Neonatal hematological and biochemical complications in TTTS and TAPS

Verbeek, L.I.

Neonatal Hematological and Biochemical Complications in TTTS and TAPS

Lianne Verbeek

Neonatal hematological and biochemical complications in TTTS and TAPS

ISBN: 978-90-9030370-3
Cover design: Lianne Verbeek, Maikel Rolvink
Design: Robert Huiberts
Printing: Zalsman Print B.V.

The research presented in this thesis was performed at the Department of Pediatrics, Leiden University Medical Center, The Netherlands.
The publication of this thesis was financially supported by afdeling kindergeneeskunde LUMC, afdeling verloskunde LUMC, BMA B.V. (Mosos), Chiesi pharmaceuticals B.V., KARL STORZ Endoscopie Nederland B.V., Toshiba Medical Systems Nederland, Vaco2Med en Vygon Nederland B.V.

Neonatal hematological and biochemical complications in TTTS and TAPS

Proefschrift
ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 13 juni 2017
klokke 13:45 uur

door

Lianne Irene Verbeek
geboren te Rijswijk in 1990

Promotoren:	Prof. Dr. E. Lopriore
	Prof. Dr. D. Oepkes
Leden promotiecommissie:	Prof. Dr. A. Lankester
	Prof. Dr. C.M. Fijnvandraat *Emma Children's Hospital, Academic Medical Center, Amsterdam,The Netherlands*
	Dr. A. Khalil *St George's Medical School, University of London, London, United Kingdom*

Contents

General introduction

Over the past decades the incidence of monozygotic and dizygotic twin pregnancies has increased, largely as a result of assisted reproductive technologies.[1] The number of spontaneously conceived twins has increased as well due to increased maternal age in pregnant women.[2] Dizygotic twins are more common than monozygotic twins, 70 percent of twins is dizygotic and 30 percent is monozygotic.[3] Monozygotic twinning occurs in 3.5-4 per 1000 births across the world.[4] However, the incidence of dizygotic twinning variates among countries.[5] In the United States the twin birth rate was 33.9 twins per 1000 births in 2014.[6] In low and middle income countries the average twinning rate is approximately 13.1 per 1000 births.[5]

Dizygotic twins occur from fertilization of two oocytes, resulting in two embryos with each their own placenta and their own amniotic sac, named therefore dichorionic diamniotic twinning. Monozygotic twins are identical since these twins result from fertilization of a single oocyte. The type of placentation in monozygotic twins varies and depends on the timing of cleavage of the monozygotic blastomere. Cleavage during the first three days after fertilization results in two placentas, two chorions and two amnions, thus dichorionic diamniotic twinning (such as in dizygotic twins). When cleavage occurs between the fourth to the eighth day after fertilization, monozygotic twins will share their placenta and have one chorion and two amnions, henced called monochorionic diamniotic twinning. Cleavage between the ninth till twelfth day after fertilization results in a monochorionic monoamniotic twin, where fetuses share not only their placenta but also their amniotic sac. Cleavage after the twelfth day results in conjoined twins.[4;7] Approximately 75% of monozygotic twins are monochorionic, with an overall incidence of one in 400 pregnancies.[8] Twin pregnancies are at a higher risk of perinatal and neonatal morbidity and mortality compared to singleton pregnancies.[9-11] This is partly due to the fact that twins are more likely to be born prematurely and small for gestational age.[12-15] In addition, one of the most important risk factors for the increased rate of perinatal complications in twins is due to the type of placentation. Monochorionic twins (which occur only after monozygoc twinning) are at increased risk of complications compared to dichorionic twins due to the differences in placentas. Nearly all monochorionic twins (99%) have placental vascular anastomoses connecting the blood circulation of both twins[16;17] whereas dichorionic twins have always two separate placentas without vascular connections. Only a few rare cases of dichorionic twins with placental anastomoses have been described.[18;19]

Vascular anastomoses lead to inter-twin blood transfusion, which is 'balanced' in uncomplicated monochorionic twins during pregnancy. However, vascular anastomoses play may also lead to unbalanced inter-twin transfusion and play a key role in the development of severe complications such as twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia (TAPS).

TTTS occurs in approximately 9-15% of monochorionic pregnancies.[20;21] TTTS is diagnosed prenatally based on the presence of twin oligo-polyhydramnios sequence (TOPS). Oligohydramnios in the donor is defined as a cut-off at a deepest vertical pocket of amniotic fluid ≤ 2 cm and polyhydramnios in the recipient as ≥ 8 cm within the first 20 weeks of

gestation or, according to the Eurofoetus criteria, ≥ 10 cm after 20 weeks of gestation.[22;23] In 1999, Quintero et al. defined a classification which consists of 5 stages. Staging depends on bladder filling, abnormal Doppler studies, hydrops fetalis and fetal demise.[23] TTTS is associated with a high fetal and neonatal mortality rate of 73-100% if left untreated.[24] The best treatment for TTTS is fetoscopic laser coagulation of the anastomoses during pregnancy.[25]
Another complication in monochorionic twins is TAPS, first described by our research group in 2007.[26] TAPS may occur spontaneously in 3-5% of monochorionic twins or in 2-16% after laser surgery in TTTS ('post-laser TAPS').[27-31] TAPS is characterized by slow inter-twin blood transfusion resulting in large inter-twin hemoglobin differences at birth, without signs of TOPS during pregnancy. The pathogenesis is based on the presence of minuscule (<1 mm) vascular anastomoses. TAPS can be diagnosed prenatally or postnatally. Prenatal diagnosis of TAPS is based on Doppler ultrasound measurements with an increased middle cerebral artery-peak systolic velocity (MCA-PSV) > 1.5 multiples of median (MoM) in the donor, suggestive for fetal anemia and a decreased MCA-PSV (MoM < 1.0) in the recipient suggestive for polycythemia.[32] In 40-63% of cases, TAPS is diagnosed after birth.[32] Postnatal diagnosis is based on inter-twin hemoglobin difference of ≥ 8 g/dL (i.e. ≥ 5 mmol/L). In addition, to distinguish from acute peripartum TTTS, at least one of the following criteria are required: reticulocyte count ratio > 1.7 or minuscule placental anastomoses.[33]

Outline of the thesis

The Leiden University Medical Center (LUMC) is a tertiary care center and the national referral center for the treatment of complicated monochorionic twins with TTTS and TAPS. Improved antenatal management in TTTS and TAPS has led to an increase in perinatal survival. Attention is now shifting towards postnatal complications in survivors. The aim of this thesis is to evaluate the hematological and biochemical complications in twins affected by TTTS or TAPS.

A. Hematological complications: Most previous studies reported on differences in hemoglobin values in *complicated* monochorionic twins. To compare the hematological values with *uncomplicated* twins, we analyzed hemoglobin levels in dichorionic twins and monochorionic twins without TTTS or TAPS. Since hemoglobin differences may be due to a multitude of factors, we studied the impact of various risk factors including birth order, mode of delivery and delivery time interval between the first-born and second-born twin.
B. Biochemical complications: Only a few small studies in TTTS reported on inter-twin biochemical differences.[34;35] Knowledge on biochemical abnormalities in twins with TAPS is also sparse. We hypothesized that inter-twin blood flow through the placental anastomoses not only lead to transfer of red blood cells from one twin to the other, but also allow inter-twin transfer from nutrients and proteins, and focused mainly on differences in albumin and total protein, and renal function. Hypoalbuminemia is an independent risk factor for morbidity and mortality in neonates.[36;37] In addition, inter-twin blood flow may have an important impact on renal function due to impaired renal perfusion. To date, only a few small studies on renal function have been performed, mainly in TTTS treated with amniocentesis.[38-41]

The following chapters describe the objectives in more detail:

Chapter 2 – Literature review on hematological disorders in twins with TTTS, TAPS, acute peripartum TTTS, acute perimortem TTTS and uncomplicated monochorionic twins.

Chapter 3 – Study on hemoglobin differences in a large cohort of uncomplicated monochorionic twins in relation to birth order, mode of delivery and delivery time interval between the first-born and second-born twin.

Chapter 4 – Study on hemoglobin levels in a large cohort of dichorionic twins compared to uncomplicated monochorionic twins and in relation to birth order and mode of delivery.

Chapter 5 – Study on albumin levels in donor and recipient twins with TTTS treated conservatively or with fetoscopic laser surgery.

Chapter 6 – Study on albumin levels in donor and recipient twins with TAPS compared to a matched group of uncomplicated monochorionic twins.

Chapter 7– Evaluation of short-term renal function in neonates with TAPS admitted to three European centers.

Chapter 8 – Study on short-term renal function in twins with TTTS treated with fetoscopic laser surgery or conservative treatment.

Chapter 9 – General discussion concerning the results of these studies and future perspectives and proposals for future studies.

Chapter 10 – Summary of the thesis.

Chapter 11 - Appendices.

References

1 Derom C, Vlietinck R, Derom R, Van den Berghe H, Thiery M: Increased monozygotic twinning rate after ovulation induction. Lancet 1987;1:1236-1238.
2 Martin JA, Park MM: Trends in twin and triplet births: 1980-97. Natl Vital Stat Rep 1999;47:1-16.
3 Cameron AH, Edwards JH, Derom R, Thiery M, Boelaert R: The value of twin surveys in the study of malformations. Eur J Obstet Gynecol Reprod Biol 1983;14:347-356.
4 Hall JG: Twinning. Lancet 2003;362:735-743.
5 Smits J, Monden C: Twinning across the Developing World. PLoS One 2011;6:e25239.
6 Hamilton BE, Martin JA, Osterman MJ, Curtin SC, Matthews TJ: Births: Final Data for 2014. Natl Vital Stat Rep 2015;64:1-64.
7 McNamara HC, Kane SC, Craig JM, Short RV, Umstad MP: A review of the mechanisms and evidence for typical and atypical twinning. Am J Obstet Gynecol 2016;214:172-191.
8 Lewi L, Van SD, Gratacos E, Witters I, Timmerman D, Deprest J: Monochorionic diamniotic twins: complications and management options. Curr Opin Obstet Gynecol 2003;15:177-194.
9 Kiely JL: The epidemiology of perinatal mortality in multiple births. Bull N Y Acad Med 1990;66:618-637.
10 McCarthy BJ, Sachs BP, Layde PM, Burton A, Terry JS, Rochat R: The epidemiology of neonatal death in twins. Am J Obstet Gynecol 1981;141:252-256.
11 Grothe W, Ruttgers H: Twin pregnancies: an 11-year review. Acta Genet Med Gemellol (Roma) 1985;34:49-58.
12 Rode L, Tabor A: Prevention of preterm delivery in twin pregnancy. Best Pract Res Clin Obstet Gynaecol 2014;28:273-283.
13 Biggio JR, Anderson S: Spontaneous Preterm Birth in Multiples. Clin Obstet Gynecol 2015;58:654-667.
14 Ananth CV, Vintzileos AM, Shen-Schwarz S, Smulian JC, Lai YL: Standards of birth weight in twin gestations stratified by placental chorionicity. Obstet Gynecol 1998;91:917-924.
15 Lewi L, Devlieger R, De CL, Deprest J: Growth discordance. Best Pract Res Clin Obstet Gynaecol 2014;28:295-303.
16 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;182:417-426.
17 Zhao D, Lipa M, Wielgos M, Cohen D, Middeldorp JM, Oepkes D, Lopriore E: Comparison Between Monochorionic and Dichorionic Placentas With Special Attention to Vascular Anastomoses and Placental Share. Twin Res Hum Genet 2016;19:191-196.
18 Biran V, Bornes M, Aboura A, Masmoudi S, Drunat S, Baumann C, Osimani S, Dalle JH, Sterkers G, Verloes A, Farnoux C, Maury L, Schmitz T, Khung S, Baud O: A long-term competent chimeric immune system in a dizygotic dichorionic twin. Pediatrics 2011;128:e458-e463.
19 Lage JM, Vanmarter LJ, Mikhail E: Vascular anastomoses in fused, dichorionic twin placentas resulting in twin transfusion syndrome. Placenta 1989;10:55-59.
20 Lutfi S, Allen VM, Fahey J, O'Connell CM, Vincer MJ: Twin-twin transfusion syndrome: a population-based study. Obstet Gynecol 2004;104:1289-1297.
21 Baschat A, Chmait RH, Deprest J, Gratacos E, Hecher K, Kontopoulos E, Quintero R, Skupski DW, Valsky DV, Ville Y: Twin-to-twin transfusion syndrome (TTTS). J Perinat Med 2011;39:107-112.
22 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

23 Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M: Staging of twin-twin transfusion syndrome. J Perinatol 1999;19:550-555.
24 Berghella V, Kaufmann M: Natural history of twin-twin transfusion syndrome. J Reprod Med 2001;46:480-484.
25 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, Klumper FJ, DeKoninck P, Devlieger R, Kilby MD, Rustico MA, Deprest J, Favre R, Oepkes D: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144-2151.
26 Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP: Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47-51.
27 Casanova J, Paiva C, Carvalho C, Cunha AC: Twin anemia polycythemia sequence: a report of three cases. J Reprod Med 2014;59:596-598.
28 Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R, Crombleholme T: Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201:417.
29 Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP: Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;201:66-4.
30 Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van MT, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J: The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-518.
31 Gucciardo L, Lewi L, Vaast P, Debska M, De CL, Van MT, Done E, Devlieger R, Deprest J: Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438-442.
32 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.
33 Lopriore E, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Walther FJ: Hematological characteristics in neonates with twin anemia-polycythemia sequence (TAPS). Prenat Diagn 2010;30:251-255.
34 Berry SM, Puder KS, Bottoms SF, Uckele JE, Romero R, Cotton DB: Comparison of intrauterine hematologic and biochemical values between twin pairs with and without stuck twin syndrome. Am J Obstet Gynecol 1995;172:1403-1410.
35 Okamura K, Murotsuki J, Kosuge S, Tanigawara S, Yajima A: Diagnostic use of cordocentesis in twin pregnancy. Fetal Diagn Ther 1994;9:385-390.
36 Vincent JL, Dubois MJ, Navickis RJ, Wilkes MM: Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. Ann Surg 2003;237:319-334.
37 Morris I, McCallion N, El-Khuffash A, Molloy EJ: Serum albumin and mortality in very low birth weight infants. Arch Dis Child Fetal Neonatal Ed 2008;93:F310-F312.
38 Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, Ville Y: Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. Am J Obstet Gynecol 2007;196:450-457.
39 Beck M, Graf C, Ellenrieder B, Bokenkamp A, Huber A, Hecher K, Bartmann P: Long-term outcome of kidney function after twin-twin transfusion syndrome treated by intrauterine laser coagulation. Pediatr Nephrol 2005;20:1657-1659.
40 De Paepe ME, Stopa E, Huang C, Hansen K, Luks FI: Renal tubular apoptosis in twin-to-twin transfusion

syndrome. Pediatr Dev Pathol 2003;6:215-225.
41 Lopriore E, Nagel HT, Vandenbussche FP, Walther FJ: Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1314-1319.

Chapter 2
Hematological disorders at birth in complicated monochorionic twin

Lianne Verbeek
Femke Slaghekke
Marieke Sueters
Johanna M. Middeldorp
Frans J. Klumper
Monique C. Haak
Dick Oepkes
Enrico Lopriore

Expert Rev Hematol. 2016 Dec. In Press

Abstract

Introduction: Monochorionic twins are at risk of severe complications including twin-twin transfusion syndrome (TTTS), twin anemia-polycythemia sequence (TAPS) and acute peripartum TTTS. The pathophysiology is based on inter-twin blood transfusion through placental vascular anastomoses.

Areas covered: This review focuses on the incidence, management and outcome of neonatal hematological complications at birth in TTTS, TAPS and acute peripartum TTTS.

Expert commentary: Hematological disorders are often present at birth in monochorionic twins and include acute or chronic anemia, polycythemia and thrombocytopenia. Routine measurement of complete blood counts in all complicated monochorionic twins is strongly recommended. Increased awareness on these disorders and correct diagnostic tests will lead to prompt and adequate management at birth.

1. Introduction

Twin pregnancies are associated with higher morbidity and mortality rates compared to singletons. This increased risk is partly due to premature birth and lower birth weight, but chorionicity is also an important risk factor.[1;2] Monochorionic (MC) twins carry a higher risk for severe complications than dichorionic (DC) twins due to their unique placenta architecture. In DC twins, each fetus has its own placenta while in MC twins both fetuses share a single placenta. These MC placentas are characterized by the presence of vascular anastomoses, allowing blood to flow between the fetuses during pregnancy or during delivery.[3;4] Different types of anastomoses are known. Artery to vein (AV) anastomoses or also called 'deep anastomoses', are mainly responsible for complications, since blood flow through AV anastomoses is unidirectional from the artery of one twin to the vein of the co-twin. Arterio-arterial (AA) anastomoses are superficial anastomoses that allow bi-directional blood flow. AA anastomoses are detected in the majority of uncomplicated MC placentas and are considered to have a protective effect against unbalanced inter-twin blood flow. Veno-venous (VV) anastomoses are also superficial anastomoses. However, the clinical consequences of VV anastomoses are not well known and association with increased risk of morbidity and mortality has been reported.[5]

In the majority of cases, inter-twin blood flow is in balance, not causing complications. However, in up to 20% of cases net inter-twin blood flow is unbalanced and may lead to disorders such as twin-twin transfusion syndrome (TTTS) or twin anemia-polycythemia sequence (TAPS) during pregnancy. In addition, acute inter-twin blood transfusion can occur during delivery. The differences between TTTS, TAPS and acute peripartum TTTS are shown in table 1.

TABLE 1 Differences between TTTS, TAPS and acute TTTS

	Pathogenesis	Timing of diagnosis and criteria	Hematological disorders at birth
TTTS	Chronic inter-twin transfusion through anastomoses, usually during 2nd trimester of pregnancy	*Antenatal:* oligo-polyhydramnios sequence on ultrasound	Inter-twin Hb discordance might occur depending on antenatal treatment
TAPS	Chronic inter-twin transfusion through small (diameter < 1 mm) anastomoses, may occur during 2nd or 3rd trimester of pregnancy	*Antenatal:* discordant MCA-PSV values on Doppler ultrasound or *postnatal:* inter-twin Hb discordance and large inter-twin reticulocyte count ratio or small AV-anastomoses <1mm	- Large inter-twin Hb difference (> 8 g/dL) - Reticulocytosis in the donor and inter-twin reticulocyte count ratio > 1.7 - Polycythemia in the recipient - Increased risk of thrombocytopenia in recipient
Acute peripartum TTTS	Acute inter-twin transfusion through anastomoses during delivery	*Postnatal:* inter-twin Hb discordance without inter-twin difference in reticulocyte count	- Large inter-twin Hb difference > 8 g/dL - No reticulocytosis in donor - Reticulocyte count ratio < 1.7

Hb = hemoglobin

In this review we focus on the hematological disorders in MC twin neonates with various forms of inter-twin blood transfusion such as TTTS and TAPS. In addition, we will discuss the incidence, risks and management of anemia, polycythemia and thrombocytopenia in these high-risk twins. Given the paucity of published data on hematological complications at birth, we evaluated the incidence and severity of these complications in MC twins delivered at our center. Since 2002, we routinely perform a complete blood count in all MC twins admitted to our neonatal ward (standard of care), and information of the need for blood transfusion due to anemia or partial exchange transfusion due to polycythemia is prospectively recorded in a dedicated database.

Based on our clinical experience and in agreement with the literature, white blood cell disorders are not a relevant clinical complication in monochorionic twins at birth. Information on leukocytopenia and on its possible clinical implications will not be a part of this review.

