. However, no inter-twin hemoglobin (Hb) difference was found between placentas with and without deep-hidden anastomoses. Deep-hidden anastomoses are believed to have no clinical consequences [12].

Although it would seem that the placental angioarchitecture plays the largest role, the development of TTTS is most probably also related to hormonal factors. On this front more research is necessary to obtain a fuller picture of the influence of vasoactive and hormonal factors, including the renin-angiotensin system, insulin-like growth factor (IGF)-II, leptin, and endothelin-1. It has been hypothesized that the release of vasoactive substances and oliguria in the donor could cause hypertension and renal damage, while transfer of these substances could cause hypertension in the recipient [13]. However, when related to the expected blood pressure for birth weight, donors had a lower than expected blood pressure and recipients a higher [14]. It would be interesting to look further at the blood pressure and possibly at the relative renal function of MC twins with TTTS.

Lastly, one of the most important roles of placenta injection studies with color dye is related to the detection of residual anastomoses in TTTS placentas treated with laser. This is important for a number of reasons, including, to evaluate the success of the operation and the operator performance as a type of quality control. This is not only necessary for the outcome of a single operation, but also to improve the techniques of fetal surgery in general and eventually create a benchmark or standard of practice [15].

Twin anemia-polycythemia sequence (TAPS)

In 2007, our research group discovered and described for the first time a new disease in MC twins and used the term ‘twin anemia-polycythemia sequence’. Since then our understanding of this new type of chronic feto-fetal transfusion has increased dramatically and in 2010 diagnostic and staging criteria were proposed [16]. TAPS, whether it

occurs spontaneously or iatrogenically after laser coagulation, is based on an imbalance in blood flow through a few minuscule AV anastomoses. On average spontaneous TAPS placentas have only 5 anastomoses compared to 10 in uncomplicated MC placentas [17]. The small size and small number of these anastomoses allow a very slow transfer of blood from the donor to the recipient, without the associated hormonal imbalance as seen in TTTS and, most importantly, without the development of polyhydramnios in the recipient and oligohydramnios in the donor. The lack of fluid imbalance implies that TAPS pregnancies do not result in premature contractions due to symptomatic polyhydramnios as often seen in TTTS. TAPS may go undetected unless Doppler ultrasound measurements are performed to detect fetal anemia and polycythemia.

In the first small cases series with TAPS placentas, all initial cases were characterized by the absence of AA anastomoses, which seemed to be a striking and unique finding. However, as TAPS started to become better known and the detection of TAPS cases started to increase, new findings emerged. One of these findings was that in sporadic cases, small AA anastomoses were detected, suggesting that TAPS may also develop in the presence of an AA anastomosis. To accurately evaluate the incidence of AA anastomoses, we analyzed our placental data after several years of prospective registration. In addition, we measured the diameter of the AA anastomoses (*Chapter 6*). This study was performed in a cohort of MC pregnancies with spontaneous TAPS. The incidence of AA anastomoses in spontaneous TAPS placentas was 20% (3/15). The median diameter of the AA anastomosis when present was 0.4 mm in the TAPS placentas and 2.2 mm in the normal MC placentas ($p = 0.01$). The significantly higher rate of AA anastomoses in uncomplicated MC placentas supports the hypothesis that AA anastomoses have a protective effect and prevent not only the development of TTTS but also of TAPS. In the sporadic cases in which TAPS develops in the presence of an AA anastomosis, the anastomosis is very small, ≤ 1 mm preventing intertwin blood equilibration.

Since TAPS may occur spontaneously as well as after laser treatment for TTTS, we hypothesized that the angioarchitecture in these two different forms of TAPS may not be similar. We therefore compared the placental characteristics of spontaneous TAPS and iatrogenic post-laser TAPS (*Chapter 7)*. We found that post-laser TAPS placentas were characterized by the presence of significantly fewer anastomoses compared to the spontaneous TAPS group, with a median of 2 and 4 anastomoses, respectively ($p = 0.003$). Interestingly, in both groups the majority of anastomoses were detected close to the placental margin. In the post-laser group this is thought to be because of increased technical difficulties to visualize the entire vascular equator, especially toward the edge of the placenta. However, in spontaneous TAPS, the reason for the majority of anastomoses to be close to the placental margin is as yet unknown. A more recent study from our group has shown that this appears only in TAPS placentas and that AV anastomoses are usually evenly distributed along the vascular equator in other MC placentas [10]. AA

anastomoses were detected in 14% of all TAPS placentas and all AA anastomoses were minuscule, ≤ 1mm.