2.1 TTTS

TTTS is a severe complication caused by chronic unbalanced inter-twin blood transfusion through placental anastomoses that occurs in 10% of MC twins.[6;7] Diagnosis is based on prenatal ultrasound detection of large inter-twin amniotic fluid difference, so called twin oligo-polyhydramnios sequence (TOPS). Oligohydramnios in the donor is defined as a deepest vertical pocket of amniotic fluid ≤ 2 cm, and polyhydramnios in the recipient is defined as ≥ 8 cm within the first 20 weeks of gestation or, according to the Eurofoetus criteria, ≥ 10 after 20 weeks of gestation.[8] A classification system was defined by Quintero et al.[9] The five stages are based on ultrasound findings and Doppler velocimetry in the umbilical artery and the umbilical vein. In all stages TOPS is present. In stage I the bladder of the donor is still visible and the Doppler measurements in both twins are normal. In stage II is the bladder of the donor twin not visible anymore. In stage III the donor's bladder is not visible and Doppler measurements are abnormal consisting of absent/reversed end-diastolic velocity in the umbilical artery or pulsatile flow in the umbilical vein in either fetuses. In the 'atypical' presentation of stage III the bladder of the donor twin is visible. In stage IV one of the fetuses shows signs of hydrops (mainly due to progressive cardiac failure). Stage V is characterized by intrauterine fetal demise of one or both fetuses.
The reported mortality rate in TTTS is very high (up to 73-100%) if left untreated.[10] Several antenatal treatment options have been proposed during the past decades including serial amnioreduction and fetoscopic laser surgery. Amnioreduction is mainly a symptomatic treatment aiming to treat the severe polyhydramnios in the recipient and reduce the risk of premature delivery due to premature uterine distension, contractions or rupture of the membranes. Fetoscopic laser surgery is the only causal treatment and aims to separate the fetal circulations by coagulating all vascular anastomoses. A randomized controlled trial proved that fetoscopic laser coagulation results in higher survival rates and lower rates of neurologic complications compared to amnioreduction.[8]

2.2 Hematological disorders in TTTS

Hematological disorders in TTTS are already present antenatally. Some small studies examining fetal blood samples showed that hematocrit, hemoglobin and red blood cell count levels are significantly lower in donor twins compared to recipient twins.[11;12] In the past, large inter-twin difference in hemoglobin level at birth was considered diagnostic for TTTS, often in combination with large inter-twin birth weight discordances.[13;14]

These criteria were abandoned several decades ago, when it became clear that birth weight and/or hemoglobin discordance are relatively common in MC twins and that the prenatal criteria described above were more useful to reach the diagnose of TTTS.[15] Donors in TTTS may be severely anemic, whereas recipients may suffer from severe hyperviscosity-polycythemia. In addition, several case reports described vascular ischemic limb necrosis in recipient twins, mainly in TTTS cases not treated with laser surgery. The pathogenesis of limb necrosis is related to vascular occlusion due to severe hyperviscosity and polycythemia and secondary thrombosis, though hypertension and vasoconstriction may also play a role.[16;17]

At birth, neonates with TTTS often have hematological disorders. The presence of hematologic complications and inter-twin hemoglobin differences depends on the type of antenatal treatment. In TTTS twins treated conservatively (expectant management or serial amnioreduction), we found that donors had a significant lower hemoglobin level at birth compared to recipient twins. Median inter-twin hemoglobin difference was 3.6 (1.6-6.0) g/dL. In contrast, in TTTS treated with laser coagulation surgery no significant difference in hemoglobin levels was found.[18]

Between 2002 and 2016, 62 TTTS twin pairs treated conservatively were delivered at our center. Due to missing values and fetal or postnatal demise, the total number of recruited twins and described twins varies between the different variables throughout this article. For each variable the number of twins with complete information was stated as total number (n). The percentage of donors requiring a blood transfusion on day 1 was 33% (14 of a total twin number of 43). Recipients required a partial exchange transfusion at birth in 24% (10/42). In the group of TTTS twin pairs treated with complete laser coagulation and born alive at our center, only 5% (13/251) of the ex-donors needed a blood transfusion on day 1 and 1% (2/252) of the recipients needed a partial exchange transfusion (table 2). Coagulation of all vascular anastomoses thus allows re-equilibration of the hematological imbalance: after surgery the hemoglobin level in the donor twin will gradually increase due to cessation of blood loss, while the hemoglobin level in the recipient twin will gradually decrease as transfusion from its co-twin stops. However, fetoscopic laser surgery is not always complete and (small) residual anastomoses may be left patent in up to a third of cases, allowing persistence of TTTS or development of post-laser TAPS (see next paragraph).[19] In both situations large inter-twin hemoglobin differences may still be found at birth.[20] Between 2002 and 2016, 47 TTTS twin pairs were treated with laser coagulation surgery and had residual anastomoses, resulting in post-laser TAPS or recurrent TTTS. The percentage of donors requiring a blood transfusion on day 1 was 55% (26/47) and the percentage of recipients requiring a partial exchange transfusion on day 1 was 35% (16/46) (table 2). As shown in the recently published Solomon trial, the use of the Solomon laser technique (in which a laser coagulation line is drawn along the vascular equator) reduces the risks of post-laser TAPS or recurrent TTTS from 21% (in the classic selective laser technique) to 4% in the Solomon technique.[21]

Since presence of residual anastomoses may not always be predicted antenatally, we strongly advise to measure hemoglobin levels in TTTS twins at birth in all cases, whether treated with laser surgery or not. Blood transfusion in donors and partial exchange transfusion in recipients may be required depending on the local, national protocols for the man-

agement of anemia and polycythemia. Figure 1 shows the incidence of anemia and polycythemia in complicated MC twins delivered at our center between 2002 and 2016.

TABLE 2 Hemoglobin levels and reticulocyte counts at birth, and presence of anemia or polycythemia

	Hb level – g/dL		Reticulocyte count – g/dL		Anemia – n (%)[a]	Polycythemia – n (%)[b]
	Donors	Recipients	Donors	Recipients		
TTTS – conservative management N = 62	13.9 ± 3.5 *n = 43*	18.4 ± 3.6 *n = 42*	83.9 ± 50.1 *n = 33*	76.7 ± 31.0 *n = 33*	14 (33%) *n = 43*	10 (24%) *n = 42*
TTTS – complete laser N = 357	16.8 ± 2.9 *n = 245*	17.1 ± 2.5 *n = 257*	67.3 ± 35.1 *n = 198*	64.6 ± 28.4 *n = 201*	13 (5%) *n = 251*	2 (1%) *n = 252*
TTTS – incomplete laser N = 47	11.6 ± 5.0 *n = 46*	20.1 ± 3.6 *n = 45*	127.7 ± 88.6 *n = 40*	56.8 ± 31.1 *n = 39*	26 (55%) *n = 47*	16 (35%) *n = 46*
Spontaneous TAPS N = 27	9.4 ± 4.7 *n = 25*	21.6 ± 3.7 *n = 26*	175.2 ± 128.8 *n = 24*	46.3 ± 21.5 *n = 24*	19 (83%) *n = 23*	13 (54%) *n = 24*
Acute peripartum TTTS N = 12	13.5 ± 4.5 *n = 12*	20.7 ± 4.0 *n = 12*	52.4 ± 10.5 *n = 9*	53.6 ± 12.3 *n = 9*	3 (27%) *n = 11*	3 (27%) *n = 11*

Values given as mean ± SD
[a]Anemia at birth defined as low hemoglobin level requiring a blood transfusion on day 1, [b]Polycythemia at birth defined as an increased hematocrit requiring a partial exchange transfusion on day 1, N; Refers to numbers of twin pairs, n; refers to number of neonates

3.1 TAPS

TAPS is a newly described form of chronic inter-twin blood transfusion leading to anemia in the donor and polycythemia in the recipient, without signs of oligo-polyhydramnios as seen in TTTS. TAPS is caused by the presence of only few small placental vascular anastomoses (diameter < 1 mm). These minuscule anastomoses allow slow transfusion of blood from one twin to the other, without the hemodynamic imbalance and discordant fetal urine production, as in TTTS. In a few cases, we were able to measure the blood flow over the anastomoses and found it be approximately 5 to 10 ml/24 hours.[22]

Two types of TAPS have been described. The form which occurs sometimes after laser treatment for TTTS, 'post-laser TAPS', was first described in 2006 by Robyr et al.[23] The 'spontaneously' occuring form of TAPS, as well as the acronym TAPS, was first described in 2007 by Lopriore et al.[24] The close relationship between minuscule anastomoses and TAPS was then clearly established. The incidence of spontaneous TAPS is 3-5% of MC twin pregnancies while post-laser TAPS can occur in 2-16% of TTTS twins after laser surgery. [23;25-27]

TAPS can be diagnosed antenatally and postnatally. The antenatal diagnosis depends on Doppler ultrasound abnormalities and includes increased middle cerebral artery peak systolic velocity (MCA-PSV) of > 1.5 multiples of the median (MoM), predicting anemia in the donor, and reduced MCA-PSV of < 1.0 MoM in the recipient, a sign of polycythemia. Absence of TOPS is a *conditio sine qua non*, since the presence of TOPS is pathognomonic for TTTS.[23;25;28] The postnatal diagnosis is made based on hematological criteria at birth. Several definitions for postnatal TAPS have been proposed, such as a hemoglobin level < 11 g/dL in the anemic twin and > 20 g/dL in the polycythemic twin, by Lewi et al.[6] The most important limitation of this definition is the fact that it does not take into account that hemoglobin is known to increase linearly with gestation.[29;30] Currently accepted postnatal criteria include an inter-twin hemoglobin difference > 8.0 g/dL and at least one of the following: reticulocyte count ratio > 1.7 or placenta with only small (diameter < 1 mm) placental vascular anastomoses.[23;25] Reticulocyte count ratio can be measured by dividing the reticulocyte count of the donor with those of the recipient. Detection of the minuscule anastomoses can only accurately be assessed by careful placental injections studies using color dye.

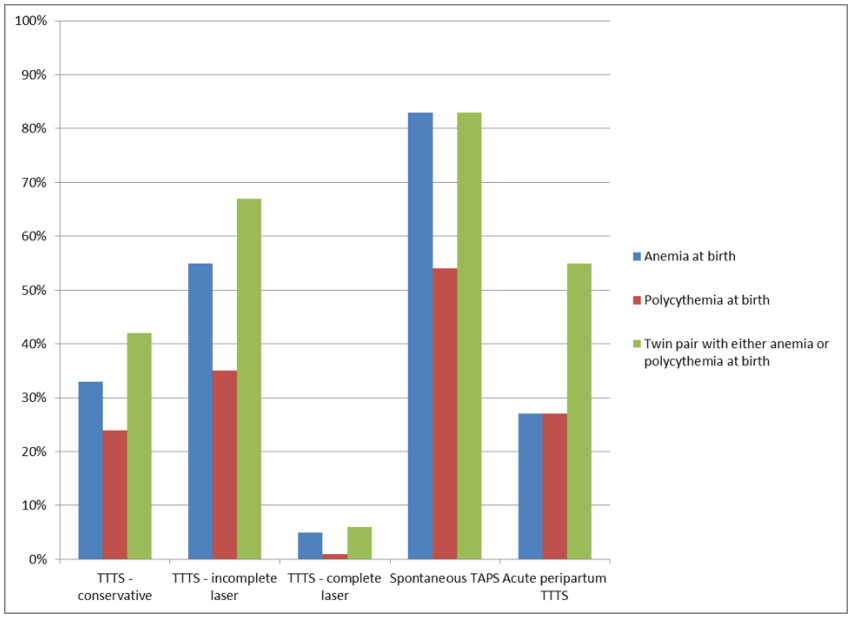
In analogy with TTTS, a TAPS staging system has been developed, subdivided in an antenatal and postnatal classification. In the antenatal stage I and II there are no signs of fetal compromise except for MCA-PSV in the donor > 1.5 MoM in stage I and > 1.7 in stage II and MCA-PSV in the recipient < 1.0 MoM in stage I and < 0.8 MoM in stage II. In stage III, the MCA-PSV is as in stage I or II and besides the donor suffers from cardiac compromise defined as critically abnormal flow (absent or reversed end-diastolic flow in umbilical artery, pulsatile flow in the umbilical vein, increased pulsatility index or reversed flow in ductus venosus). In stage IV, hydrops of the donor is seen due to severe anemia and in stage V intrauterine demise of one or both fetuses occurred. The postnatal classification is based on the inter-twin hemoglobin difference starting at > 8.0 g/dL in stage I, > 11.0 g/dL in stage II, >14.0 g/dL in stage III, > 17.0 g/dL in stage IV and > 20.0 g/dL in stage V.[25]
The optimal antenatal treatment for TAPS is still not clear. Although the pathophysiology of TAPS is also based on vascular anastomoses and fetoscopic laser surgery or delivery would be the only curative treatment, this is more challenging than in TTTS because of the absence of the polyhydramnios-oligohydramnios situation. The uterus is less distended, making insertion of instruments more risky, and the wavering inter-twin membrane prevents visualization of vessels behind it. In addition, the anastomoses in TAPS are minuscule and therefore more difficult to detect during fetoscopic laser coagulation. Moreover, laser surgery is associated with complications as premature rupture of the membranes, disruption of the inter-twin membrane and fetal demise. Besides (repeat) fetoscopic laser coagulation, possible management options are induction of labor, intrauterine transfusion (IUT) (intravascular or intraperitoneal) in the donor with or without partial exchange transfusion (PET) in the recipient, selective termination, pregnancy termination or expectant management since spontaneous resolution has been described as well. Some authors describe a combination of treatments.[25;31-35] Prevention of post-laser TAPS can be successfully achieved by using the Solomon laser coagulation technique, as previously mentioned.[21]

3.2 Hematological disorders in TAPS

Neonates with TAPS have mainly short-term hematologic complications. Donor twins have chronic anemia with highly increased reticulocyte counts, reflecting chronic blood loss. A blood transfusion is often needed in the first 24 hours of birth in 57-80% of cases.[36-39] However, given the chronic nature of the anemia, immediate transfusion at birth or in the delivery room is not necessary. In contrast, recipients may be severely polycythemic and require PET in 40-71% of cases.[36-39] Severe hyperviscosity in recipients may lead to necrosis of the skin and multiple limb ischemia or severe cerebral injury.[23;40] Prompt detection and treatment at birth with partial exchange transfusion is of paramount importance. Some studies report blood transfusion in the donor with the blood concomitantly obtained from the polycythemic co-twin during PET.[41] Between 2002 and 2016 27 spontaneous TAPS twin pairs were delivered in our center. A blood transfusion on day 1 was required in 83% (19/23) of the donor twins and 54% (13/24) of the recipient twins required partial exchange transfusion on day 1 (see table 2 and figure 1).

Thrombocytopenia (platelet count < 150 x 10^9/L) occurs more often in twins affected by TAPS compared to MC twins. In a study of Lopriore et al. with 38 TAPS neonates 1 recipient with severe thrombocytopenia required a platelet transfusion at birth. However, thrombocytopenia in TAPS is mostly self-limiting.[37] Polycythemia is generally associated with thrombocytopenia.[42] Recipient twins are by definition polycythemic and several studies reported lower platelet count in recipients than in donors. Platelet count in recipient twins is also negatively correlated to hemoglobin level at birth. Low platelet count in polycythemic recipients might be explained by impaired production secondary to tissue hypoxia, slow spleen blood flow and decreased plasma fraction with normal concentrations.[37;42]

FIGURE 1 Anemia (needing blood transfusion) and/or polycythemia (needing PET) at birth in complicated monochorionic twins



4. Acute peripartum TTTS

Acute peripartum TTTS, sometimes referred to as 'acute perinatal TTTS', is a rare form of inter-twin transfusion which may occur due to acute shifts of blood between the two fetuses during delivery. Knowledge on acute peripartum TTTS is limited and mainly based on casuistic reports and small, often incomplete, cases series. The incidence is reported to range from 1.5 to 2.5% in all MC twins.[39;43;44] The pathogenesis is not clear and several hypothetic mechanisms have been suggested. Reports suggest that acute peripartum TTTS may be due to acute shifts of relatively large volumes of blood from one twin to the other due to blood pressure differences following uterine contractions or changes in fetal positions. This is likely only possible through large AA or VV anastomoses. Mode of delivery and birth order could play a role, since acute peripartum TTTS mostly occurs in twins born vaginally and first-born twins are usually the ones with the lower hemoglobin levels. [44-47] However, Mabuchi et al. described 5 twins with acute peripartum TTTS born through emergency cesarean section.[39] The diagnostic criteria for acute peripartum TTTS are not clear and most reports use a hemoglobin difference of more than 8 g/dL at birth in the absence of TTTS or TAPS. Characteristically, twins with acute peripartum TTTS have a large color difference at birth and large differences in hemoglobin levels and may thus resemble cases with TAPS. Differentiation with TAPS can be made after birth by measuring reticulocyte counts. In acute peripartum TTTS, the reticulocyte count in the donor twins should be low since increased erythropoiesis (and increased reticulocytes) cannot occur in a short instant of time. In contrast in TAPS, the donor twin always has a highly increased reticulocyte count as a sign of chronic blood loss.

In addition, placental injection studies should also be performed to help differentiate between the various complications. As mentioned above, acute shifts of blood between two fetuses could only occur through superficial AA or VV anastomoses. These superficial anastomoses have a large diameter and a low vascular resistance allowing rapid shifts of blood. The absence of such large anastomoses should prompt investigators to envisage other causes. In most case reports on acute peripartum TTTS published in the past, measurement of reticulocyte count or placental injection studies were not performed hampering the differentiation with TAPS. We hypothesize that most case reports of acute peripartum TTTS in the past were probably TAPS cases, wrongly diagnosed due to limited knowledge on TAPS.

Importantly, the donor twin in acute peripartum TTTS may suffer from acute blood loss resulting in severe anemia and hypovolemic shock. Management in case of acute peripartum TTTS warrant prompt use of volume expanders and blood transfusion in the donor twin, and possibly partial exchange transfusion in the recipient in case of polycythemia hyperviscosity. The donor twins described in the case reports needed a blood transfusion in 33-40% of cases and recipients needed partial exchange transfusion in 17-20%.[39;44] Between 2002 and 2016 12 twin pairs with acute peripartum TTTS were born at our center. In 27% (3/11) of the donors a blood transfusion was needed and in 27% (3/11) of the recipients a blood transfusion was needed (see table 2 and figure 1).

5. Acute perimortem TTTS due to acute exsanguination

Acute perimortem TTTS occurs during intrauterine fetal demise of a co-twin and is due to acute exsanguination from the surviving twin into the low-pressure circulation of the dying co-twin who fails to maintain its sympatic tone. It is thought to be mainly mediated by large, superficial, AA or VV anastomoses, since these anastomoses have a low resistance and therefore allow blood to flow rapidly from one twin to the other.[48] The acute hypovolemic shock and ischemia occurs so rapidly that treatment using a blood transfusion practically always comes too late. Similarly, an emergency cesarean section would require detecting the impending fetal demise as it happens or very shortly after, a rare situation. Whether intrauterine transfusion at a later stage, when the surviving fetus remains anemic as diagnosed by MCA Doppler measurements, is useful is unclear. The acute blood loss during the process of demise of the co-twin likely stops quickly after its death. The resulting anemia cannot be reversed by a late blood transfusion. The resulting anemia likely improves by itself, however this may take several days or more. Transfusion may help restore normal hemoglobin levels. More data on this situation is needed, and several studies are on their way.[48-50]

Acute exsanguination may also occur in specific conditions such as in cases of ruptured vasa previa, one of the most dramatic complications in MC twins. In the literature 10 cases of MC twins with antenatally undetected ruptured vasa previa and a velamentous cord insertion are described, with a perinatal mortality rate of 55%.[51]

A velamentous umbilical cord insertion is characterized by membranous umbilical vessels at the placental insertion site. In vasa previa one or more fetal blood vessels run through the amniotic membranes and cross or run near the external orifice of the uterus.[52] Rupture of the membranes can lead to tearing of these vessels and thereby cause acute fetal exsanguination.

Vasa previa is more common in placenta previa, conception by assisted reproductive technologies, velamentous cord insertion and bilobed placenta.[53] Velamentous cord insertion with vasa previa occurs more frequently in twin pregnancies compared to singletons, with a higher prevalence in MC twins than in DC twins.[54] In singletons or DC twins, rupture of vasa previa can result in acute exsanguination and subsequently severe hypoxic-ischemic injury. In MC twins, due to placental vascular anastomoses, rupture of vasa previa of one of the twins can lead to exsanguination in both twins. This can lead to hypovolemic shock and severe anemia requiring prompt management with rapid iv bolus and blood transfusion. Hypovolemic shock can lead to hypoxic-ischemic injury mainly to the brain and kidneys, and result in severe cerebral injury or terminal kidney failure or double fatal outcome. Prevention is the key to reduce the risks of acute exsanguination and is based on prenatal detection of vasa previa and delivery through cesarean section before spontaneous rupture of membranes.[55]

6. Uncomplicated monochorionic twins

Various studies showed that hemoglobin levels are higher in term and preterm neonates born after vaginal delivery compared to neonates delivered through cesarean section.[56-58] These studies also show that hemoglobin levels are often higher in second-born twins compared to their first-born co-twin. The cause of this discordance may be due to various reasons. First, higher hemoglobin levels in second-borns may result from unbalanced inter-twin transfusion during delivery. It might be that due to differences in pressure gradients and uterine contractions relatively more blood flows from the first-born twin to the second-born twin. Another hypothesis could be that after delivery and umbilical cord clamping of the first twin, the yet unborn second twin receives blood from both placental shares through placento-fetal transfusion. An alternative explanation could be that the timing of umbilical cord clamping differs in the first and second twin. Since delayed cord clamping (> 30 seconds) leads to higher hemoglobin levels due to increased placento-fetal transfusion, it might be that the umbilical cord of the first-born twin is clamped relatively early compared to the second-born co-twin. In this case we theorize that once the first twin is born, obstetricians may tend to clamp the cord relatively early in order to focus on the delivery of the second twin. The obstetrician may leave a longer interval for clamping the cord of the second twin, resulting in a higher hemoglobin level in the second twin.[59;60]

Stagnati et al. describe that third-trimester inter-twin MCA-PSV discrepancy in uncomplicated MC is associated with selective intrauterine growth restriction (sIUGR) at birth and an inter-twin birth weight discrepancy of > 25%.[26] MCA-PSV values are highly correlated with hemoglobin levels.[61] One would therefore also expect to find hemoglobin discordances at birth in sIUGR twins. However, hemoglobin levels were not reported in this study. In another small group of sIUGR twins, Chang et al. found increased umbilical plasma erythropoietin concentrations. Increased levels of erythropoietin reflect hypoxia and increased hemoglobin levels are expected. However, erythropoietin ratio was not paralleled by alterations in hemoglobin levels.[62] Further research to investigate hemoglobin levels in MC twins with sIUGR in a larger cohort is warranted.

7. Expert commentary

MC twins are at risk for severe complications including TTTS, acute peripartum TTTS and TAPS. The pathophysiology of these complications is based in all cases on the presence of placental vascular anastomoses. Differentiation between these various disorders is important. TTTS and TAPS are based on chronic unbalanced inter-twin blood transfusion during pregnancy (usually 2nd trimester), while acute peripartum TTTS is based on acute inter-twin blood transfusion during delivery. Untreated or incompletely treated TTTS often results in hematological complications in neonates. After complete laser surgery, hematological complications are rare. In TAPS and in acute peripartum TTTS, large inter-twin hemoglobin differences are found and measurement of reticulocyte counts is of paramount importance to differentiate between the two disorders. In addition, TAPS recipient twins are also at risk for thrombocytopenia probably due to hyperviscosity.

In uncomplicated vaginally delivered MC twins , the second-born twins often have a higher hemoglobin level than the first-born co-twins. Whether this is due to vascular anastomoses in the placenta or differences in umbilical cord clamping interval is not yet known. Lastly, the most dramatic hematologic complication in MC twins occurs in case of rupture of vasa

previa. Acute exanguination in both twins due to transfusion through vascular anastomoses may occur and which may lead to a double fatal outcome. Increased knowledge and awareness of the characteristic placental angioarchitecture and associated complications is important for prompt and adequate management and to reduce the risk of adverse outcome in MC twins.

8. Five-year view

Improved antenatal management in complicated MC twins with TTTS or TAPS has led to an increased perinatal survival. Attention is now shifting towards postnatal morbidity in survivors, including hematological complications after delivery. Despite the improvements in perinatal care in complicated MC twins, hematological disorders are frequently detected. Given the important effect of timing of cord clamping on hemoglobin values at birth, more research is needed to study this effect in specific subgroups of MC twins. Delayed cord clamping could be beneficial to reduce the risk in anemia in donors, and early cord clamping to reduce the risks of hyperviscosity in recipients.

References

1 Kiely JL: The epidemiology of perinatal mortality in multiple births. Bull N Y Acad Med 1990;66:618-637.

2 Biggio JR, Anderson S: Spontaneous Preterm Birth in Multiples. Clin Obstet Gynecol 2015;58:654-667.

3 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;182:417-426.

4 Zhao D, Lipa M, Wielgos M, Cohen D, Middeldorp JM, Oepkes D, Lopriore E: Comparison Between Monochorionic and Dichorionic Placentas With Special Attention to Vascular Anastomoses and Placental Share. Twin Res Hum Genet 2016;19:191-196.

5 Slaghekke F, Zhao DP, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D, Lopriore E: Antenatal management of twin-twin transfusion syndrome and twin anemia-polycythemia sequence. Expert Rev Hematol 2016;9:815-820.

6 Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van MT, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J: The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-518.

7 Baschat A, Chmait RH, Deprest J, Gratacos E, Hecher K, Kontopoulos E, Quintero R, Skupski DW, Valsky DV, Ville Y: Twin-to-twin transfusion syndrome (TTTS). J Perinat Med 2011;39:107-112.

8 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

9 Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M: Staging of twin-twin transfusion syndrome. J Perinatol 1999;19:550-555.

10 Berghella V, Kaufmann M: Natural history of twin-twin transfusion syndrome. J Reprod Med 2001;46:480-484.

11 Denbow M, Fogliani R, Kyle P, Letsky E, Nicolini U, Fisk N: Haematological indices at fetal blood sampling in monochorionic pregnancies complicated by feto-fetal transfusion syndrome. Prenat Diagn 1998;18:941-946.

12 Berry SM, Puder KS, Bottoms SF, Uckele JE, Romero R, Cotton DB: Comparison of intrauterine hematologic and biochemical values between twin pairs with and without stuck twin syndrome. Am J Obstet Gynecol 1995;172:1403-1410.

13 Tan KL, Tan R, Tan SH, Tan AM: The twin transfusion syndrome. Clinical observations on 35 affected pairs. Clin Pediatr (Phila) 1979;18:111-114.

14 Rausen AR, Seki M, Strauss L: Twin transfusion syndrome. A review of 19 cases studied at one institution. J Pediatr 1965;66:613-628.

15 Wenstrom KD, Tessen JA, Zlatnik FJ, Sipes SL: Frequency, distribution, and theoretical mechanisms of hematologic and weight discordance in monochorionic twins. Obstet Gynecol 1992;80:257-261.

16 Schrey S, Huber A, Hecher K, Quintero R, Alkazaleh F, Moise KJ, Jr., Allbert JR, Schneider C, Palermo M, Yinon Y, Chmait RH, Windrim R, Ryan G: Vascular limb occlusion in twin-twin transfusion syndrome (TTTS): case series and literature review. Am J Obstet Gynecol 2012;207:131-10.

17 Carr SR, Luks F, Tracy T, Plevyak M: Antenatal necrotic injury in severe twin-to-twin transfusion syndrome. A case and review. Fetal Diagn Ther 2004;19:370-372.

18 Verbeek L, Middeldorp JM, Hulzebos CV, Oepkes D, Walther FJ, Lopriore E: Hypoalbuminemia in donors with twin-twin transfusion syndrome. Fetal Diagn Ther 2013;33:98-102.

19 Slaghekke F, Lewi L, Middeldorp JM, Weingertner AS, Klumper FJ, Dekoninck P, Devlieger R, Lanna MM, Deprest J, Favre R, Oepkes D, Lopriore E: Residual anastomoses in twin-twin transfusion syndrome after laser: the Solomon randomized trial. Am J Obstet Gynecol 2014;211:285-287.

20 Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ, Vandenbussche FP: Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome. Placenta 2007;28:204-208.
21 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, Klumper FJ, DeKoninck P, Devlieger R, Kilby MD, Rustico MA, Deprest J, Favre R, Oepkes D: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144-2151.
22 Lopriore E, van den Wijngaard JP, Pasman SA, Oepkes D, Walther FJ, van Gemert MJ, Vandenbussche FP: Quantification of feto-fetal transfusion rate through a single placental arterio-venous anastomosis in a monochorionic twin pregnancy. Placenta 2009;30:223-225.
23 Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J, Ville Y: Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194:796-803.
24 Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP: Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47-51.
25 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.
26 Stagnati V, Pagani G, Fichera A, Prefumo F: Intertwin discrepancy in middle cerebral artery peak systolic velocity and third-trimester fetal growth restriction in monochorionic-diamniotic twin pregnancy. Ultrasound Obstet Gynecol 2016;48:66-71.
27 Gucciardo L, Lewi L, Vaast P, Debska M, De CL, Van MT, Done E, Devlieger R, Deprest J: Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438-442.
28 Tollenaar LS, Slaghekke F, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D, Lopriore E: Twin Anemia Polycythemia Sequence: Current Views on Pathogenesis, Diagnostic Criteria, Perinatal Management, and Outcome. Twin Res Hum Genet 2016;19:222-233.
29 Jopling J, Henry E, Wiedmeier SE, Christensen RD: Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. Pediatrics 2009;123:e333-e337.
30 Nicolaides KH, Thilaganathan B, Mibashan RS: Cordocentesis in the investigation of fetal erythropoiesis. Am J Obstet Gynecol 1989;161:1197-1200.
31 Slaghekke F, van den Wijngaard JP, Akkermans J, van Gemert MJ, Middeldorp JM, Klumper FJ, Oepkes D, Lopriore E: Intrauterine transfusion combined with partial exchange transfusion for twin anemia polycythemia sequence: modeling a novel technique. Placenta 2015;36:599-602.
32 Abdel-Sattar M, Platt LD, DeVore G, Porto M, Benirschke K, Chmait RH: Treatment of Complicated Spontaneous Twin Anemia-Polycythemia Sequence via Fetoscopic Laser Ablation of the Vascular Communications. Fetal Diagn Ther 2015;38:233-237.
33 Genova L, Slaghekke F, Klumper FJ, Middeldorp JM, Steggerda SJ, Oepkes D, Lopriore E: Management of twin anemia-polycythemia sequence using intrauterine blood transfusion for the donor and partial exchange transfusion for the recipient. Fetal Diagn Ther 2013;34:121-126.
34 Lopriore E, Hecher K, Vandenbussche FP, van den Wijngaard JP, Klumper FJ, Oepkes D: Fetoscopic laser treatment of twin-to-twin transfusion syndrome followed by severe twin anemia-polycythemia sequence with spontaneous resolution. Am J Obstet Gynecol 2008;198:e4-e7.
35 Guenot C, Robyr R, Jastrow N, Vial Y, Raio L, Baud D: Fetal Intra-Peritoneal Transfusion for the Management of Very Early Spontaneous Twin Anemia-Polycythemia Sequence in an Obese Patient With a Whole Anterior Placenta. Twin Res Hum Genet 2016;19:154-157.

36 Lopriore E, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Walther FJ: Clinical outcome in neonates with twin anemia-polycythemia sequence. Am J Obstet Gynecol 2010;203:54-55.
37 Lopriore E, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Walther FJ: Hematological characteristics in neonates with twin anemia-polycythemia sequence (TAPS). Prenat Diagn 2010;30:251-255.
38 Ashwal E, Yinon Y, Fishel-Bartal M, Tsur A, Chayen B, Weisz B, Lipitz S: Twin Anemia-Polycythemia Sequence: Perinatal Management and Outcome. Fetal Diagn Ther 2016;40:28-34.
39 Mabuchi A, Ishii K, Yamamoto R, Taguchi T, Murata M, Hayashi S, Mitsuda N: Clinical characteristics of monochorionic twins with large hemoglobin level discordance at birth. Ultrasound Obstet Gynecol 2014;44:311-315.
40 Stranak Z, Korcek P, Hympanova L, Kyncl M, Krofta L: Prenatally Acquired Multiple Limb Ischemia in a Very Low Birth Weight Monochorionic Twin. Fetal Diagn Ther 2015.
41 Yarci E, Alyamac DE, Oncel MY, Kose CA, Derme T, Canpolat FE, Oguz SS, Dilmen U: Successful management of twin anemia/polycythemia sequence by syngeneic partial exchange transfusion. Fetal Diagn Ther 2014;36:251-254.
42 Sarkar S, Rosenkrantz TS: Neonatal polycythemia and hyperviscosity. Semin Fetal Neonatal Med 2008;13:248-255.
43 Suzuki S, Iwasaki N, Ono S, Igarashi M, Murata T: Fetal heart rate patterns in monochorionic twins following acute twin-twin transfusion. Obstet Gynecol Int 2009;2009:498530.
44 Lopriore E, Holtkamp N, Sueters M, Middeldorp JM, Walther FJ, Oepkes D: Acute peripartum twin-twin transfusion syndrome: incidence, risk factors, placental characteristics and neonatal outcome. J Obstet Gynaecol Res 2014;40:18-24.
45 Klebe JG, Ingomar CJ: The fetoplacental circulation during parturition illustrated by the interfetal transfusion syndrome. Pediatrics 1972;49:112-116.
46 Faxelius G, Raye J, Gutberlet R, Swanstrom S, Tsiantos A, Dolanski E, Dehan M, Dyer N, Lindstrom D, Brill AB, Stahlman M: Red cell volume measurements and acute blood loss in high-risk newborn infants. J Pediatr 1977;90:273-281.
47 Galea P, Scott JM, Goel KM: Feto-fetal transfusion syndrome. Arch Dis Child 1982;57:781-783.
48 Lopriore E, Oepkes D: Fetal and neonatal haematological complications in monochorionic twins. Semin Fetal Neonatal Med 2008;13:231-238.
49 Senat MV, Bernard JP, Loizeau S, Ville Y: Management of single fetal death in twin-to-twin transfusion syndrome: a role for fetal blood sampling. Ultrasound Obstet Gynecol 2002;20:360-363.
50 Senat MV, Loizeau S, Couderc S, Bernard JP, Ville Y: The value of middle cerebral artery peak systolic velocity in the diagnosis of fetal anemia after intrauterine death of one monochorionic twin. Am J Obstet Gynecol 2003;189:1320-1324.
51 van SA, Zhao DP, Steggerda SJ, Kist WJ, Haak MC, Oepkes D, Lopriore E: Double fatal outcome after ruptured vasa previa in monochorionic twins: case report and review of the literature. J Matern Fetal Neonatal Med 2016;29:2523-2526.
52 Bohiltea RE, Cirstoiu MM, Ciuvica AI, Munteanu O, Bodean O, Voicu D, Ionescu CA: Velamentous insertion of umbilical cord with vasa praevia: case series and literature review. J Med Life 2016;9:126-129.
53 Ruiter L, Kok N, Limpens J, Derks JB, de Graaf IM, Mol B, Pajkrt E: Incidence of and risk indicators for vasa praevia: a systematic review. BJOG 2016;123:1278-1287.
54 Gandhi M, Cleary-Goldman J, Ferrara L, Ciorica D, Saltzman D, Rebarber A: The association between vasa previa, multiple gestations, and assisted reproductive technology. Am J Perinatol 2008;25:587-589.
55 Oyelese Y, Catanzarite V, Prefumo F, Lashley S, Schachter M, Tovbin Y, Goldstein V, Smulian JC: Vasa

previa: the impact of prenatal diagnosis on outcomes. Obstet Gynecol 2004;103:937-942.
56 Lubetzky R, Ben-Shachar S, Mimouni FB, Dollberg S: Mode of delivery and neonatal hematocrit. Am J Perinatol 2000;17:163-165.
57 Sheffer-Mimouni G, Mimouni FB, Lubetzky R, Kupferminc M, Deutsch V, Dollberg S: Labor does not affect the neonatal absolute nucleated red blood cell count. Am J Perinatol 2003;20:367-371.
58 Wu JH, Chou HC, Chen PC, Jeng SF, Chen CY, Tsao PN, Hsieh CJ, Huang HM, Hsieh WS: Impact of delivery mode and gestational age on haematological parameters in Taiwanese preterm infants. J Paediatr Child Health 2009;45:332-336.
59 Lopriore E, Sueters M, Middeldorp JM, Vandenbussche FP, Walther FJ: Haemoglobin differences at birth in monochorionic twins without chronic twin-to-twin transfusion syndrome. Prenat Diagn 2005;25:844-850.
60 Verbeek L, Zhao DP, Te Pas AB, Middeldorp JM, Hooper SB, Oepkes D, Lopriore E: Hemoglobin Differences in Uncomplicated Monochorionic Twins in Relation to Birth Order and Mode of Delivery. Twin Res Hum Genet 2016;1-5.
61 Teixeira JM, Duncan K, Letsky E, Fisk NM: Middle cerebral artery peak systolic velocity in the prediction of fetal anemia. Ultrasound Obstet Gynecol 2000;15:205-208.
62 Chang YL, Chao AS, Peng HH, Chang SD, Su SY, Chen KJ, Cheng PJ, Wang TH: Increased Fetal Plasma Erythropoietin in Monochorionic Twin Pregnancies With Selective Intrauterine Growth Restriction and Abnormal Umbilical Artery Doppler. Twin Res Hum Genet 2016;19:383-388.

Chapter 3
Hemoglobin differences in uncomplicated monochorionic twins in relation to birth order and mode of delivery

Lianne Verbeek
Depeng P. Zhao
Arjan B. te Pas
Johanna M. Middeldorp
Stuart B. Hooper
Dick Oepkes
Enrico Lopriore

Twin Res Hum Genet. 2016 Jun;19(3):241-5

Abstract

Aim: To determine the differences in hemoglobin (Hb) levels in the first two days after birth in uncomplicated monochorionic twins in relation to birth order and mode of delivery.

Methods: All consecutive uncomplicated monochorionic pregnancies with two live-born twins delivered at our center were included in this retrospective study. We recorded Hb levels at birth and on day 2 and analyzed Hb levels in association with birth order, mode of delivery, and time interval between delivery of twin 1 and 2.

Results: A total of 290 monochorionic twin pairs were analyzed, including 171 (59%) twins delivered vaginally and 119 (41%) twins born by cesarean section (CS). In twins delivered vaginally, mean Hb levels at birth and on day 2 were significantly higher in second-born twins compared to first-born twins: 17.8 versus 16.1 g/dL and 18.0 versus 14.8 g/dL, respectively ($p < 0.01$). Polycythemia was detected more often in second-born twins (12%, 20/166) compared to first-born twins (1%, 2/166; $p < 0.01$). Hb differences within twin pairs delivered by CS were not statistically or clinically significant. We found no association between inter-twin delivery time intervals and Hb differences.

Conclusions: Second-born twins after vaginal delivery have higher Hb levels and more often polycythemia than their co-twin, but not when born by CS.

Introduction

Placental vascular anastomoses are extremely rare in dichorionic twins, but almost ubiquitous in monochorionic twins. These vascular anastomoses allow antenatal inter-twin blood transfusion and may lead to severe complications such as twin-twin transfusion syndrome (TTTS) or twin anemia-polycythemia sequence (TAPS). However, in most monochorionic twins, inter-twin blood transfusion between the two fetuses is 'balanced' and the pregnancy evolves without complications until birth. During birth, acute shifts of blood through the vascular anastomoses may also occur and lead to large hemoglobin (Hb) differences between the two neonates.[1;2] A few small studies in uncomplicated monochorionic twins reported significantly higher Hb levels in second-born twins compared to first-borns, suggesting a possible role for intrapartum placento-fetal transfusion.[3-5] However, these studies are limited by small sample sizes, hampering the interpretation of the results and the evaluation of possible risk factors.

The aim of this study was to evaluate the Hb differences at birth in a large cohort of uncomplicated monochorionic twins and determine the effect of birth order, mode of delivery and time-interval to delivery between twin 1 and twin 2.

Methods

All consecutive monochorionic twins delivered at our tertiary care center between May 2002 and October 2015 were included in this retrospective study. We excluded monochorionic twins affected by TTTS, TAPS, twin reversed arterial perfusion sequence (TRAP) or single or double fetal demise. Similarly, when the first twin was delivered vaginally and the co-twin through secondary cesarean section (CS), the twin pair was also excluded. We defined TTTS according to the Eurofoetus criteria, with a cut-off at a deepest vertical pocket of amniotic fluid in the donor at ≤2 cm and in the recipient of ≥8 cm within the first 20 weeks of gestation or ≥10 cm after gestational week 20.[6] The definition of TAPS was based on the presence of inter-twin Hb difference > 8 g/dL and at least two of the following criteria: reticulocyte count ratio > 1.7 or placenta injection with colored dye showing only minuscule anastomoses (diameter < 1 mm).[7]

Hb levels were routinely assessed at birth from umbilical cord blood or venous blood directly from the neonate when umbilical cord blood could not be used. Since Hb levels are subject to significant change in the first few hours after birth, and in particular in cases with acute large shifts due to intra-partum blood transfusion, we also recorded Hb levels on day 2.[3] The following perinatal data was collected: gestational age at birth, mode of delivery (CS or vaginal delivery), and time interval between delivery of the first twin and the second twin (in minutes). We were not able to record the timing of cord clamping as this is not routinely registered in deliveries occurring at our center. Local guidelines recommend cord clamping at 60-90 seconds, but this varies in practice.

The following neonatal data were collected: gender, birth weight, birth weight discordance, occurrence of polycythemia, treatment with partial exchange transfusion or red blood cell transfusion in the first two days of life and neonatal mortality. Birth weight discordance was calculated by dividing the difference in birth weight between twins by the birth weight of the larger twin. Polycythemia was defined as a venous hematocrit level > 65%. Neonatal mortality was defined as death within 28 days after birth. Acute peripartum

TTTS was defined as an Hb difference of at least 8 g/dL at birth, without signs of TAPS or chronic TTTS.[1] In case of polycythemia, partial exchange transfusion was performed when venous hematocrit > 70% in asymptomatic patients or hematocrit > 65% in symptomatic patients.[8]

The primary outcome of the study was inter-twin Hb differences at birth and day 2. Data were analyzed in association with birth order, mode of delivery and time interval between delivery of twin 1 and twin 2.

The hospitals' Research Ethics Committee approved the study (protocol number: P15.325).

Statistics
Data are reported as means and standard deviations (SD) or as medians and interquartile ranges (IQR), as appropriate. Continuous values within twin pairs were analyzed using the Paired t-test. Paired nominal data was analyzed using the McNemar test. Unpaired continuous data were analyzed using the Mann-Whitney Test. For comparing unpaired nominal data, the Chi-square test was used. The Spearman correlation coefficient was used to study the relationship between time interval at birth between the first-born twin and the second-born twin and Hb levels . A p-value <0.05 was considered to be statistically significant. SPSS version 20 was used for performing the analysis (SPSS, Inc., Chicago, Illinois, USA).

Results

A total of 290 monochorionic twin pairs were analyzed in this retrospective study, including 171 (59%) twin pairs delivered vaginally and 119 (41%) pairs born through CS. Baseline characteristics of the population are shown in Table 1.

TABLE 1 Baseline characteristics

	Twin pairs delivered vaginally (n = 171)	Twin pairs delivered through CS (n = 119)
Gestational age at birth – wk[a]	34.6 ± 3.0	33.2 ± 2.8
Birth weight – gr[a]	2167 ± 610	1821 ± 650
Birth weight discordance - %[a]	9.8 (3.8 – 17.8)	16.7 (7.8 – 30.2)
Female – no. (%)	89 (52%)	58 (49%)
Delivery time interval - min[a]	9 (5 – 16)	2 (1 – 2)

[a] *Value given as mean ± SD or median (IQR)*

At birth, paired Hb samples were available in 143 (84%) twin pairs in the vaginal delivery group and 115 (97%) twin pairs in the CS group. On day 2, paired Hb samples were available in 89 (52%) vaginally born twin pairs and 67 (56%) in the CS group. Hb levels in relation to birth order and mode of delivery are presented in Table 2. Mean Hb levels at birth

were significantly higher in second-born twins when delivered vaginally, but no differences were detected between twin 1 and 2 when delivered through CS. Inter-twin Hb differences were significantly larger in twins delivered vaginally compared to CS, 1.9 (IQR: 0.6 – 3.9) g/dL versus 1.1 (IQR: 0.4 – 3.1) g/dL (p =0.02).