Another interesting aspect of TAPS placentas is related to the placental sharing. In MC twin pregnancies in general, discrepancies in placental share lead to similar discrepancies in birth weight. The twin infant with a lower placental share often has more impaired growth and a lower birth weight, whereas the co-twin with the larger placental share has a higher birth weight. In contrast, with TAPS, birth weights seemed to be inversely related to the placental shares. To investigate this intriguing finding, we recently evaluated the placental share and hemoglobin level in TAPS twins in regards to birth weight. Interestingly, hemoglobin (Hb) level at birth was associated with birth weight, but placental share was not associated with birth weight. In other words, the recipient twin with the larger Hb level often had a larger birth weight but a smaller placental share, whereas the donor twin with the lower hemoglobin level had a larger placental share. This would seem to indicate that fetal growth in TAPS is determined by feto-fetal blood transfusion and not by placental share [18]. One could speculate that this chronic anemia and the resulting loss of nutrients as well as the associated chronic hypoxia could lead to diminished fetal growth. However the conversely larger placental share has yet to be explained. One explanation is that the chronic anemia, chronic hypoxia and loss of nutrients stimulate expansion of the placental territory and therefore a relatively larger placental share. Another explanation is that there is a form of selection bias at play and that in TAPS cases where the donor has a smaller placenta share there is also an increased risk of intra uterine mortality. Cases with fetal demise in utero lead to maceration of the placenta which were excluded from the study. We therefore only studied cases with double fetal survival which might explain the underreporting of donor twin with smaller placental shares.

Another element with regards to TAPS is the striking difference in color between the dark and plethoric placental share of the recipient and the pale placental share of the donor. This seems, intuitively, to be related to the large difference in Hb content in both placental shares. Microscopic confirmation of this explanation has not been published yet. The striking difference in color may also play a role as an additional diagnostic criteria for TAPS. The diagnosis of TAPS is based on the large Hb difference (>8g/dL) in association with at least one of the following criteria: intertwin reticulocyte count ratio > 1.7 and/or placenta injection studies showing only minuscule anastomoses. However, in various cases reticulocyte counts are not determined and placentas are not injected. In these cases, confirmation of the striking color difference could play an additional role. More research is needed to determine if the color difference can be measured easily and objectively and prove if this is indeed characteristic of TAPS.

Future research can focus on other placental characteristics of TAPS placentas which may or may not influence the pathogenesis and that have not yet been determined, e.g. the role of VV anastomoses and the type of umbilical cord insertion.

As our understanding of TAPS and TTTS continues to grow, so too are we better able to treat Exciting solutions are being investigated, especially with regards to the optimal treatment for TAPS. Further trials must determine if fetoscopic laser surgery in TAPS should be considered as the treatment of choice in TAPS, in analogy with TTTS.

The success of fetoscopic laser surgery in improving outcome in TTTS and possibly also in TAPS, has a drawback in terms of placenta examination and investigation. Since the goal of fetal surgery in these cases is to coagulate all anastomoses, the angioarchitecture in the majority of TTTS and TAPS cases is disrupted. We therefore foresee that since the vast majority of TTTS and TAPS placentas will be treated with laser surgery, evaluation of the vascular anastomoses will only be possible in the minority of untreated cases. To further investigate the role of these anastomoses in untreated placentas, international multicenter collaboration must be initiated.

It is therefore of paramount importance that all centers routinely perform placental injection studies with colored dye. This is not only of advantage to the center itself, but will also enable multicenter participation. To achieve this the placenta should be refrigerated in a sealed container and should not be frozen or fixed in formalin. This renders the placenta unviable for placental injection studies. The placenta should be examined preferably in the first few days, maximally within a week. The vessels should then be carefully catheterized for injection with contrasting colors of dye. Standard meticulous notes of the different anastomoses and types of cord insertions, as well as photographic evidence to later determine the diameter of anastomoses and placental share, will enable future research.