TABLE 2 Hb levels in relation to birth order in monochorionic twin pairs delivered vaginally or through CS

	Twin pairs delivered vaginally (n = 171)			Twin pairs delivered through CS (n = 119)			
	Twin 1	**Twin 2**	**P-value**	**Twin 1**	**Twin 2**	**P-value**	**P-value**
Hb level at birth – g/dL[a, b]	16.1 ± 2.4	17.8 ± 2.5	<0.01	15.9 ± 2.2	16.3 ± 2.5	0.10	
Hb level on day 2 – g/dL[a, c]	14.8 ± 2.7	18.0 ± 3.0	<0.01	15.5 ± 2.6	16.0 ± 2.8	0.23	
Hb difference at birth – g/dL[a, b]	1.9 (0.6 – 3.9)			1.1 (0.4 – 3.1)			0.02
Hb difference on day 2 – g/dL[a, c]	3.7 (2.7 – 6.3)			2.4 (0.9 – 4.9)			<0.01
Hb difference > 8g/dL at birth – n (%)[b]	9 (5.2%)			0 (0.0%)			<0.01
Hb difference > 8g/dL on day 2 – n (%)[c]	11 (6.4%)			0 (0.0%)			<0.01

[a] Value given as mean ± SD or median (IQR)

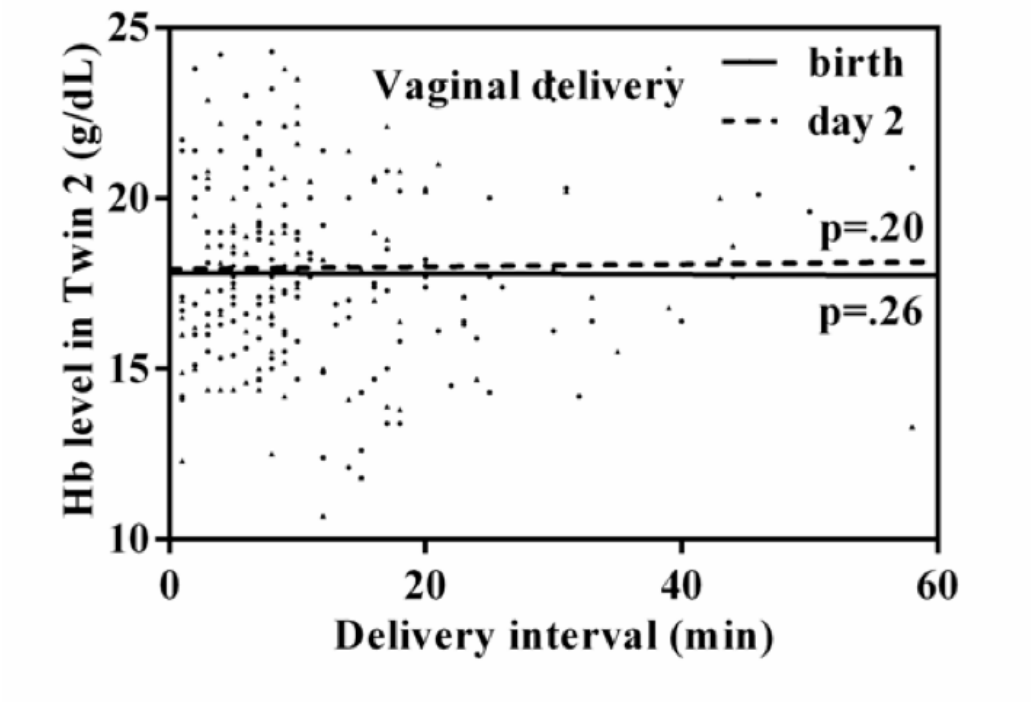
[b] Hb levels at birth were available in 143 twin pairs delivered vaginally and in 115 twin pairs delivered through CS

[c] Hb levels on day 2 were available in 91 twin pairs delivered vaginally and in 64 twin pairs delivered through CS

Abbreviations: Hb = hemoglobin, CS = cesarean section

On day 2, inter-twin Hb differences increased and became more evident in twins born vaginally. Second-born twins had a higher Hb level in 70% (100/143) of cases at birth and 80% (74/93) on day 2 after vaginal birth. In the CS group, second-born twins had a higher Hb level in 62% (70/113) of cases at birth and 54% (35/65) of cases on day 2. The median time interval between the birth of twin 1 and twin 2 by means of vaginal delivery was 9 (IQR: 5 - 16) minutes. The median delivery time interval in the twins born through CS was 2 (IQR: 1 – 2) minutes. In both groups no association between prolonged inter-twin delivery time interval and Hb level differences were found. We found no association between delivery time interval and Hb levels at birth or day 2 in second born twins after vaginal delivery, as shown in Figure 1.

FIGURE 1 Hemoglobin level at birth and on day 2 in twin 2 in relation to delivery time interval



The rate of polycythemia was significantly higher in second-born twins compared to first-borns after vaginally delivery, 12% (20/166) versus 1% (2/166) ($p < 0.01$). No difference was detected in the CS group, 1% (1/119) versus 0% (0/199), ($p = 0.99$).
Acute peripartum TTTS (Hb difference > 8 g/dL at birth) occurred in 5.2% pregnancies (9/166) and was detected only in twin pairs after vaginal delivery. Second-born twins were always the recipient twins in case of acute peripartum TTTS. Further details on the clinical characteristics are listed in Table 3.

TABLE 3 Clinical outcome in twins born through vaginally delivery and CS delivery

	Twins delivered through vaginal delivery			Twins delivered through CS		
	Twin 1 n = 171	Twin 2 n = 171	P-value	Twin 1 n = 119	Twin 2 n = 119	P-value
Mortality – no. (%)	2 (1%)	4 (2%)	0.63	2 (2%)	5 (4%)	0.45
Polycythemia – no. (%)[a]	2 (1%)	20 (12%)	<0.01	0 (0%)	1 (1%)	0.99
Partial exchange transfusion – no. (%)[a]	1 (1%)	4 (3%)	0.38	0 (0%)	1 (1%)	0.99
Blood transfusion – no. (%)[a]	4 (3%)	2 (1%)	0.63	5 (5%)	2 (2%)	0.33

[a] *In first two days after birth*

Abbreviation: CS = cesarean section

Discussion

This study showed that Hb differences at birth are a common finding in monochorionic twins born vaginally, even in the absence of TTTS or TAPS. We found that Hb differences are only present after vaginal delivery but not after CS, highlighting an important effect of mode of delivery. In addition, Hb levels are usually higher in second-born twins suggesting an important role of birth order. Our data suggest that inter-twin blood shifts may occur during vaginal delivery particularly towards the second twin.

Our findings confirm observations from a few small studies [3-5;9], although they differ from the findings of Bhide et al. While they found a trend towards a lower Hb level in a group of 20 second-born monochorionic twins, the small sample size may have hampered their conclusions.[10] In a previous similar study from our research group, we found no association between mode of delivery and Hb differences.[3] Again, the small study groups (28 twin pairs delivered vaginally and 17 pairs delivered through CS) prevented accurate statistical analyses. The large sample size in our present study reduces the risks of bias and may allow unraveling of the complex mechanisms that regulate inter-twin blood shifts during delivery. The other important strength of this study is that we were able to collect data for Hb levels at birth as well as on day 2. In general, Hb levels in neonates (whether singletons or twins) increase in the first few hours after birth. The initial short-term increase is due to hemoconcentration as plasma moves towards the extravascular space to compensate for the placental transfusion and increase in circulating red cell volume at the time of delivery. After several hours, Hb levels in neonates gradually decreases.[11;12] In addition, in case of acute shifts of blood, initial Hb level measurements are known to be unreliable. When acute blood loss occurs, Hb levels initially remain stable due to vasoconstriction.[13] Hb levels reflecting the true blood loss can only be clearly detected after several hours due to a compensatory mechanism of equilibration termed hemodilution. Hemodilution is a gradual increase in intravascular volume and plasma volume which takes place to compensate for acute blood loss.[14] Conversely, when an infant receives an

acute transfusion of blood, the expected rise in Hb levels is only seen after several hours once equilibration between the intravascular and extravascular spaces is achieved. This is why we chose in this study to record Hb levels at birth as well as on day 2.

Inter-twin Hb differences in monochorionic twins are known to be mediated through the invariable presence of placental vascular anastomoses connecting the two fetal circulations. Inter-twin Hb differences may already be present during pregnancy and can be associated with several disorders such as TAPS or TTTS. Inter-twin Hb differences may also occur during delivery in uncomplicated monochorionic twins. The exact pathophysiologic mechanism leading to inter-twin blood transfusion during delivery is not well known. Some authors suggested that uterine contractions or changes in fetal positioning may lead to inter-twin blood pressure differences and exacerbated inter-twin blood transfusion through the vascular anastomoses.[15-17] Evidence for this hypothesis is however lacking. Various other alternatives may explain the inter-twin Hb differences. In this study, we found a similar decrease in Hb levels (0.3-0.4 g/dL) between birth and day 2 in both first and second-born twins delivered through CS. In contrast, in twins born vaginally, the decrease in Hb level in first-born twins is more prominent (1.3 g/dL) and is associated with a concomitant increase in Hb levels in second-born twins. The increase in Hb levels in second-born twins suggests that twin 2 may receive more blood during vaginal delivery. One hypothesis is that this increase in Hb level in twin 2 may result from blood transfusion from twin 1 to twin 2 during delivery (which could explain the significant decrease in Hb level in twin 1). Alternatively, the increase in Hb level in twin 2 may result from transfusion of blood from both placental shares into the circulation of the second twin (placento-fetal transfusion) through the patent vascular anastomoses.[2;18] A third hypothesis is that increase in Hb levels in second born twins could be related to the time interval between delivery of twin 1 and twin 2. Theoretically, prolonged time interval until delivery of twin 2 could be associated with increased placenta-fetal transfusion towards twin 2 and explain the higher Hb levels in second born twins. However, our data do not support this theory since we found no association between prolonged delivery time interval and Hb levels in twin 2. Last, a fourth hypothesis is that the large Hb differences between twin 1 and twin 2 could be related to the timing of cord clamping. As shown in various studies, Hb levels at birth are strongly related with the timing of cord clamping. Late cord clamping (> 30 s after birth) leads to higher Hb levels due to increased placento-fetal transfusion compared to early cord clamping.[19-21] Unfortunately, the timing of cord clamping was not recorded at our center and may vary between caregivers. Although it is tempting to assume that the time taken to clamp each cord was not different during delivery of each twin pair, it is possible that a difference could have occurred. Obstetricians may theoretically be tempted to clamp the cord of the first-born twin more quickly to focus their attention towards the sometimes more complicated delivery of the second twin. The significant drop in Hb level in twin 1 may be the result of relatively very early cord clamping resulting in reduced placento-fetal transfusion towards twin 1.

Although our study was not designed to assess the impact of timing of cord clamping, our data may have implications concerning the timing of cord clamping. Since second-born twins delivered vaginally have increased Hb levels and are more often at risk of polycythemia, delayed cord clamping may not be advisable in second-born twins. In contrast, since first-born twins have a significant decrease in Hb levels early cord clamping should be

avoided in first-born twins. Evidently, these suggestions must be tested in appropriately designed studies to evaluate the benefits of different cord clamping strategies in this particular subgroups of monochorionic twins delivered vaginally.

Although our study is the largest study so far analyzing Hb levels in uncomplicated monochorionic twins, care should be taken when interpreting due to the retrospective nature of the study. Another limitation of our study is that Hb levels on day 2 were only measured in 53% of the included neonates and that timing of cord clamping was not recorded. Both findings are inherent to the retrospective nature of the study and should be taken into account when designing a prospective study in the future.

In conclusion, twins born second generally have higher Hb levels at birth and on day 2 when delivered vaginally, but no inter-twin Hb differences are detected after CS. Whether Hb differences result primarily from increase in Hb levels in twin 2 due to placento-fetal transfusion, decrease in Hb levels in twin 1 due to early cord clamping, or inter-twin blood shifts from twin 1 to twin 2 during delivery is not clear. Targeted studies to evaluate the optimal timing of cord clamping in monochorionic twins delivered vaginally are strongly required. Further studies are also necessary to determine the association between Hb differences in monochorionic twins and the number, type, and size of placental vascular anastomoses. These studies may improve our understanding of the pathophysiological mechanisms leading to blood shifts in monochorionic twins at birth.

References

1 Lopriore E, Holtkamp N, Sueters M, Middeldorp JM, Walther FJ, Oepkes D: Acute peripartum twin-twin transfusion syndrome: incidence, risk factors, placental characteristics and neonatal outcome. J Obstet Gynaecol Res 2014;40:18-24.

2 Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Vandenbussche FP, Walther FJ: Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses. J Pediatr 2005;147:597-602.

3 Lopriore E, Sueters M, Middeldorp JM, Vandenbussche FP, Walther FJ: Haemoglobin differences at birth in monochorionic twins without chronic twin-to-twin transfusion syndrome. Prenat Diagn 2005;25:844-850.

4 Klebe JG, Ingomar CJ: The fetoplacental circulation during parturition illustrated by the interfetal transfusion syndrome. Pediatrics 1972;49:112-116.

5 Faxelius G, Raye J, Gutberlet R, Swanstrom S, Tsiantos A, Dolanski E, Dehan M, Dyer N, Lindstrom D, Brill AB, Stahlman M: Red cell volume measurements and acute blood loss in high-risk newborn infants. J Pediatr 1977;90:273-281.

6 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

7 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.

8 Rosenkrantz TS: Polycythemia and hyperviscosity in the newborn. Semin Thromb Hemost 2003;29:515-527.

9 Galea P, Scott JM, Goel KM: Feto-fetal transfusion syndrome. Arch Dis Child 1982;57:781-783.

10 Bhide A, Prefumo F, Sairam S, Cobian-Sanchez F, Thilaganathan B: Effect of inter-twin delivery interval on neonatal haemoglobin concentration. J Obstet Gynaecol 2006;26:759-762.

11 Brans YW, Shannon DL, Ramamurthy RS: Neonatal polycythemia: II. Plasma, blood and red cell volume estimates in relation to hematocrit levels and quality of intrauterine growth. Pediatrics 1981;68:175-182.

12 Gairdner D, Marks J, Roscoe JD, Brettell RO: The fluid shift from the vascular compartment immediately after birth. Arch Dis Child 1958;33:489-498.

13 Drucker WR, Chadwick CD, Gann DS: Transcapillary refill in hemorrhage and shock. Arch Surg 1981;116:1344-1353.

14 Saito F, Shimazu T, Miyamoto J, Maemura T, Satake M: Interstitial fluid shifts to plasma compartment during blood donation. Transfusion 2013;53:2744-2750.

15 Lehndorff H: [Intrauterine hemorrhage from one twin into the other]. Neue Osterr Z Kinderheilkd 1961;6:163-172.

16 Sherer DM, Sinkin RA, Metlay LA, Woods JR, Jr.: Acute intrapartum twin-twin transfusion. A case report. J Reprod Med 1992;37:184-186.

17 Wenstrom KD, Tessen JA, Zlatnik FJ, Sipes SL: Frequency, distribution, and theoretical mechanisms of hematologic and weight discordance in monochorionic twins. Obstet Gynecol 1992;80:257-261.

18 Lopriore E, Oepkes D: Fetal and neonatal haematological complications in monochorionic twins. Semin Fetal Neonatal Med 2008;13:231-238.

19 Mercer JS: Current best evidence: a review of the literature on umbilical cord clamping. J Midwifery Womens Health 2001;46:402-414.

20 McDonald SJ, Middleton P, Dowswell T, Morris PS: Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. Cochrane Database Syst Rev 2013;7:CD004074.

21 Rabe H, Diaz-Rossello JL, Duley L, Dowswell T: Effect of timing of umbilical cord clamping and other

strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. Cochrane Database Syst Rev 2012;8:CD003248.

Chapter 4
Hemoglobin discordances in twins: due to differences in timing of cord clamping?

Lianne Verbeek
Depeng P. Zhao
Johanna M. Middeldorp
Dick Oepkes
Stuart B. Hooper
Arjan B. te Pas
Enrico Lopriore

Arch. Dis Child. Fetal Neonatal Ed. 2016 Dec 9.

Abstract

Objective: Our objective was to study the differences in Hb at birth in dichorionic (DC) versus monochorionic (MC) twins in relation to birth order and mode of delivery.

Methods: All consecutive DC twin pregnancies and uncomplicated MC twin pregnancies with two live-born twins delivered at our center were included in this retrospective cohort study. Hb levels at birth and on day 2 were evaluated in association with birth order and mode of delivery. The occurrence of polycythemia (venous hematocrit > 65%) was also recorded.

Results: A total of 300 DC and 290 MC twin pairs were included. In DC and MC twins delivered vaginally, second-born twins had a higher Hb level at birth compared to their co-twin (mean Hb level 16.7 versus 15.9 g/dL ($p<0.01$) in DC twins and 17.8 versus 16.1 g/dL ($p<0.01$) in MC twins). In twins delivered through cesarean section, no inter-twin differences in Hb levels were detected. Polycythemia occurred significantly more often in second-born twins compared to first-born twins delivered vaginally, 10 (5%) versus 2 (1%) ($p=0.02$) in DC twins and 20 (12%) versus 2 (1%) ($p<0.01$) in MC twins.

Conclusion: Second-born DC and MC twins delivered vaginally have higher Hb levels at birth compared to first-born twins. Inter-twin Hb differences in MC twins may partly be related to blood transfusion through the vascular anastomoses. Since DC twins do not have anastomoses, other factors may lead to Hb differences including differences in timing of umbilical cord clamping.

Introduction

The main difference between monochorionic (MC) and dichorionic (DC) twins is based on the placenta. MC twins share a single placenta and are almost invariably connected with each other through placental vascular anastomoses whereas DC twins have two separate placentas without connecting anastomoses. Vascular anastomoses lead to inter-twin blood transfusion, which is 'balanced' in uncomplicated MC twins during pregnancy. When 'unbalanced', serious complications such as twin-twin transfusion syndrome (TTTS) or twin anemia-polycythemia sequence (TAPS) can occur. In uncomplicated MC twins, several studies have shown that second-born twins have higher hemoglobin (Hb) levels compared with the first-born twins, when delivered vaginally.[1-4] The cause of the inter-twin Hb differences was thought to be solely related to the presence of the vascular anastomoses allowing either intrapartum inter-twin blood transfusion or large placento-fetal transfusion. Recently, we hypothesized that Hb differences in MC twins may also partly be due to differences in the timing of cord clamping (early cord clamping in first-born twins vs late cord clamping in second born-twins).[5] Unfortunately, timing of cord clamping is often not well recorded. An alternative and indirect method to test this hypothesis is to evaluate the Hb differences in DC twins. Since DC twins do not have vascular anastomoses, eventual inter-twin Hb differences could be explained by differences in the timing of cord clamping.
The aim of this study was to evaluate Hb levels at birth and on day 2 in DC and uncomplicated MC twins and determine the effect of birth order and mode of delivery.

Methods

All consecutive uncomplicated MC twins and DC twins born between May 2002 and January 2016 and delivered at our tertiary care center were included in this retrospective cohort study. Uncomplicated MC twins were defined as MC twins unaffected by TTTS, TAPS, twin reversed arterial perfusion sequence or single or double fetal demise. Data on uncomplicated MC twins analyzed in this study were also analyzed in our previous study on Hb levels in uncomplicated MC twins.[5] TTTS was defined according to the Eurofoetus criteria, with a cut-off at a deepest vertical pocket of amniotic fluid in the donor at ≤ 2 cm and in the recipient at ≥8 cm within the first 20 weeks of gestation or ≥ 10 cm after 20 weeks of gestational age.[6] TAPS was defined as an inter-twin Hb difference > 8 g/dL and at least one of the following criteria: reticulocyte count ratio > 1.7 or placenta injection with colored dye showing only minuscule anastomoses (diameter < 1 mm).[7]
We excluded twin pairs with acute exsanguination due to ruptured vasa previa, ruptured velamentous vessels or placental abruption. We also excluded twin pairs when the first twin was delivered vaginally and the co-twin through secondary caesarean section (CS).
Chorionicity was determined antenatally through ultrasound evaluation during the first trimester of pregnancy and postnatally through macroscopic examination of the placenta and inter-twin membrane directly after birth.
The following baseline characteristics were collected: gestational age, birth weight, birth weight discordance, gender, mode of delivery and delivery time interval in minutes between delivery of twin 1 and twin 2. Birth weight discordance was calculated by dividing the difference in birth weight between the twins by the birth weight of the largest twin and multiplied by 100. Timing of cord clamping was not recorded in this study. Although our local guideline underscores the importance of late cord clamping (> 30 s after delivery), timing of cord clamping is left to the discretion of the attending obstetrician and the timing of cord clamping is unfortunately not routinely registered.

Hb levels were routinely assessed at birth from umbilical cord blood or venous blood directly from the neonate. Hb levels were recorded as well on day 2, since Hb levels are subject to significant change in the first few hours after birth, especially after acute (large) blood shifts.[4;8] The inter-twin Hb differences were calculated, and the occurrence of an inter-twin Hb difference ≥ 8 g/dL was recorded and defined as 'acute peripartum TTTS' in case there were no signs of TAPS or chronic TTTS. The occurrence of polycythemia and treatment with partial exchange transfusion during admission on the neonatal intensive care unit was recorded as well. Polycythemia was defined as a venous hematocrit level > 65%. Partial exchange transfusion was performed in case venous hematocrit > 65% in symptomatic patients or venous hematocrit > 70% in asymptomatic patients. Lastly neonatal mortality, defined as death within 28 days after birth, was recorded.
Primary outcome was the inter-twin Hb differences in relation to chorionicity, birth order and mode of delivery. We hypothesized that second-born twins in DC twins delivered vaginally have a higher Hb level compared with their first-born co-twin.
The hospitals' research ethics committee approved this study.

Statistics
Sample size calculation was based on the number of DC twins required to detect a difference in Hb levels in DC twins delivered vaginally. We calculated that group size of at least 190 DC twin pairs born vaginally was required to demonstrate an inter-twin Hb difference of 1 g/dL (17 g/dL in second-born twins vs 16 g/dL in first-born twins, with a SD of 3 g/dL), with significance of 0.05 and a power of 90%. Data are reported as medians and IQRs or as means and SDs, according to the type of data. Paired nominal data were analyzed using the Mc Nemar test. For paired continuous values, the Paired t-test was used. Unpaired continuous data were analyzed using the Mann-Whitney U test. A value of $p<0.05$ was considered to be statistically significant. Statistical analysis was performed by using SPSS version 20 (SPSS, Inc., Chicago, Illinois, USA).

Results
A total of 590 twin pairs were included in this study, including 300 DC twin pairs and 290 MC twin pairs. In the DC twins group, 66% (197/300) of the twin pairs were delivered vaginally, and 34% (103/300) were delivered by CS. In the MC twins group, 59% (171/290) of the twin pairs were born through vaginal delivery and 41% (119/290) were born by CS. As expected, median delivery time interval between the birth of twin 1 and twin 2 was shorter in both DC and MC twins when born through CS compared with vaginal delivery. Characteristics of the included patients are presented in Table 1.

TABLE 1 Baseline characteristics

	MC twin pairs (n = 290)		DC twin pairs (n = 300)	
	Pairs delivered vaginally (n = 171)	**Pairs delivered through CS (n = 119)**	**Pairs delivered vaginally (n = 197)**	**Pairs delivered through CS (n = 103)**
Gestational age at birth – wk[a]	34.6 ± 3.0	33.2 ± 2.8	31.2 ± 3.6	32.8 ± 2.9
Birth weight – gr[a]	2167 ± 610	1821 ± 650	1656 ± 614	1858 ± 618
Birth weight discordance - %[a]	9.8 (3.8 – 17.8)	16.7 (7.8 – 30.2)	9.1 (4.5 – 15.5)	12.9 (4.8 – 24.0)
Female – no. (%)	89 (52%)	58 (49%)	191 (49%)	96 (47%)
Delivery time interval - min[s]	9 (5 – 16)	2 (1 – 2)	13 (7 – 30)	2 (1 – 2)

[a] Value given as mean ± SD or median (IQR)
Abbreviations: CS = cesarean section, DC = dichorionic, MC = monochorionic

Table 2 shows Hb levels at birth and on day 2 in DC twins, distinguishing between twins born through vaginal delivery and twins born through CS and comparing the first-born twins with the second-born twins. Paired Hb samples at birth were available in 152 (78%) twin pairs born vaginally and in 82 (85%) twin pairs delivered through CS. On day 2, paired Hb samples were available in 138 (71%) twin pairs delivered vaginally and in 59 (61%) twin pairs delivered through CS. In the vaginally born DC group, second-born twins had significantly higher Hb levels compared with first-born twins, 16.7 vs 15.9 g/dL at birth ($p<0.01$) and 15.8 vs 15.0 g/dL on day 2 ($p<0.01$). In DC twins born through CS no significant differences in mean Hb levels were found. However, the inter-twin Hb difference at birth and on day 2 did not differ significantly between the vaginally born group and the CS group.

TABLE 2 Hb levels in relation to birth order in DC twin pairs delivered vaginally or through CS

	Twin pairs delivered vaginally (n = 194)			Twin pairs delivered through CS (n = 96)			
	Twin 1	**Twin 2**	**P-value**	**Twin 1**	**Twin 2**	**P-value**	**P-value**
Hb level at birth – g/dL[a,b]	15.9 ± 2.6	16.7 ± 2.6	<0.01	16.7 ± 2.6	16.8 ± 2.7	0.98	
Hb level on day 2 – g/dL[a,c]	15.0 ± 2.7	15.8 ± 2.8	<0.01	16.4 ± 2.8	17.0 ± 2.8	0.63	
Hb difference at birth – g/dL[a,b]	1.8 (0.8 – 3.3)			1.9 (0.9 – 3.0)			0.75
Hb difference on day 2 – g/dL[a,c]	1.8 (0.8 – 3.0)			1.3 (0.5 – 2.6)			0.08
Hb difference > 8 g/dL at birth – n (%)[b]	0 (0%)			0 (0%)			0.99
Hb difference > 8 g/dL on day 2 – n (%)[c]	0 (0%)			0 (0%)			0.99

[a] Value given as mean ± SD or median (IQR). [b] Hb levels at birth were available in 152 twin pairs delivered vaginally and in 82 twin pairs delivered through CS. [c] Hb levels on day 2 were available in 138 twin pairs delivered vaginally and in 59 twin pairs delivered through CS
Abbreviations: CS = cesarean section, DC = dichorionic, Hb = hemoglobin

Table 3 shows Hb levels in relation to birth order in MC twin pairs delivered vaginally or through CS. Paired Hb samples at birth were available in 143 (84%) twin pairs in the vaginal delivery group and in 155 (97%) twin pairs in de CS group. On day 2, paired Hb levels were available in 89 (52%) vaginally born twin pairs and 67 (56%) pairs in the CS group. As in DC twins, mean Hb levels were significantly higher in second-born twins compared to first-born twins when born through vaginal delivery: 17.8 vs 16.1 g/dL at birth (p<0.01) and 18.0 vs 14.8 g/dL on day 2 (p<0.01). When delivered through CS, no differences in Hb levels between twin 1 and twin 2 were found. In contrast to the DC twins, the inter-twin Hb differences in MC twins were significantly larger in the vaginally born group compared with the CS group, both at birth as on day 2.

TABLE 3 Hb levels in relation to birth order in MC twin pairs delivered vaginally or through CS

	Twin pairs delivered vaginally (n = 171)			Twin pairs delivered through CS (n = 119)			
	Twin 1	**Twin 2**	**P-value**	**Twin 1**	**Twin 2**	**P-value**	**P-value**
Hb level at birth – g/dL[a, b]	16.1 ± 2.4	17.8 ± 2.5	<0.01	15.9 ± 2.2	16.3 ± 2.5	0.10	
Hb level on day 2 - g/dL [a, c]	14.8 ± 2.7	18.0 ± 3.0	<0.01	15.5 ± 2.6	16.0 ± 2.8	0.23	
Hb difference at birth – g/dL[a, b]		1.9 (0.6 – 3.9)			1.1 (0.4 – 3.1)		0.02
Hb difference on day 2 – g/dL[a, c]		3.7 (2.7 – 6.3)			2.4 (0.9 – 4.9)		<0.01
Hb difference > 8 g/dL at birth – n (%)[b]		9 (5.2%)			0 (0.0%)		<0.01
Hb difference > 8 g/dL on day 2 – n (%)[c]		11 (6.4%)			0 (0.0%)		<0.01

[a] *Value given as mean ± SD or median (IQR).* [b] *Hb levels at birth were available in 143 twin pairs delivered vaginally and in 115 twin pairs delivered through CS* [c] *Hb levels on day 2 were available in 91 twin pairs delivered vaginally and in 64 twin pairs delivered through CS*
Abbreviations: CS = cesarean section, Hb = hemoglobin, MC = monochorionic

Table 4 shows that on day 2, inter-twin Hb differences were significantly larger in vaginally born MC twins compared with vaginally born DC twins: respectively 3.7 (2.7 – 6.3) g/dL and 1.8 (0.8 – 3.0) g/dL (p<0.01), respectively.

TABLE 4 Inter-twin Hb differences in vaginally born MC and DC twins

	MC twins (n = 171)	DC twins (n = 194)	P-value
Hb difference a birth – g/dL[a,b]	1.9 (0.6 – 3.9)	1.8 (0.8 – 3.3)	0.83
Hb difference on day 2 – g/dL[a,c]	3.7 (2.7 – 6.3)	1.8 (0.8 – 3.0)	<0.01

[a] Value given as median (IQR)
[b] Paired Hb levels at birth were available in 143 MC twin pairs and 152 DC twin pairs
[c] Paired Hb levels at birth were available in 91 MC twin pairs and 138 DC twin pairs
Abbreviations: DC = dichorionic, Hb = hemoglobin, MC = monochorionic

Acute peripartum TTTS, defined as Hb difference > 8 g/dL without signs of TAPS or chronic TTTS, only occurred in MC twin pairs born through vaginal delivery. In all cases, the second-born twin had the highest Hb level. In DC twins, no twin pair had a Hb difference > 8 g/dL.

Clinical outcome in MC and DC twins are presented in Table 5. In both twin groups, polycythemia occurred significantly more often in second-born twins compared with first-born twins when born through vaginal delivery.

TABLE 5 Clinical outcome in MC and DC twins born through vaginally delivery and CS delivery

	MC twins (n = 580)						DC twins (n = 600)					
	Vaginal delivery			Delivered through CS			Vaginal delivery			Delivered through CS		
	Twin 1 n = 171	Twin 2 n = 171	P-value	Twin 1 n = 119	Twin 2 n = 119	P-value	Twin 1 n = 194	Twin 2 n = 194	P-value	Twin 1 n = 96	Twin 2 n = 96	P-value
Mortality – no. (%)	2 (1%)	4 (2%)	0.63	2 (2%)	5 (4%)	0.45	11 (6%)	11 (6%)	0.99	1 (1%)	2 (2%)	0.99
Polycythemia – no. (%)[a]	2 (1%)	20 (12%)	<0.01	0 (0%)	1 (1%)	0.99	2 (1%)	10 (5%)	0.02	3 (3%)	6 (6%)	0.45
Partial exchange transfusion – no. (%)[a]	1 (1%)	4 (3%)	0.38	0 (0%)	1 (1%)	0.99	0 (0%)	1 (1%)	0.99	0 (0%)	0 (0%)	0.99

[a] In first two days after birth
Abbreviations: CS = cesarean section, DC = dichorionic, MC = monochorionic

Discussion

This study shows that DC twins born through vaginal delivery also have significant differences in Hb levels at birth, which is similar to that observed in MC twins. Several small studies have previously shown that uncomplicated MC twins have significant Hb differences at birth. In MC twins, second-born twins have significantly higher Hb levels than first-born co-twins, mainly after vaginal delivery.[1-4] Our findings demonstrate that in DC twins delivered vaginally, second-born twins also have significantly higher Hb levels. Since DC twins do not have inter-twin placental vascular anastomoses, these Hb differences cannot be explained by inter-twin blood transfusion but must mainly be due to other factors such as differences in timing of cord clamping. This hypothesis on the important role of timing of cord clamping on Hb differences in twins was recently postulated in a large study from our research group in uncomplicated MC twins.[5] In that study we described several theories that might explain the differences in Hb levels at birth. The first hypothesis was that the higher Hb level in twin 2 may result from unbalanced inter-twin blood transfusion through the anastomoses during delivery. Due to differences in pressure gradients and uterine contractions relatively more blood might flow from twin 1 to twin 2 resulting in a higher Hb level in twin 2. Since DC twins do not have placental anastomoses, this first hypothesis cannot explain the higher Hb levels in second-born DC twins. The second hypothesis was related to a larger placentofetal transfusion towards the second-born twin due to the shared placenta and the vascular anastomoses. After birth of twin 1, twin 2 might receive blood from both placental shares through the anastomoses, resulting in a higher Hb level. Once more, for DC twins, this is not an appropriate explanation, due to the separate placentas. A third theory was that the differences in Hb levels are related to the timing of cord clamping after birth. Early cord clamping (< 30 s after birth) is known to lead to lower Hb levels due to reduced placentofetal transfusion.[9-11] We hypothesized that once the first-born twin is delivered, gynecologist may tend to clamp the cord at an earlier stage in order to focus on the delivery of the second twin. Once the second twin is delivered, the gynecologist may experience less hurry to clamp the cord and could tend to delay the clamping (>30 s). Delayed cord clamping may then result in increased placentofetal transfusion and higher Hb levels in second-born twins. Although the timing of cord clamping was not registered in this study, our data comparing the Hb levels in first-born and second-born twins may provide indirect evidence on the possible effect of differences in the timing of cord clamping. In addition, no Hb differences were detected between first and second-born twins either in MC or DC twins when delivered through SC. This could again theoretically be due to a similar cord clamping attitude by the gynecologist for both twins.

In the current study, Hb levels were collected both at birth and on day 2. The reason for measurements at two different time points is that in case of acute blood loss or blood transfusion, Hb levels measured directly after such an event are unreliable and measurements should be repeated after several hours to allow for compensatory mechanisms to lead to equilibration of the Hb levels.[2;8;12;13] Furthermore, several hours after birth Hb levels in all neonates gradually decrease, including in both singletons or twins.[14] The drop in Hb levels between birth and day 2 found in all twins (first-born MC and DC twins and second-born DC twins) in this study confirms this. The only exception is an increase in Hb levels in second-born MC twins on day 2.

Table 4 shows that median inter-twin Hb differences are significantly larger on day 2 in vaginally born MC twins compared with vaginally born DC twins. As stated before, the main difference between MC and DC twins are the vascular anastomoses in the MC placenta. This could mean that the larger inter-twin Hb difference in vaginally delivered MC twins is also related to the presence of the vascular anastomoses. In MC twins the significantly higher Hb levels in second-born twins could result from a combination of a difference in timing of cord clamping between first-born and second-born twins, in combination with unbalanced intrapartum transfusion and/or placentofetal transfusion from both placental shares towards twin 2.

Finally, this study also has important clinical implications. In both twin groups, polycythemia occurred more often in second-born twins when delivered through vaginal birth, although the incidence of polycythemia was only particularly increased in second-born MC twins.[15;16] Since polycythemia is associated with a higher risk of complications such as hypoglycemia and jaundice,[17] delayed cord clamping may not be advisable in second-born MC twins after vaginal birth.

Our findings should be interpreted with care due to the retrospective nature of the study. An additional limitation of our study is that Hb levels on day 2 were only measured in 60% of all included neonates. However, Hb differences were already present on day 1. Although local discussions with obstetricians at our center confirm our hypothesis of a difference in the timing of cord clamping in twins, information on the timing of the umbilical cord clamping was not available, making it difficult to confirm our hypothesis. Our theory on the relation between Hb levels and the timing of cord clamping must be tested in appropriately designed studies to evaluate the benefits of different cord clamping strategies in MC and DC twins. Our data can provide important information for a future study design.

To summarize, twins born second through vaginal delivery have significantly higher Hb levels, both in MC and in DC twins. Given the absence of vascular anastomoses in DC twins, this study provides indirect evidence that higher Hb levels in second-born twins are partly due to relatively earlier cord clamping in twin 1 compared with twin 2, resulting in more time for placentofetal transfusion in twin 2. Since inter-twin Hb differences in MC are larger, there might also be a role for intrapartum transfusion and/or placentofetal transfusion from both placenta shares towards twin 2 in MC twins. Targeted studies to evaluate the optimal timing of cord clamping in twins delivered vaginally are warranted.

References

1 Klebe JG, Ingomar CJ. The fetoplacental circulation during parturition illustrated by the interfetal transfusion syndrome. *Pediatrics* 1972;49:112-116.

2 Faxelius G, Raye J, Gutberlet R, *et al.* Red cell volume measurements and acute blood loss in high-risk newborn infants. *J Pediatr* 1977;90:273-281.

3 Galea P, Scott JM, Goel KM. Feto-fetal transfusion syndrome. *Arch Dis Child* 1982;57:781-783.

4 Lopriore E, Sueters M, Middeldorp JM, *et al.* Haemoglobin differences at birth in monochorionic twins without chronic twin-to-twin transfusion syndrome. *Prenat Diagn* 2005;25:844-850.

5 Verbeek L, Zhao DP, Te Pas AB, *et al.* Hemoglobin Differences in Uncomplicated Monochorionic Twins in Relation to Birth Order and Mode of Delivery. *Twin Res Hum Genet* 2016;1-5.

6 Senat MV, Deprest J, Boulvain M, *et al*. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. *N Engl J Med* 2004;351:136-144.

7 Slaghekke F, Kist WJ, Oepkes D, *et al*. Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. *Fetal Diagn Ther* 2010;27:181-190.

8 Lubin B. Neonatal anaemia secondary to blood loss. *Clin Haematol* 1978;7:19-34.

9 McDonald SJ, Middleton P, Dowswell T, *et al*. Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. *Evid Based Child Health* 2014;9:303-397.

10 Tiemersma S, Heistein J, Ruijne R, *et al.* Delayed cord clamping in South African neonates with expected low birthweight: a randomised controlled trial. *Trop Med Int Health* 2015;20:177-183.

11 Raju TN. Timing of umbilical cord clamping after birth for optimizing placental transfusion. *Curr Opin Pediatr* 2013;25:180-187.

12 Saito F, Shimazu T, Miyamoto J, *et al.* Interstitial fluid shifts to plasma compartment during blood donation. *Transfusion* 2013;53:2744-2750.

13 Drucker WR, Chadwick CD, Gann DS. Transcapillary refill in hemorrhage and shock. *Arch Surg* 1981;116:1344-1353.

14 Brans YW, Shannon DL, Ramamurthy RS. Neonatal polycythemia: II. Plasma, blood and red cell volume estimates in relation to hematocrit levels and quality of intrauterine growth. *Pediatrics* 1981;68:175-182.

15 Dempsey EM, Barrington K. Short and long term outcomes following partial exchange transfusion in the polycythaemic newborn: a systematic review. *Arch Dis Child Fetal Neonatal Ed* 2006;91:F2-F6.

16 Pappas A, Delaney-Black V. Differential diagnosis and management of polycythemia. *Pediatr Clin North Am* 2004;51:1063-10xi.

17 Alsafadi TR, Hashmi SM, Youssef HA, *et al.* Polycythemia in neonatal intensive care unit, risk factors, symptoms, pattern, and management controversy. *J Clin Neonatol* 2014;3:93-98.

Chapter 5
Hypoalbuminemia in donors with twin-twin transfusion syndrome

Lianne Verbeek
Johanna M. Middeldorp
Christian V. Hulzebos
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Fetal Diagn Ther. 2013;33(2):98-102

Abstract

Objective: To estimate the differences in albumin levels between donors and recipients with twin-twin transfusion syndrome (TTTS).

Methods: We performed a matched case control study including twin pairs with TTTS treated conservatively (conservative group) or with fetoscopic laser surgery (laser group) and analyzed the albumin levels at birth in donor and recipient twins.

Results: We included 18 twin pairs in the conservative group and 36 control twin pairs (laser group), matched for gestational age at birth. Median albumin levels in donor twins in the conservative group were significantly lower than in recipient twins, 25.0 versus 33.0 g/l, respectively ($p = 0.001$). In the laser group, albumin levels in donors and recipients were similar, 32.0 versus 32.0 g/l, respectively ($p = 0.633$). Hypoalbuminemia (albumin level < 20 g/l) occurred in 22% (4/18) of donor twins in the conservative group

Conclusions: Hypoalbuminemia occurs frequently in donor twins with TTTS treated conservatively. In TTTS treated with laser, donor twins have similar and normal albumin levels compared to recipients, confirming a successfully performed fetoscopic laser procedure.

Introduction

Twin-twin transfusion syndrome (TTTS) is a severe complication of monochorionic twinning and is due to inter-twin blood transfusion via placental vascular anastomoses. TTTS affects 10% of monochorionic twin gestations and usually develops during the second trimester of pregnancy, leading to hypovolemia and oligohydramnios in the donor twin and hypervolemia and polyhydramnios in the recipient twin.[1;2] Several studies have reported significant inter-twin hematological differences, showing that the hemoglobin levels in donor twins are often significantly lower than in recipient twin.[3;4] Few small studies have reported on other inter-twin differences including biochemical variables and showed that conservatively treated donor twins may also have lower levels of albumin and total protein at birth.[3;5] Hypoalbuminemia in neonates is an independent risk factor for mortality and morbidity and has been associated with various adverse clinical conditions, including necrotizing enterocolitis, intracranial hemorrhage, sepsis, respiratory distress syndrome, chronic lung disease and edema.[6–10] Treatment with fetoscopic laser surgery ameliorates the clinical condition at birth, but it is unknown whether albumin levels are concomitantly higher.

Given the clinical importance of albumin in neonates and the high morbidity rates in TTTS, we analyzed the differences in albumin at birth between donors and recipients with TTTS treated with or without fetoscopic laser surgery.

Methods

We performed a retrospective analysis in all consecutive liveborn monochorionic twins with TTTS admitted to our neonatal nursery at the Leiden University Medical Center over a 10-year period, between August 2003 and August 2012. The Leiden University Center is a tertiary medical center and serves as the national referral center for fetoscopic laser treatment in TTTS pregnancies in the Netherlands.

Diagnosis of TTTS was reached using the standard ultrasound criteria.[11] TTTS was staged according to the classical staging system.[12] Included in the conservative group were all consecutive TTTS cases treated conservatively either with expectant management or with serial amnioreduction. Each twin pair in the conservative group was compared with 2 control twins pairs with TTTS treated with laser surgery (laser group), matched by gestational age at birth ±(1 week of gestation). We excluded TTTS cases with single or double intrauterine death, recurrent or reversal of TTTS after laser surgery, twin anemia-polycythemia sequence and triplet gestations (or higher order). The following obstetrical data were collected: TTTS stage, type of treatment for TTTS, mode of delivery, gestational age at birth, birth weight and inter-twin birth weight discordance. Birth weight discordance was assessed and calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100. Birth weight discordance was defined as more than 20% difference in birth weight. The following neonatal data were collected: respiratory distress syndrome, necrotizing enterocolitis, patent ductus arteriosus, neonatal sepsis (defined as a clinically ill neonate with positive bacterial culture), and cerebral injury detected with cranial ultrasound (defined as any of the following: cystic periventricular leukomalacia or intraventricular hemorrhage grade 3–4) and neonatal mortality. Cranial ultrasound is routinely performed in all TTTS cases during the neonatal period according to our previously published protocol.[13] At birth, the following hematological and biochemical parameters

are routinely analyzed in all TTTS twins: levels of hemoglobin, albumin and total protein. Blood samples were obtained primarily from umbilical cord blood or from venous blood collected within 12 h after birth. In this study we defined hypoalbuminemia at birth as albumin level < 20 g/l and total protein was considered too low when < 40 g/l.[14–16] The primary outcome measure was albumin level at birth. We compared albumin differences between donors and recipients within the conservative group and within the laser group and between both groups. We hypothesized that albumin levels would be lower in donor twins in the conservative group.