Hopefully, close collaboration between fetal surgeons, neonatologists and pathologists with experience in placental injection studies will further improve our understanding of TTTS and TAPS.

REFERENCE LIST

[1] Taylor MJ, Denbow ML, Duncan KR, Overton TG, Fisk NM. Antenatal factors at diagnosis that predict outcome in twin-twin transfusion syndrome. Am J Obstet Gynecol 2000;183:1023-8.

[2] Denbow ML, Cox P, Taylor M, Hammal DM, Fisk NM. Placental angioarchitecture in monochorionic twin pregnancies: relationship to fetal growth, fetofetal transfusion syndrome, and pregnancy outcome. Am J Obstet Gynecol 2000;182:417-26.

[3] Fries MH, Goldstein RB, Kilpatrick SJ, Golbus MS, Callen PW, Filly RA. The role of velamentous cord insertion in the etiology of twin-twin transfusion syndrome. Obstet Gynecol 1993;81:569-74.

[4] Machin GA. Velamentous cord insertion in monochorionic twin gestation. An added risk factor. J Reprod Med 1997;42:785-89.

[5] Saunders NJ, Snijders RJ, Nicolaides KH. Therapeutic amniocentesis in twin-twin transfusion syndrome appearing in the second trimester of pregnancy. Am J Obstet Gynecol 1992;166:820-24.

[6] Bruner JP, Anderson TL, Rosemond RL. Placental pathophysiology of the twin oligohydramnios-polyhydramnios sequence and the twin-twin transfusion syndrome. Placenta 1998;19:81-6.

[7] De Paepe ME, DeKoninck P, Friedman RM. Vascular distribution patterns in monochorionic twin placentas. Placenta 2005;26:471-75.

[8] Bajoria R. Vascular anatomy of monochorionic placenta in relation to discordant growth and amniotic fluid volume. Hum Reprod 1998;13:2933-40.

[9] Lopriore E, Sueters M, Middeldorp M, et al. Velamentous cord insertion and unequal placental territories in monochorionic twins with and without twin-to-twin-transfusion syndrome. Am J Obstet Gynecol 2007;196:159.e1-159.e5.

[10] Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D et al. Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta 2013; 34(7):589-593.

[11] Zhao DP, Cohen D, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D et al. The role of veno-venous anastomoses in twin-twin transfusion syndrome. Placenta 2014; 35(5):334-336.

[12] van den Wijngaard JP, Lopriore E, van der Salm SM, Schaap AH, Vandenbussche FP, Deruiter MC et al. Deep-hidden anastomoses in monochorionic twin placentae are harmless. Prenat Diagn 2007; 27(3):233-239.

[13] Gratac+¦s E, Ortiz JU, Martinez JM. A Systematic Approach to the Differential Diagnosis and Management of the Complications of Monochorionic Twin Pregnancies. Fetal Diagnosis and Therapy 2012; 32(3):145-155.

[14] Mercanti I, Boivin A, Wo B, Vlieghe V, Le RC, Audibert F et al. Blood pressures in newborns with twin-twin transfusion syndrome. J Perinatol 2011; 31(6):417-424.

[15] Peeters SH, Devlieger R, Middeldorp JM, DeKoninck P, Deprest J, Lopriore E et al. Fetal surgery in complicated monoamniotic pregnancies: case series and systematic review of the literature. Prenat Diagn 2014; 34(6):586-591.

[16] Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ et al. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010; 27(4):181-190.

[17] De VS, Slaghekke F, Middeldorp JM, Klumper FJ, Walther FJ, Oepkes D et al. Arterio-arterial vascular anastomoses in monochorionic twin placentas with and without twin anemia-polycythemia sequence. Placenta 2012; 33(3):227-229.

[18] Zhao D, Slaghekke F, Middeldorp JM, Duan T, Oepkes D, Lopriore E. Placental share and hemoglobin level in relation to birth weight in twin anemia-polycythemia sequence. Placenta 2014; 35(12):1070-1074.