Statistics

Data are reported as medians and interquartile range (IQR). Descriptive analyses on data of the conservative group and laser group were performed. Results of continuous variables within twin pairs were analyzed using paired Student's t test and categorical variables were compared using McNemar's test. Unpaired Student's t test was used to compare continuous variables between the conservative group and the laser group. For statistical analyses, two-sided tests were used and a p value ! 0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 17.0 (SPSS, Inc., Chicago, Ill., USA).

Results

A total of 216 live-born twin pairs with TTTS were admitted to our neonatal nursery during the 10-year study period. 25 (12%) TTTS twin pairs were treated conservatively and included in the conservative group. Seven eligible twin pairs in the conservative group were excluded because of incomplete biochemical data at birth. Twelve TTTS pregnancies (66%) were treated with serial amnioreduction and the other six pregnancies (34%) were managed expectantly because of asymptomatic stage 1 TTTS. We were able to match each twin pair in the conservative group (n = 18) with 2 control TTTS twin pairs (n = 36) treated with laser surgery. Baseline characteristics of both groups are shown in table 1.

TABLE 1 Baseline characteristics in the TTTS group treated conservatively (conservative group) and TTTS group treated with laser surgery (laser group)

	Conservative group (n^a = 36)	Laser group (n^a = 72)
TTTS stage at diagnosis - n (%)		
Stage 1	14 (39)	10 (14)
Stage 2	10 (28)	18 (25)
Stage 3	8 (22)	42 (58)
Stage 4	4 (11)	2 (3)
Treated with serial amnioreduction - n (%)	24 (67)	
Gestational age at diagnosis - weeks[b]	27.0 (24.25 – 29.0)	19.5 (17.25 – 23.75)
Gestational age at birth - weeks[b]	30 (29 – 32)	30 (29 – 32)
Birth weight difference - %[b,c]	15.9 (10.9 – 27.5)	15.9 (5.9 – 26.4)
Caesarean delivery - n (%)	24 (67)	35 (49)
Female - n (%)	24 (67)	46 (64)

[a]Refers to the number of fetuses vs. neonates
[b]Value given as median (interquartile range (IQR))
[c]Birth weight difference was calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100

In the conservative group, median albumin levels in donor twins were significantly lower than in recipient twins, 25.0 versus 33.0 g/l, respectively (p = 0.001) (table 2). Hypoalbuminemia occurred in 22% (4/18) of donor twins. A co-twin (recipient) of 1 of these 4 donor twins had also an albumin level < 20 g/l. In 1 of the donors with severe hypoalbuminemia, skin edema was present. This patient had an albumin level of 17 g/l at birth and received two albumin transfusions, while his twin brother had an albumin level of 36 g/l at birth. Total protein levels < 40 g/l were detected in 50% (9/18) of the donors and 16.6% (3/18) of the recipient twins in the conservative group (p = 0.031). Hemoglobin levels at birth were also significantly lower in donors compared to recipients.

We found no relationship between albumin levels and TTTS stage in the conservatively managed cases.

TABLE 2 Hematological and biochemical differences at birth between donors and recipients

	Conservative group			Laser group		
	Donors (n = 18)	**Recipients (n = 18)**	**p-value**	**Donors (n = 36)**	**Recipients (n = 36)**	**p-value**
Hemoglobin – g/dL[a]	13.6 (11.6-15.7)	16.1 (13.8-20.2)	0.018	16.7 (15.1-18.7)	16.8 (15.2-17.7)	0.640
Albumin – g/L[a]	25.0 (19.5-30.8)	33.0 (28.0-38.0)	0.001	32.0 (30.0-35.0)	32.0 (30.0-34.0)	0.633
Albumin < 25 g/L – n (%)	9 (50)	1 (5.6)	0.008	0 (0)	0 (0)	1.000
Albumin < 20 g/L – n (%)	4 (22)	1 (5.6)	0.250	0 (0)	0 (0)	1.000
Total protein – g/L[a]	40.0 (31.0-48.0)	48.0 (45.0-58.3)	0.002	48.0 (44.3-52.8)	48.0 (44.0-52.5)	0.721
Total protein < 40 g/L – n (%)	9 (50)	3 (16.6)	0.031	4 (11.2)	1 (2.8)	0.250
Inter-twin hemoglobin difference – g/dL[a]		3.6 (1.6-6.0)		1.2 (0.5-2.1)		0.007
Inter-twin albumin difference – g/L[a]		5.0 (2.0-15.3)		2.0 (1.0-4.8)		0.009
Inter-twin total protein difference – g/L[a]		6.5 (2.0-23.8)		3.5 (1.0-6.0)		0.011

[a]*Data given as median (IQR)*

In the laser group, albumin levels in donors and recipients were similar, 32.0 versus 32.0 g/l, respectively (p = 0.633). No differences in levels of hemoglobin, total protein were noted between donors and recipients.

No differences were found in neonatal morbidity or mortality between donors and recipients in the conservative group and laser group (table 3). In the conservative group, neonatal sepsis occurred more frequently in donor twins compared to recipient twins 38.8% (7/18) versus 16.6% (3/18), but the difference did not reach statistical significance.

TABLE 3 Clinical differences between donors and recipients

	Conservative group			Laser group		
	Donors (n = 18)	**Recipients (n = 18)**	**p-value**	**Donors (n = 36)**	**Recipients (n = 36)**	**p-value**
Birth weight – g[a]	1231 (925-1688)	1459 (1265-1819)	0.002	1293 (1008-1697)	1550 (1247-1783)	0.007
RDS – n (%)	10 (56)	10 (56)	1.000	13 (36)	14 (38)	1.000
NEC– n (%)	1 (5.6)	1 (5.6)	1.000	2 (5.6)	2 (5.6)	1.000
PDA– n (%)	1 (5.6)	1 (5.6)	1.000	0 (0)	1 (2.8)	1.000
Cerebral injury – n (%)[b]	1 (2.8)	3 (16.6)	0.500	1 (5.6)	5 (13.8)	0.125
Sepsis – n (%)[c]	7 (38.8)	3 (16.6)	0.344	5 (13.8)	4 (11.2)	1.000
Mortality– n (%)	0 (0.0)	0 (0.0)	1.000	1 (2.8)	1 (2.8)	1.000

RDS = Respiratory distress syndrome, NEC = necrotizing enterocolitis, PDA = patent ductus ateriosus,
a Value given as median (IQR)
b Cerebral injury is defined as any of the following: cystic periventricular leukomalacia or intraventricular hemorrhage grade 3-4
c Sepsis is defined as blood-culture proven clinical sepsis

Discussion

This study shows that donor twins with TTTS treated conservatively have significantly lower levels of albumin and total protein compared to recipient twins and compared TTTS twins treated with fetoscopic laser surgery. In addition, lower hemoglobin levels were only found in donors of the conservative group. Our findings may suggest that patent placental vascular anastomoses allow not only significant loss of hemoglobin from donors to recipients, but also result in loss of albumin. Once placental anastomoses are completely coagulated with laser surgery, levels of albumin or total protein in donors are restored and no inter-twin differences are detected.

Interestingly, albumin levels in recipients in the conservative group were only slightly increased compared to the lasered cases (33 vs. 32 g/l, respectively). Albumin levels in recipient twins could be lower than expected due to hemodilution following (cardiac) overload. Alternatively, our finding could suggest that the inter-twin difference in albumin levels depend on more factors than simply a loss of albumin from donors to recipients. Hypothetically, hypoalbuminemia in donor twins may also result from a decreased production in the liver.
The pathophysiology of TTTS is still not completely understood. The validity of the traditional theory of TTTS being based solely on unbalanced blood shunting from the donor to

the recipient has frequently been questioned [17]. In contrast with twin anemia-polycythemia sequence, which is primarily a transfusion process, the pathophysiology of TTTS seems to be based on several factors including hormonal dysregulation and circulatory imbalance. Nevertheless, the inter-twin transfusion process probably also plays an important role in the development of TTTS.

Inter-twin hematological differences in TTTS have frequently been reported in various studies [3,4]. However, only two small studies have reported on other inter-twin differences including biochemical variables [3,5]. In a small study with cordocentesis in 8 TTTS cases, Berry et al. [3] found significant inter-twin albumin difference between donors and recipients with a median difference of 12.1 g/l, which is higher compared to the inter-twin albumin difference of 8.11 g/l found in our study. In another small study with cordocentesis in 6 TTTS cases, Okamura et al. [5] found normal albumin levels in all twins. Although albumin levels were lower in donor twins, the difference did not reach statistical significance, probably due to the small sample size. Different results between the studies are probably due to differences in methodology, sample sizes, time of measurements (intrauterine vs. postnatal) and cohort-differences.

Albumin has several important physiological properties, including protein binding and transport, maintenance of colloid osmotic pressure, free radical scavenging, platelet function inhibition, antithrombosis and -alterations of vascular permeability [6]. Albumin is -produced in the liver, but not stored [18]. It is immediately excreted into the hepatic lymph system or the sinusoids and circulates from the vascular space across the capillary wall into the interstitium and returns to the vascular space via the lymphatic system [6,18]. This circulation half-life of albumin is approximately 16-18 h and degradation half-life is 17-20 days [6]. Hypothetically, the loss of albumin and total protein, and possibly other important nutrients, may also play a role in the deterioration of the intrauterine clinical condition of the donor twin in TTTS and may prevent adequate growth. In this study we did not detect an increased risk of neonatal morbidities or mortality in donor twins, but our study was not designed to detect such differences.

Our results should be interpreted with care due to the retrospective nature of the study. In addition, a selection towards milder TTTS cases was present in the conservative group, which is inherent with the conservative treatment. Most severe TTTS cases in our country are treated primarily with laser surgery. This means that our research might not be truly representative for most twins with TTTS. Nevertheless, our results show that 50% (9/18) of the donors in the conservative group has an albumin level <25 g/l, while this never occurred in the laser group. Albumin levels appear thus to restore after fetoscopic laser surgery, which could partly explain the catch-up growth detected in donor twins after laser surgery [19].

In conclusion, significant albumin differences are found between donors and recipients with TTTS treated conservatively but not after laser surgery. Our data do not support that hypoalbuminemia per se is associated with an increased risk of neonatal morbidity and mortality in donor twins, but larger studies are required to detect differences in neonatal outcome.

References

1 Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J: The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-518.

2 Lewi L, Gucciardo L, Van Mieghem T, De Koninck P, Beck V, Medek H, Van Schoubroeck D, Devlieger R, De Catte L, Deprest J: Monochorionic diamniotic twin pregnancies: natural history and risk stratification. Fetal Diagn Ther 2010;27:121-133.

3 Berry SM, Puder KS, Bottoms SF, Uckele JE, Romero R, Cotton DB: Comparison of intrauterine hematologic and biochemical values between twin pairs with and without stuck twin syndrome. Am J Obstet Gynecol 1995;172:1403-1410.

4 Denbow M, Fogliani R, Kyle P, Letsky E, Nicolini U, Fisk N: Haematological indices at fetal blood sampling in monochorionic pregnancies complicated by feto-fetal transfusion syndrome. Prenat Diagn 1998;18:941-946.

5 Okamura K, Murotsuki J, Kosuge S, Tanigawara S, Yajima A: Diagnostic use of cordocentesis in twin pregnancy. Fetal Diagn Ther 1994;9:385-390.

6 Margarson MP, Soni N: Serum albumin: touchstone or totem? Anaesthesia 1998;53:789-803.

7 Vincent JL, Dubois MJ, Navickis RJ, Wilkes MM: Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. Ann Surg 2003;237:319-334.

8 Morris I, Molloy EJ: Albumin administration in the management of neonatal hypoalbuminaemia. Arch Dis Child Fetal Neonatal Ed 2008;93:F326.

9 Morris I, McCallion N, El-Khuffash A, Molloy EJ: Serum albumin and mortality in very low birth weight infants. Arch Dis Child Fetal Neonatal Ed 2008;93:F310-F312.

10 Atkinson SD, Tuggle DW, Tunell WP: Hypoalbuminemia may predispose infants to necrotizing enterocolitis. J Pediatr Surg 1989;24:674-676.

11 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

12 Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M: Staging of twin-twin transfusion syndrome. J Perinatol 1999;19:550-555.

13 Lopriore E, Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ: Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006;194:1215-1220.

14 Reading RF, Ellis R, Fleetwood A: Plasma albumin and total protein in preterm babies from birth to eight weeks. Early Hum Dev 1990;22:81-87.

15 Cartlidge PH, Rutter N: Serum albumin concentrations and oedema in the newborn. Arch Dis Child 1986;61:657-660.

16 Moniz CF, Nicolaides KH, Bamforth FJ, Rodeck CH: Normal reference ranges for biochemical substances relating to renal, hepatic, and bone function in fetal and maternal plasma throughout pregnancy. J Clin Pathol 1985;38:468-472.

17 Lopriore E, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ: Twin-to-twin transfusion syndrome: from placental anastomoses to long-term neurodevelopmental outcome. Curr Pediatr Rev 2005;1:191-203.

18 Doweiko JP, Nompleggi DJ: Role of albumin in human physiology and pathophysiology. JPEN J Parenter Enteral Nutr 1991;15:207-211.

19 Maschke C, Franz AR, Ellenrieder B, Hecher K, Diemert A, Bartmann P: Growth after intrauterine laser coagulation for twin-twin transfusion syndrome. Arch Dis Child Fetal Neonatal Ed 2010;95:F115-F117.

Chapter 6
Hypoalbuminemia in donors with twin anemia-polycythemia sequence: a matched case-control study

Lianne Verbeek
Femke Slaghekke
Christian V. Hulzebos
Dick Oepkes
Frans J. Walther
Enrico Lopriore

Fetal Diagn Ther. 2013;33(4):241-5

Abstract

Objective: To determine the differences in albumin levels between donors and recipients with twin anemia-polycythemia sequence (TAPS).

Methods: We included all consecutive monochorionic twins with TAPS with double survivors. Each twin pair was matched for gestational age at birth with 2 control monochorionic twin pairs unaffected by TAPS or twin-twin transfusion syndrome. We measured levels of albumin, total protein, and hemoglobin on the first day of life in donors and recipients (TAPS group) and the control group.

Results: A total of 25 TAPS twin pairs and 50 control twin pairs were included in the study. The median gestational age at birth was 32 weeks in both groups. In the TAPS group, median levels (IQR) of albumin in donor twins were significantly lower than in recipient twins, i.e. 28.0 g/l (24.0-32.0) versus 32.0 g/l (30.0-34.5) ($p = 0.008$). Median levels (IQR) of total protein in donor twins were also lower than in recipients, i.e. 44.0 g/l (36.5-49.0) versus 49.0 g/l (46.5-51.0), respectively ($p = 0.004$). The median (IQR) intertwin albumin difference was significantly higher in the TAPS group than in the control group, i.e. 4.0 g/l (2.5-10.5) versus 2.0 g/l (1.0-4.0) ($p = 0.003$). The rate of hypoalbuminemia (<20 g/l) and hypoproteinemia (<40 g/l) in donor twins with TAPS was 20% (5/25) and 32% (8/25).

Conclusions: In addition to lower hemoglobin levels, donor twins with TAPS also have significantly lower albumin and total protein levels compared to recipient twins.

Introduction

Vascular anastomoses are almost invariably present in all monochorioric twin placentas and may lead to several complications including twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) [1]. TTTS occurs in 9% of monochorionic twins and is characterized by the development of twin oligo-polyhydramnios sequence (TOPS) [2]. In contrast, TAPS is characterized by large intertwin hemoglobin differences without signs of TOPS [3]. TAPS can be detected in 2-13% of TTTS pregnancies treated with laser surgery (postlaser TAPS) [4,5] and can also occur spontaneously in about 3-5% of monochorionic twin pregnancies (spontaneous TAPS) [6]. TAPS placentas are characterized by the presence of only few minuscule arteriovenous placental anastomoses allowing slow but chronic intertwin blood transfusion, resulting in chronic anemia (with reticulocytosis) in donors and polycythemia in recipients [3].

Knowledge on biochemical differences between donors and recipients in TAPS is sparse but important. Hypoalbuminemia in neonates is an independent risk factor for mortality and morbidity [7,8] and has been associated with various adverse clinical conditions like necrotizing enterocolitis, respiratory distress syndrome, intracranial hemorrhage, sepsis, chronic lung disease, and edema [9,10,11]. In analogy to hypoproteinemia in donors with TTTS, we hypothesize that similar differences may be detected in TAPS twins.

The aim of this study was to determine the differences in albumin and total protein between donor and recipient twins with TAPS compared to a control group of uncomplicated monochorionic twins matched for gestational age at birth.

Methods

We conducted a retrospective analysis of consecutive monochorionic twin pairs with TAPS (TAPS group) and uncomplicated monochorionic twins (control group) delivered at the Leiden University Medical Center (The Netherlands) between August 2003 and August 2012. The Leiden University Medical Center is a tertiary care center managing all types of complications of monochorionic pregnancies and a national referral center for fetoscopic laser treatment for TTTS.

For the purpose of this study, TAPS was diagnosed using the following proposed postnatal criteria: an intertwin Hb difference >8.0 g/dl and at least one of the following: reticulocyte count ratio >1.7 or placenta with only small (diameter <1 mm) vascular anastomoses [3]. Each twin pair with TAPS was compared with 2 uncomplicated monochorionic twin pairs unaffected by TAPS or TTTS and matched for gestational age at birth (±1 week). We excluded all twin pregnancies with single or double intrauterine death, TAPS cases treated with fetoscopic laser surgery, and cases with incomplete placental injection study. At birth, levels of hemoglobin, reticulocyte count, albumin, and total protein were measured routinely in all twins. Blood samplings were primarily obtained from umbilical cord. If umbilical cord blood was not available, venous blood samplings were obtained on day 1. We defined hypoalbuminemia at birth as an albumin level <20 g/l and hypoproteinemia as a total protein level <40 g/l [12,13,14].

We recorded the following perinatal variables: gestational age at birth, birth weight, birth weight discordance, individual placental territory, and placental territory discordance.

Birth weight discordance was assessed and calculated as follows: [(birth weight of the larger twin - birth weight of the smaller twin)/birth weight of the larger twin] × 100. The definition of small for gestational age was a birth weight <2 SD according to the growth charts for the Dutch population [15]. The percentage of individual placental territory was determined by dividing each individual placental territory by the sum of both territories. The following neonatal data were collected: respiratory distress syndrome, necrotizing enterocolitis, patent ductus arteriosus, sepsis, mortality, and cerebral injury. Cerebral injury was defined as the presence of at least 1 of the following findings: cystic periventricular leukomalacia ≥grade 2, intraventricular hemorrhage ≥grade 3, ventricular dilatation, arterial or venous infarct, or other severe cerebral dilatation detected with cranial ultrasound. Sepsis was defined as a clinically ill neonate with positive bacterial culture. Neonatal mortality was defined as death within 28 days after birth.

Primary outcome was the level of albumin and total protein at birth which was compared between donors and recipients in the TAPS group and between the smaller one (lowest birth weight) and the larger one in the control group. We also calculated the intertwin difference in levels of albumin, total protein, and hemoglobin and compared the results between the TAPS group and the control group.

Statistics

Data are reported as medians and IQR. Results of continuous variables within twin pairs were analyzed using a related-samples Wilcoxon signed rank test (when not normally distributed). Similarly, a Mann-Whitney test was used to compare continuous variables between the TAPS group and the control group. For analyses of paired nominal variables, the McNemar test was used. For statistical analyses, two-sided tests were used and $p < 0.05$ was considered statistically significant. Analysis was performed using SPSS version 17 (SPSS, Inc., Chicago, Ill., USA).

Results

During the 10-year study period, 216 monochorionic twins were born at our center. TAPS was diagnosed in 32 (15%) twins. Seven eligible twin pairs (22%) in the TAPS group were excluded because of incomplete biochemical data at birth. Of the remaining 25 TAPS twin pairs, 6 (24%) twins were spontaneous TAPS cases and 19 (76%) were postlaser TAPS cases. In the 19 postlaser TAPS twins the mean gestational age at laser treatment for TTTS was 21 weeks (IQR 18-25). Each TAPS twin pair (n = 25) was matched with 2 uncomplicated monochorionic twins (n = 50). The baseline characteristics in the TAPS and control groups are listed in table 1.

TABLE 1 Baseline characteristics in the twins with TAPS (TAPS group) and monochorionic twins uncomplicated by TAPS or TTTS (control group)

	TAPS group (N[c] = 50)	Control group (N[c] = 100)
Caesarean delivery - no. (%)	28 (56%)	44 (44%)
Female – no. (%)	24 (48%)	52 (52%)
Gestational age at birth – wk[a]	32 (29 – 34)	32 (29 – 33)
Birth weight difference - %[a,b]	10.4 (4.5 – 18.5)	10.4 (4.3 – 24.7)
Small for gestational age – no. (%)	2 (4%)	4 (4%)

[a]*Value given as median (IQR)*
[b]*Birth weight difference was calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100*
[c]*Refers to the number of fetuses cq neonates*

In the TAPS group, median albumin levels at birth in donors were significantly lower compared to those of the recipient twins, i.e. 28.0 versus 32.0 g/l (p = 0.008) (table 2). Hypoalbuminemia (albumin level <20 g/l) was detected in 5 donors (20%). Skin edema was present in 2 of the donors with hypoalbuminemia. The first infant had an albumin level of 16 g/l (and required several albumin infusions) while her cotwin sister had an albumin level of 27 g/l. In the second case the donor had an albumin level of 11 g/l, and received several albumin infusions, while his cotwin brother had an albumin level of 33 g/l. On day 8 the infant died of severe respiratory and circulatory failure and persistent pulmonary hypertension of the newborn. In another donor with hypoalbuminemia (albumin of 13 g/l), fetal hydrops at birth was diagnosed. His cotwin brother had an albumin of 34 g/l. Albumin infusions were given in combination with diuretics to treat the hypoalbuminemia. The donor died due to multiorgan failure. In the 2 other infants with hypoalbuminemia no fetal hydrops was present and treatment with albumin transfusions was not administered.

TABLE 2 Hematological and biochemical differences at birth between donors and recipients (TAPS group) and smaller and larger twins (control group)

	TAPS group			Control group		
	Donors (N = 25)	Recipients (N = 25)	p-value	Smaller twins (N = 50)	Larger twins (N = 50)	p-value
Hemoglobin – g/dL[a]	9.5 (7.7 – 11.2)	22.6 (20.6 - 24.5)	0.000	16.3 (14.7 – 18.5)	16.5 (15.3 – 18.1)	0.882
Albumin – g/L[a]	28.0 (24.0 – 32.0)	32.0 (30.0 - 34.5)	0.008	31.0 (29.0 – 33.0)	31.0 (28.8 – 35.0)	0.692
Albumin < 25 g/L – no. (%)	6 (24%)	0 (0%)	0.031	1 (2%)	5 (10%)	0.375
Albumin < 20 g/L – no. (%)	5 (20 %)	0 (0%)	0.063	1 (2%)	0 (0%)	1.000
Total protein – g/L[a]	44.0 (36.5 – 49.0)	49.0 (46.5– 51.0)	0.004	46.0 (43.0– 1.5)	47.0 (42.3–53.0)	0.656
Total protein < 40 g/L – no. (%)	8 (32%)	1 (4%)	0.039	4 (8%)	7 (14%)	0.453

[a]*Data given as median (IQR)*

All of the recipients in the TAPS group had an albumin level >25 g/l. One infant in the control group had a albumin level <20 g/l.

Median levels of total protein in donors were lower than in recipients, i.e. 44.0 g/l versus 49.0 g/l (p = 0.004). Hypoproteinemia was detected in 32% (8/25) of the donor twins and in 4% (1/25) of the recipient twins (p = 0.039). Intertwin hematological and biochemical differences at birth between the TAPS group and control group are shown in table 3.

TABLE 3 Inter-twin hematological and biochemical differences at birth between the TAPS group and the control group

	TAPS group	Control Group	p-value
Inter-twin hemoglobin difference – g/dL[a]	12.2 (9.7–15.3)	1.2 (0.3–3.7)	0.000
Inter-twin albumin difference – g/L[a]	4.0 (2.5–10.5)	2.0 (1.0–4.0)	0.003
Inter-twin total protein difference – g/L[a]	7.0 (3.0–14.0)	3.0 (2.0–6.8)	0.012

[a]*Data given as median (IQR)*

Clinical and placental characteristics in twins in the TAPS group and control group are presented in table 4. We found no differences in neonatal mortality and morbidity between donors and recipients in the TAPS group. Donors in the TAPS group had a lower birth weight than recipients, but larger individual placental territories, whereas the lower birth weight in the smaller twins in the control group was correlated with a smaller placental share.

TABLE 4 Clinical outcome and placental characteristics in the TAPS group and control group

	TAPS group			Control group		
	Donors (N = 25)	Recipients (N = 25)	p-value	Smaller twins (N = 50)	Larger twins (N = 50)	p-value
Birth weight – gr[a]	1459 (1117–1815)	1695 (1208–1893)	0.016	1435 (1141–1733)	1671 (1385–2013)	0.000
Individual placental territory - %[a]	53.6 (47.2–64.0)	46.5 (36.0–52.8)	0.107	42.4 (36.0–51.7)	57.6 (48.4–64.0)	0.007
RDS– no. (%)	5 (20%)	6 (24%)	1.000	15 (30%)	20 (40%)	0.180
NEC– no. (%)	0 (0%)	0 (0%)	1.000	5 (10%)	4 (8%)	1.000
PDA– no. (%)	2 (8%)	0 (0%)	0.500	2 (4%)	2 (4%)	1.000
Cerebral injury – no. (%)[b]	1 (4%)	1 (4%)	1.000	3 (6%)	2 (4%)	1.000
Sepsis – no. (%)[c]	4 (16%)	4 (16%)	1.000	6 (12%)	6 (12%)	1.000
Mortality– no. (%)	2 (8%)	1 (4%)	1.000	1 (2%)	3 (6%)	0.500

[a]Data given as median (IQR)
[b]Cerebral injury is defined as any of the following: cystic periventricular leukomalacia grade 2 or higher, intraventricular hemorrhage grade 3-4, ventricular dilatation, arterial or venous infarct or other severe cerebral dilatation [c]Sepsis is defined as blood-culture proven clinical sepsis

Abbreviations: RDS = Respiratory distress syndrome, NEC = necrotizing enterocolitis, PDA = patent ductus arteriosus

Discussion

TAPS was first described in 2007 [16] and data on the pathophysiology of TAPS is mostly limited to reports on hematological features of TAPS [17]. This is the first study evaluating differences in biochemical variables in TAPS twins and showing that donor twins with TAPS have significantly lower levels of albumin and total protein levels at birth compared to recipients, in addition to lower hemoglobin levels. In a previous study, we and others found similar differences in albumin and total protein between donors and recipients with TTTS [18,19]. The intertwin albumin difference in the TTTS group in our previous study was slightly larger (5.0 g/l) compared to the TAPS group in the current study (4 g/l) [20].

Our data may suggest that placental vascular anastomoses in TAPS, such as in TTTS, allow intertwin transfusion not only of hemoglobin but also of albumin and total protein [16]. Another explanation could be that the lower albumin and total protein levels in donors may not only be due to loss of proteins into the circulation of the recipients but could also be due to a reduced production of albumin in donors.

An additional finding of this study concerns the correlation between birth weight and individual placental share. The smaller twins in the control group have a significantly lower individual placental territory, confirming the well-known association between placental function and birth weight [1]. In contrast, although donors in the TAPS group have larger placental shares than recipients, we found a trend in lower birth weights compared to recipients. This paradoxical finding was previously described by Lewi et al. [21]. It is tempting to speculate that the lower levels of albumin and total protein may play a role in the reduced growth of donors despite the larger placental share.

Albumin, which makes up 50% of the normal intravascular protein mass and is responsible for 75-80% of the plasma colloid pressure, has several important physiological properties. Albumin is involved in protein binding and transport of many endogenous and exogenous substances (e.g. drugs, bilirubin) and acts as a free radical scavenger. Furthermore, albumin inhibits platelet function and has antithrombotic effects and finally affects vascular permeability [9,22]. Serum albumin concentrations increase with gestational age. Possible explanations for this could be increased synthesis by the fetal liver [23] or the greater placental transfer of albumin towards term [24]. Serum levels of albumin may fall during periods of stress, trauma, or sepsis [25] despite its long half-life of 17-19 days [22].

Although hypoalbuminemia is associated with an increased risk of neonatal morbidity and mortality [7,8], we found no differences in morbidity or mortality between donors and recipients. However, our study was not designed to detect such differences.

The data in this study should be interpreted with care due to the known methodological limitations associated with retrospective study designs.

In conclusion, our data add to the understanding of the pathophysiologic characteristics of TAPS. Our findings confirm that placental vascular anastomoses in TAPS may allow transport not only of hemoglobin from donors to recipients but also of albumin and total protein.

References

1 Lewi L, Cannie M, Blickstein I, Jani J, Huber A, Hecher K, Dymarkowski S, Gratacos E, Lewi P, Deprest J: Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. Am J Obstet Gynecol 2007;197:587-588.

2 Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, Done E, Boes AS, Hecher K, Gratacos E, Lewi P, Deprest J: The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514-518.

3 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.

4 Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R, Crombleholme T: Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201:417.

5 Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J, Ville Y: Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194:796-803.

6 Gucciardo L, Lewi L, Vaast P, Debska M, De CL, Van MT, Done E, Devlieger R, Deprest J: Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438-442.

7 Vincent JL, Dubois MJ, Navickis RJ, Wilkes MM: Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. Ann Surg 2003;237:319-334.

8 Morris I, McCallion N, El-Khuffash A, Molloy EJ: Serum albumin and mortality in very low birth weight infants. Arch Dis Child Fetal Neonatal Ed 2008;93:F310-F312.

9 Margarson MP, Soni N: Serum albumin: touchstone or totem? Anaesthesia 1998;53:789-803.

10 Morris I, Molloy EJ: Albumin administration in the management of neonatal hypoal buminaemia. Arch Dis Child Fetal Neonatal Ed 2008;93:F326.

11 Atkinson SD, Tuggle DW, Tunell WP: Hypoalbuminemia may predispose infants to necrotizing enterocolitis. J Pediatr Surg 1989;24:674-676.

12 Reading RF, Ellis R, Fleetwood A: Plasma albumin and total protein in preterm babies from birth to eight weeks. Early Hum Dev 1990;22:81-87.

13 Cartlidge PH, Rutter N: Serum albumin concentrations and oedema in the newborn. Arch Dis Child 1986;61:657-660.

14 Moniz CF, Nicolaides KH, Bamforth FJ, Rodeck CH: Normal reference ranges for biochemical substances relating to renal, hepatic, and bone function in fetal and maternal plasma throughout pregnancy. J Clin Pathol 1985;38:468-472.

15 Kloosterman GJ: Intrauterine growth and intrauterine growth curves. Ned Tijdschr Verloskd Gynaecol 1969;69:349-365.

16 Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP: Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47-51.

17 Lopriore E, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Walther FJ: Hematological characteristics in neonates with twin anemia-polycythemia sequence (TAPS). Prenat Diagn 2010;30:251-255.

18 Berry SM, Puder KS, Bottoms SF, Uckele JE, Romero R, Cotton DB: Comparison of intrauterine hematologic and biochemical values between twin pairs with and without stuck twin syndrome. Am J Obstet Gynecol 1995;172:1403-1410.

19 Okamura K, Murotsuki J, Kosuge S, Tanigawara S, Yajima A: Diagnostic use of cordo centesis in twin pregnancy. Fetal Diagn Ther 1994;9:385-390

20 Verbeek L, Middeldorp JM, Hulzebos CV, Oepkes D, Walther F, Lopriore E. Hypoalbuminemia in donors with twin-twin transfusion syndrome. Fetal Diagn Ther, in press.
21 Lewi L, Gucciardo L, Huber A, Jani J, Van Mieghem T, Done E, Cannie M, Gratacos E, Diemert A, Hecher K, Lewi P, Deprest J: Clinical outcome and placental characteristics of monochorionic diamniotic twin pairs with early- and late-onset discordant growth. Am J Obstet Gynecol 2008;199:511.e1-511.e7.
22 Doweiko JP, Nompleggi DJ: Role of albumin in human physiology and pathophysiology. J Parenter Enteral Nutr 1991;15:207-211.
23 Dancis J, Braverman N, Lind J: Plasma protein synthesis in the human fetus and placenta. J Clin Invest 1957;36:398-404.
24 Gitlin D, Kumate J, Urrusti J, Morales C: The selectivity of the human placenta in the transfer of plasma proteins from mother to fetus. J Clin Invest 1964;43:1938-1951.
25 Mendez CM, McClain CJ, Marsano LS: Albumin therapy in clinical practice. Nutr Clin Pract 2005;20:314-320.

Chapter 7
Short-term postnatal renal function in twin anemia-polycythemia sequence

Lianne Verbeek
Femke Slaghekke
Romain Favre
Marine Vieujoz
Francesco Cavigioli
Gianluca Lista
Dick Oepkes
Enrico Lopriore

Fetal Diagn Ther. 2016;39(3):192-7

Abstract

Objective: To evaluate the short-term renal function in neonates with twin anemia-polycythemia sequence (TAPS).

Methods: All consecutive monochorionic twins with TAPS with double survivors admitted to three European centers were included in this retrospective study. Each twin pair was matched for gestational age at birth with a control twin pair unaffected by TAPS or twin-twin transfusion syndrome. Creatinine and urea levels in the first week after birth were recorded. Short-term postnatal renal dysfunction was defined as creatinine >100 μmol/l during the first week after birth.

Results: A total of 52 TAPS twin pairs and 52 control twin pairs with a median gestational age of 31 weeks at birth were included in the study. In the TAPS group, donors had higher mean creatinine levels compared to recipients, 85 versus 71 μmol/l, respectively ($p = 0.001$). Short-term renal dysfunction was detected in 26.0% (13/50) of the donors versus 6.3% (3/48) of the recipients ($p = 0.022$). In the control group, no inter-twin differences in creatinine levels were found.

Conclusions: Donor twins with TAPS have higher creatinine levels than recipient twins, suggesting that chronic inter-twin transfusion in TAPS may also cause short-term renal dysfunction. Long-term renal consequences in TAPS donors require further investigation.

Introduction

Twin anemia-polycythemia sequence (TAPS) is a recently described disorder, which is characterized by large inter-twin hemoglobin differences without signs of twin oligo-polyhydramnios sequence [1]. TAPS can be detected in 2-16% of the twin-twin transfusion syndrome (TTTS) pregnancies treated with laser surgery (post-laser TAPS) [1,2,3,4] and can also occur spontaneously in about 3-5% of the monochorionic twin pregnancies (spontaneous TAPS) [5]. The pathophysiology of TAPS is based on the presence of few minuscule arteriovenous placental anastomoses allowing slow but chronic inter-twin blood transfusion, resulting in chronic anemia (with reticulocytosis) in donors and polycythemia in recipients [1].

In TTTS, various renal complications have been reported in donor twins, including renal cortical necrosis and fibrosis, transient renal insufficiency, acute renal failure requiring long-term peritoneal dialysis or permanent tubular dysfunction with polyuria due to renal dysgenesis [6]. In TAPS, knowledge on biochemical differences between donors and recipients is sparse. Recent studies have shown that donors in TAPS have lower albumin and total protein levels due to chronic blood transfusion into the circulation of the recipient, causing loss of nutrients [7]. Whether chronic blood loss in TAPS donors may also cause renal impairment is not known. The aim of this retrospective study is to evaluate the short-term renal function in donors and recipients with TAPS, compared to a control group of uncomplicated monochorionic twins matched for gestational age at birth.

Patients and Methods

We performed a retrospective analysis of all consecutive monochorionic twin pairs with TAPS admitted between August 2003 and December 2014 to the neonatal intensive care unit of three European tertiary care centers: the Leiden University Medical Center (The Netherlands), Strasbourg Teaching Hospital (France) and V. Buzzi Children Hospital, Milan (Italy). The three centers are tertiary care centers managing all types of complications of monochorionic pregnancies and serve as regional and national referral centers for fetoscopic laser treatment for TTTS or TAPS. TAPS was diagnosed using previously reported antenatal and/or postnatal criteria. Antenatal criteria included an increased peak systolic velocity in the middle cerebral artery >1.5 multiples of the median in the donor twin, and a reduced peak systolic velocity in the middle cerebral artery (<1.0 multiples of the median) in the recipient twin, in the absence of oligo-polyhydramnios sequence. Postnatal criteria included an inter-twin hemoglobin (Hb) difference >8.0 g/dl and at least one of the following: reticulocyte count ratio >1.7 or a placenta with only small (diameter <1 mm) placental vascular anastomoses [4].

To compensate for the lack of data on normal creatinine levels per gestational age, a control group was incorporated in the study design. Each twin pair with TAPS was compared with an uncomplicated monochorionic twin pair unaffected by TAPS or TTTS. The control pregnancies were the next uncomplicated twin pregnancy delivered at a matched gestational age (±1 week of gestation). All neonates of the control group were born at the Leiden University Medical Center. Since the vast majority of donor twins with TAPS are smaller than recipients [8], we compared the data in the control twin pairs between the smaller twin and the larger twin. We excluded all twin pregnancies with single or double intrauterine death, TAPS cases treated with fetoscopic laser surgery and cases with congenital urinary tract anomalies.

At birth, the following neonatal parameters were routinely measured and recorded in all twins: blood pressure, heart rate, Hb levels and reticulocyte count. Blood pressure was recorded noninvasively. Creatinine and urea levels were routinely measured in the first week after birth. We excluded creatinine and urea levels measured within 48 h after birth to reduce the maternal influence on creatinine and urea levels in the neonates. When multiple values were measured, we included the highest values. We also compared the creatinine and urea levels between the subgroups with spontaneous TAPS cases and post-laser TAPS cases. Creatinine measurement was performed using an enzymatic method (Roche Modular P800).

We recorded the urine output (in ml/kg/h) during the first 3 days after birth. Short-term postnatal renal dysfunction was defined as creatinine >100 μmol/l during the first week after birth.

The following perinatal variables were recorded: gender, gestational age at birth, birth weight, birth weight discordance, asphyxia, patent ductus arteriosus and neonatal mortality. Birth weight discordance was assessed and calculated as follows: [(birth weight larger twin - birth weight smaller twin)/birth weight larger twin] × 100. Asphyxia was defined as the presence of at least three of the following criteria: decelerative cardiotocogram, an arterial umbilical cord pH level <7.10, a 5-min Apgar score <5, spontaneous breathing >5 min after birth or multi-organ failure. Neonatal mortality was defined as death within 28 days after birth.

Statistics

Data were reported as means and standard deviations (SD). We calculated that a minimum of 42 TAPS twin pairs were required to demonstrate a difference in creatinine levels of 10 μmol/l with an SD of 20 μmol/l between donors and recipients, with a significance of 0.05 and a power of 80%, by two-tailed analysis. The results of the continuous variables within twin pairs were analyzed using the related-samples Wilcoxon signed-rank test. The Mann-Whitney U test was used to compare the continuous variables between the TAPS group and the control group. For analyses of paired nominal variables, the McNemar test was used. For statistical analyses, two-sided tests were employed, and a p value of <0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 17 (SPSS, Inc., Chicago, Ill., USA).

Results

A total of 52 TAPS twin pairs fulfilled the inclusion criteria, of which 39 (75%) were born in Leiden, 10 (19%) in Strasbourg and 3 (6%) in Milan. The characteristics of the included patients in the TAPS group and control group of uncomplicated MC twins are summarized in table 1. Of the 52 TAPS twin pairs, 18 (35%) pairs were spontaneous TAPS cases and 34 (65%) were post-laser TAPS cases.

TABLE 1 Baseline characteristics in study group with TAPS and control group

	TAPS group N[a] = 104	Control group N[a] = 104	P-value
Cesarean delivery – no. (%)	70 (67.3%)	45 (43.3%)	0.001
Female – no. (%)	50 (48.1%)	48 (46.2%)	0.782
Gestational age at birth - wk[b]	31 ± 2	31 ± 3	0.584
Birth weight difference - %[c]	15.9 ± 12.0	13.4 ± 13.3	0.127

[a]Refers to the number of neonates
[b]Value given as mean ± SD
[c]Birth weight difference was calculated as follows: ((birth weight larger twin – birth weight smaller twin)/ birth weight larger twin) x 100

Table 2 shows the hematological and biochemical differences at birth between donors and recipients (TAPS group) and the control group. In the TAPS group, creatinine and urea levels from the donor and recipient were measured on the same day in 63% of the cases. In 10% of the cases, there was a 1-day difference between the two measurements, in 14% a 2-day difference and in 12% a 3-day difference. In the control group, creatinine and urea levels were measured on the same day in both neonates in 68%, with a 1-day difference in 20%, a 2-day difference in 8% and a 3-day difference in 4%. Donor twins had significantly higher creatinine levels during the first week after birth compared to recipients in the TAPS group, 85 and 71 µmol/l, respectively (p = 0.001). Short-term renal dysfunction (a creatinine level >100 µmol/l) was detected in 26.0% (13/50) of the donors versus 6.3% (3/48) of the recipients (p = 0.022). None of the TAPS donors had evidence of severe renal damage of renal failure requiring treatment during the neonatal period. In the control group, no inter-twin differences in creatinine were found (table 2). Renal dysfunction was detected in 3 (5.9%) of the smaller twins and in 5 (9.8%) of the larger twins.

TABLE 2 Hematological and biochemical differences at birth between donors and recipients (TAPS group) and smaller and larger twins (control group)

	TAPS group			Control group		
	Donors (N = 52)	Recipients (N = 52)	p-value	Smaller twins (N = 52)	Larger twins (N = 52)	p-value
Hemoglobin – g/dL[a]	8.8 ± 2.8	21.6 ± 3.1	0.000	16.4 ± 3.1	16.0 ± 2.6	0.289
Reticulocyte count –promille[a]	138 ± 85[b]	51 ± 48[c]	0.000	68 ± 20[d]	68 ± 22[e]	0.228
Inter-twin hemoglobin difference – g/dL[a]	12.8 ± 4.2			2.3 ± 2.0		0.000
Inter-twin reticulocyte count - promille[a]	91 ± 59[b]			10 ± 28[d]		0.000
Creatinine week 1 - µmol/l[a]	85 ± 27[f]	71 ± 17[b]	0.001	76 ± 15[g]	78 ± 24[g]	0.727
Day after birth creatinine measured[a]	3.8 ± 1.8[f]	3.4 ± 1.5[b]	0.109	3.1 ± 1.9[g]	3.3 ± 1.3[f]	0.263
Creatinine >100 µmol/l[a] – no. (%)	13 (26.0%)[f]	3 (6.3%)[b]	0.022	3 (5.9%)[g]	5 (9.8%)[g]	0.687
Urea week 1 – mmol/l[a]	6.0 ± 3.5[f]	5.9 ± 3.4[b]	0.548	5.0 ± 2,2[g]	5.8 ± 2,4[g]	0.007
Day after birth urea measured[a]	3.8 ± 1.7[f]	3.5 ± 1.6[b]	0.553	3.3 ± 1.3[g]	3.1 ± 1.9[f]	0.263
Inter-twin creatinine difference - µmol/l[a]	20 ± 22[b]			13 ± 22[c]		0.007
Inter-twin urea difference - mmol/l[a]	2.1 ± 2.0[b]			2.1 ± 2.9[c]		0.665

[a]Value given as mean ± SD.

[b]Assessed in 48/52 twin pairs. [c]Assessed in 49/52 neonates. [d]Assessed in 39/52 twin pairs. [e]Assessed in 40/52 twin pairs. [f]Assessed in 50/52 twin pairs. [g]Assessed in 51/52 neonates.

Analyses regarding creatinine and urea levels between the spontaneous TAPS group and the post-laser TAPS group are shown in table 3. Differences between donors and recipients appeared to be slightly more prominent in the spontaneous TAPS group.

TABLE 3 Biochemical differences at birth between donors and recipients in the spontaneous TAPS group and in the post-laser TAPS group

	Spontaneous TAPS			Post-laser TAPS		
	Donors (N=18)	**Recipients (N=18)**	**p-value**	**Donors (N=34)**	**Recipients (N=34)**	**p-value**
Creatinine week 1 - µmol/l[a]	88 ± 27[b]	70 ± 17[b]	0.006	84 ± 28[c]	73 ± 18[c]	0.051
Day after birth creatinine measured[a]	3.8 ± 1.8[b]	3.1 ± 1.5[b]	0.121	3.8 ± 1.9[c]	3.5 ± 1.5[c]	0.509
Creatinine >100 µmol/l[a] – no. (%)	6 (35.3%)b	1 (5.9%)[b]	0.063	6 (18.8%)c	3 (9.4%)[c]	0.289
Urea week 1 – mmol/l[a]	5.6 ± 3.4[b]	4.9 ± 3.5[b]	0.326	6.1 ± 3.7[c]	6.4 ± 3.2[c]	0.918
Day after birth urea measured[a]	4.3 ± 1.7[d]	3.4 ± 1.7[b]	0.150	3.7 ± 1.8[c]	3.6 ± 1.6[e]	0.472
Inter-twin creatinine difference - µmol/l[a]		22 ± 20			20 ± 23	0.418
Inter-twin urea difference - mmol/l[a]		2.4 ± 2.5			1.9 ± 1.7	0.991

[a]Value given as mean ± SD. [b]Assessed in 17/18 neonates. [c]Assessed in 32/34 neonates. [d]Assessed in 16/18 neonates. [e]Assessed in 31/34 neonates.

Additional information on the hemodynamic and circulatory clinical condition of neonates in the TAPS and control groups is shown in table 4. The heart rate at birth was similar in donors and recipients, but blood pressure was significantly lower in donors compared to recipients. The mean blood pressure at birth in donors and recipients was 37 ± 9 and 43 ± 10 mm Hg, respectively (p = 0.009). Urine output on day 1 was significantly lower in donor twins compared to recipient twins, 2.1 ± 2.0 and 2.4 ± 1.4 ml/kg/h, respectively (p = 0.033). The mean urine output during the first 3 days in donors and recipients in the TAPS group was 3.2 ± 1.6 and 3.6 ± 1.5 ml/kg/h, respectively (p = 0.188).

TABLE 4 Clinical outcome differences between donors and recipients (TAPS group) and smaller and larger twins (control group)

	TAPS group			Control group		
	Donors (N = 52)	Recipients (N = 52)	p-value	Smaller twins (N = 52)	Larger twins (N = 52)	p- value
Birth weight – gr[a]	1434 ± 402	1585 ± 415	0.000	1396 ± 392	1605 ± 428	0.000
SBP at birth – mmHg[b]	53 ± 9[c]	59 ± 12[d]	0.026	50 ± 9[e]	54 ± 14[f]	0.080
DBP at birth – mmHg[b]	28 ± 9[c]	34 ± 10[d]	0.012	26 ± 8[e]	28 ± 11[f]	0.221
MBP at birth – mmHg[b]	37 ± 9[c]	43 ± 10[d]	0.009	34 ± 7[e]	37 ± 13[f]	0.061
HR at birth – bpm[b]	155 ± 12[c]	153 ± 16[d]	0.345	148 ± 19[e]	146 ± 17[f]	0.405
UO Day 1 – ml/kg/h[a]	2.1 ± 2.0[g]	2.4 ± 1.4[h]	0.033	2.1 ± 3.9[i]	2.0 ± 2.1[j]	0.285
UO Day 2 – ml/kg/h[a]	4.1 ± 2.2[g]	4.3 ± 2.1[k]	0.728	4.9 ± 5.1[l]	3.3 ± 1.6[k]	0.177
UO Day 3 – ml/kg/h[a]	3.7 ± 2.1[i]	4.4 ± 2.1[h]	0.072	4.8 ± 4.8[f]	4.3 ± 3.4[k]	0.940
PDA – no. (%)	1 (1.9%)	1 (1.9%)	0.999	2 (3.8%)	2 (3.8%)	0.999
Asphyxia – no (%)[m]	2 (3.8%)	0 (0.0%)	0.500	2 (3.8%)	1 (1.9%)	0.999
Mortality – no. (%)	4 (9.5%)[j]	0 (0.0%)[j]	0.125	1 (1.9%)	0 (0.0%)	1.000

[a]Data given as mean ± SD.
[b]First measured value.
[c]Assessed in 25/52 neonates. [d]Assessed in 26/52 neonates. [e]Assessed in 32/52 neonates. [f]Assessed in 34/52 neonates. [g]Assessed in 41/52 neonates. [h]Assessed in 37/52 neonates. [i]Assessed in 38/52 neonates. [j]Assessed in 42/52 neonates. kAssessed in 40/52 neonates. [l]Assessed in 36/52 neonates.

[m]Asphyxia was defined as the presence of at least three of the following criteria: decelerative cardiotocogram, arterial umbilical cord pH <7.10, 5-minute Apgar score < 5, spontaneous breathing > 5 min after birth or multi organ failure.

Abbreviations: SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure, HR = Heart rate, UO = urine output, PDA = patent ductus arteriosus

Discussion

This is the first study evaluating the short-term renal function in twins with spontaneous or post-laser TAPS. We found that donors have significantly higher creatinine levels compared to recipients during the first week after birth.

Our data suggest that chronic blood loss in donor twins may not only lead to anemia and hypoalbuminemia but may also affect the short-term renal function. Although none of the TAPS donors had signs of severe renal failure requiring treatment during the neonatal period, the focus of this evaluation was mainly based on the short-term outcome. Unfortunately, data on the long-term follow-up of the renal function was often incomplete as most neonates were transferred to general hospitals once intensive care treatment was not required anymore. Whether renal dysfunction may also have long-term consequences,

such as impaired renal function in adult age, requires further investigation.
Various hypotheses may be envisaged to explain the increased rate of short-term renal dysfunction in donor twins. First, increased creatinine levels may reflect a state of chronic hypovolemia due to chronic blood loss ('prerenal hypothesis'). This could also explain the lower blood pressure and lower urine output detected in TAPS donors at birth. However, the absence of increased urea levels and heart rate at birth in donor twins may not fully support the hypothesis of chronic hypovolemia. An alternative hypothesis may be that elevated creatinine levels in donors could result from chronic hypoxia and renal injury due to chronic hypoxia and anemia ('renal hypothesis'). If the renal hypothesis were to be true, the renal injury, which may occur in donor twins, may have permanent effects up to adult age, warranting further long-term investigations. This is in analogy with the 'Brenner hypothesis', which states that retardation of renal development may give rise to an increased postnatal risk for systemic hypertension as well as an enhanced risk of expression of renal disease [9]. Alternatively, short-term renal dysfunction could result from a combination of both prerenal factors (chronic hypovolemia) and renal factors (chronic hypoxia). Lastly, an 'endocrinological' hypothesis could be envisaged, driven by the renin-angiotensin-aldosterone production and resulting in hyperplasia of the juxtaglomerular renin-producing cells in the donor.

In TTTS, a few studies on the postnatal renal function have been performed both in TTTS cohorts treated with fetoscopic laser coagulation as well as in cohorts treated with (serial) amnioreduction. Beck et al. [10] evaluated the long-term outcome of kidney function in 18 twin pairs with TTTS treated by intrauterine laser coagulation and found no evidence of long-term renal function impairment. In a study in 40 TTTS pairs treated with laser at our center, we detected 1 donor with evidence of transient renal failure [11]. The risk of renal failure in donor twins TTTS treated with laser appears thus to be low, probably due to the therapeutic effect of coagulation of the vascular anastomoses and cessation of inter-twin blood flow.

In contrast, most reports in TTTS treated with (serial) amnioreduction show an increased risk of severe renal failure in donor twins. In a study of 33 TTTS twin pairs treated conservatively with amnioreduction at our center, we found severe renal morbidity in 2 (6%) donors. One donor died from terminal renal failure, while the other donor required hemodialysis [12]. Cincotta et al. [13] found that anuria or oliguria were present in 29% (4/14) of the donor twins with TTTS treated with serial amnioreduction. Lenclen et al. [14] also reported an increased incidence of renal failure in TTTS twins treated with amnioreduction (20%, 6/30) but made no distinction between donors and recipients. The etiology and pathology of renal injury in TTTS was evaluated in detail by De Paepe et al. [6] who reviewed the renal pathology in 25 fetal twin pairs. The authors showed that donor twins had a high prevalence of tubular loss, involving both proximal and medullary tubules.

In summary, overall findings in the literature show that the risk of severe renal injury is particularly increased in donors with TTTS treated with amnioreduction. The data in this study suggest that the renal dysfunction in TAPS is less severe and less frequent but still present in both subgroups (spontaneous TAPS and post-laser TAPS). Although TTTS and TAPS are both due to inter-twin blood transfusion, the effect of inter-twin blood transfusion in TTTS are more drastic and cause the rapid development of oligohydramnios and polyhydram-

nios in the donor and recipient, respectively. However, it is important to note that the definitions and criteria of renal failure used in the various studies vary greatly, preventing adequate and accurate comparisons between the different cohorts. For example, more than 30 different definitions for acute kidney injury have been published in the literature [15]. Nevertheless, several recent studies have shown that even a modest rise in creatinine levels is a risk factor for mortality in adults [16]. The data of this study should be interpreted with care due to the retrospective nature of the study and the relatively small sample size. Nevertheless, this study is larger compared to previous reports on renal function in TTTS. Longer and more accurate follow-up of the renal dysfunction would have been more valuable. Unfortunately, this data was often incomplete due to the retrospective nature of this study and the fact that neonates admitted to our tertiary care center are transferred to a general hospital once intensive care treatment is not required anymore. Another potential limitation is that the measurements of creatinine and urea levels were not always determined on the same day. In view of these limitations, our data should be viewed as a starting point for larger studies on renal dysfunction in TAPS or other complicated monochorionic twins.

In conclusion, our data show that donor twins may have an impaired short-term renal function. Our findings add to the understanding of the pathophysiologic characteristics of neonates with TAPS. In view of our findings, we suggest that routine evaluations at birth in neonates with TAPS should not only include hematological measurements but also careful monitoring of renal function. Further investigations should be performed to determine whether renal function may return to normal in donors before discharge and at an older age. Given the rarity of this disease, we have recently set up an international web-based TAPS registry (www.TAPSregistry.org) to evaluate the perinatal mortality and neonatal morbidity associated with TAPS.

References

1 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.

2 Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R, Crombleholme T: Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201:417.

3 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, Klumper FJ, Dekoninck P, Devlieger R, Kilby MD, Rustico MA, Deprest J, Favre R, Oepkes D: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144-2151.

4 Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J, Ville Y: Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194:796-803.

5 Gucciardo L, Lewi L, Vaast P, Debska M, De Catte L, Van Mieghem T, Done E, Devlieger R, Deprest J: Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438-442.

6 De Paepe ME, Stopa E, Huang C, Hansen K, Luks FI: Renal tubular apoptosis in twin-to-twin transfusion syndrome. Pediatr Dev Pathol 2003;6:215-225.

7 Verbeek L, Slaghekke F, Hulzebos CV, Oepkes D, Walther FJ, Lopriore E: Hypoalbuminemia in donors with twin anemia-polycythemia sequence: a matched case-control study. Fetal Diagn Ther 2013;33:241-245.

8 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;35:1070-1074.

9 Brenner BM: The etiology of adult hypertension and progressive renal injury: a hypothesis. Bull Mem Acad R Med Belg 1994;149:121-125.

10 Beck M, Graf C, Ellenrieder B, Bokenkamp A, Huber A, Hecher K, Bartmann P: Long-term outcome of kidney function after twin-twin transfusion syndrome treated by intrauterine laser coagulation. Pediatr Nephrol 2005;20:1657-1659.

11 Lopriore E, Sueters M, Middeldorp JM, Oepkes D, Vandenbussche FP, Walther FJ: Neonatal outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser occlusion of vascular anastomoses. J Pediatr 2005;147:597-602.

12 Lopriore E, Nagel HT, Vandenbussche FP, Walther FJ: Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1314-1319.

13 Cincotta R, Oldham J, Sampson A: Antepartum and postpartum complications of twin-twin transfusion. Aust NZ J Obstet Gynaecol 1996;36:303-308.

14 Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, Ville Y: Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. Am J Obstet Gynecol 2007;196:450-457.

15 Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P: Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204-R212.

16 Chertow GM, Burdick E, Honour M, Bonventre JV, Bates DW: Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 2005;16:3365-3370.

Chapter 8
Renal function in neonates with twin-twin transfusion syndrome treated with or without fetoscopic laser surgery

Lianne Verbeek
Faiez Joemmanbaks
Jacoba M. Quak
Rám Sukhai
Johanna M. Middeldorp
Dick Oepkes
Enrico Lopriore

Submitted

Abstract

To investigate the short-term renal function in neonates with twin-twin transfusion syndrome (TTTS), treated with fetoscopic laser surgery (laser group) or conservatively (non-laser group) creatinine and urea levels and urine output were recorded in the first week after birth. Primary outcome was short-term renal dysfunction, defined as a creatinine level > 100 μmol/L during the first week postpartum. We evaluated 312 twins (laser group, n=274; non-laser group, n=18). Median creatinine and urea levels were lower in the laser group than in the non-laser group (71 versus 82 μmol/L, p = 0.002). Short-term renal dysfunction was lower in the laser group compared to the non-laser group (7.2% versus 34.4%, p<0.001). Within the laser group, creatinine levels were significantly higher in the subgroup with incomplete laser surgery compared to twins with successful laser surgery (76 μmol/L versus 69 μmol/L, p=0.018). No differences were found between donors and recipients except for a higher incidence of oliguria in donors in the non-laser group on day 1.

Conclusion: Short-term renal dysfunction occurs less frequently in TTTS twins treated with fetoscopic laser coagulation, particularly after complete surgery, suggesting that laser surgery may have a protective effect on renal function.

Introduction

Twin-twin transfusion syndrome (TTTS) is a serious complication that occurs in 10-15% of monochorionic twin pregnancies and results from unbalanced inter-twin blood transfusion through placental vascular anastomoses.[1] TTTS is detected by prenatal ultrasound and is characterized by the presence of polyhydramnios in the recipient twin due to hypervolemia and polyuria, and oligohydramnios in the donor due to hypovolemia, renal hypoperfusion and oliguria. TTTS can be treated antenatally with serial amnioreduction, but the optimal treatment is coagulation of the vascular anastomoses with fetoscopic laser surgery.[2] Laser surgery is associated with a significant reduction in perinatal morbidity and mortality.[3] As a result of the increase in perinatal survival, attention is now shifting towards short-term and long-term morbidity in survivors. Several small studies in TTTS not treated with laser surgery reported various renal complications in donor twins after birth, caused by impaired renal perfusion, including histological renal changes such as hypovascularisation and microangiopathy.[4-8] Whether postnatal renal dysfunction also occurs in twins affected by TTTS treated with laser surgery is not well known as only few small studies have been published to date.[9;10]

The aim of this retrospective study is to investigate the short-term postnatal renal function in a large cohort of TTTS twins treated with laser surgery (laser group), compared to a group of TTTS twins treated conservatively with either serial amnioreduction or expectant management (non-laser group) and evaluate potential risk factors such as incomplete laser surgery and donor status.

Methods

Data of all consecutive monochorionic twin pairs with TTTS, born at the Leiden University Medical Center (The Netherlands) between July 2009 and June 2016, were collected. The Leiden University Medical Center is a tertiary care center and serves as the national referral center for monochorionic twin pregnancies with TTTS.

TTTS was diagnosed using antenatal ultrasound, according to the Eurofoetus criteria.[2] Pregnancies with intrauterine death of one or both fetuses or twins with congenital anomalies of the kidney and urinary tract were excluded.

Laser surgery was performed up to 26 weeks of gestation in all cases with stage 2 TTTS or higher and in cases with stage 1 associated with symptomatic polyhydramnios. All other cases were treated conservatively with either serial amnioreduction or expectant management.

Outcome of twins treated with laser surgery (laser group) was compared to twins treated conservatively (non-laser group) and between donors and recipients. In the laser group, outcome was compared between twins with successful laser surgery (complete laser group) and the twins with either recurrent or reversal of TTTS or post-laser twin anemia-polycythemia sequence (TAPS) (incomplete laser group). The following perinatal variables were recorded: gender, mode of delivery, gestational age at birth, birth weight and birth weight difference between both twins. Birth weight difference was calculated as follows: ((birth weight larger twin – birth weight smaller twin)/birth weight larger twin) x 100. At birth, blood pressure, heart rate and hemoglobin levels were routinely measured in all

twins as standard of care. In addition, creatinine and urea levels were routinely measured in the first week after birth. Values measured in the first two days after birth were excluded to reduce the influence of maternal creatinine and urea. The highest value was included if more than one value was measured. Urine output (in ml/kg/h) and the presence of oliguria (urine output < 1 ml/kg/h) during the first three days after birth were recorded.

The following neonatal data were collected as well: hypotension, respiratory distress syndrome, necrotizing enterocolitis, patent ductus arteriosus, sepsis, asphyxia, cerebral injury and neonatal mortality. Hypotension was defined as low blood pressure requiring vasopressor therapy. Cerebral injury was defined as any of the following: cystic periventricular leukomalacia or intraventricular haemorrhage grade 3-4. Sepsis was defined as blood culture-proven sepsis or clinical sepsis. Asphyxia was defined as the presence of at least three if the following criteria: decelerative cardiotocogram, arterial umbilical cord pH < 7.10, 5-minute Apgar score <5, spontaneous breathing > 5min after birth or multi organ failure.

The primary outcome in this study was short-term renal dysfunction, defined as a creatinine level > 100 μmol/L in the first week of life. We defined severe renal dysfunction as a creatinine level of > 150 μmol/L. Secondary outcomes were urine output and oliguria in the first three days of life.

The local Medical Ethics Committee provided a statement of no objection for obtaining and publishing the anonymized data.

Statistics

Data were reported as median and interquartile range (IQR). Results of continuous variables between two groups were analyzed using a Mann-Whitney-U test. In order to compare donors and recipients we used the paired sample t-test for continuous variables and the Mc Nemar test for categorical variables. Because of the limited sample size in the non-laser group a Fisher-Exact test was used for nominal variables. Two-sided tests were used for statistical analyses and a p-value < 0.05 was considered statistically significant. All analyses were performed using SPSS version 23 (SPSS, Inc., Chicago, Ill, USA).

Results

During the study period, 312 twins fulfilled the inclusion criteria, of which 274 twins were treated with fetoscopic laser surgery (laser group) and 38 twins were treated conservatively (non-laser group) and managed either expectantly (n=23) or with serial amniodrainage (n=15). The baseline characteristics of the two studied groups are summarized in table 1. The gestational age at diagnosis of TTTS was 19 (17-22) weeks in the laser group and 28 (26-29) weeks in the non-laser group. Quintero stadium at diagnosis was 2 (1-3) in the laser group and 1 (1-3) in the non-laser group. In the laser group, the median gestational age at laser surgery was 19 (17-22) weeks.

TABLE 1 Baseline characteristics in twins affected by TTTS treated with laser surgery (laser group) and treated conservatively (non-laser group)

	Laser group (n^a = 274)	Non-laser group (n^a = 38)
Quintero stage at diagnosis	2 (1-3)[c]	1 (1-3)[d]
Quintero stage 1 – n (%)	72 (26.3%)[c]	16 (42.1%)[d]
Gestational age at diagnosis - wk[b]	19 (17-22)[e]	28 (26-29)[d]
Gestational age at birth – wk[b]	32 (30-34)	30 (29-34)
Cesarean section – no (%)	114 (41.6%)	30 (78.9%)
Female – no (%)	134 (48.9%)	14 (36.8%)
Birth weight – gr[b]	1652 (1257-2046)	1548 (1039-2205)
Birth weight difference - %[b]	10.5 (5.2-20.6)	9.8 (8.1-21.7)

[a]Refers to the number of neonates

[b]Value given as median (IQR)

[c]4.4% (12) missing, [d]26% (10) missing, [e]3.6% (10) missing

Results of renal function in the laser group and non-laser group are shown in table 2. Creatinine and urea levels were significantly lower in the laser group compared to the non-laser group, respectively 71 µmol/L versus 82 µmol/L ($p = 0.002$) and 5.2 mmol/L versus 7.6 mmol/L ($p < 0.001$). Short-term renal dysfunction (creatinine level > 100µmol/L) occurred less often in the laser group (7.1%) compared to the non-laser group (37.9%) ($p < 0.001$).

The incidence of oliguria was significantly lower in the laser group, compared to the non-laser group, but only on day 2.

TABLE 2 Renal function in the laser group and non-laser group and between the subgroup with complete laser and incomplete laser treatment

				Laser group		
	Laser group (n[a] = 274)	**Non-laser group (n[a] = 38)**	**P-value**	**Complete laser (n=228)**	**Incomplete laser (n=46)**	**P-value**
Creatinine week 1 - µmol/L[b]	71 (61-81)[c]	82 (69-148)[d]	0.002	69 (59-80)[e]	76 (66-87)[f]	0.018
Creatinine > 100 µmol/L – n (%)	12 (7.1%)[c]	11 (37.9%)[d]	<0.001	6 (5%)[e]	6 (15%)[f]	0.073
Urea week 1 – mmol/L[b]	5.2 (3.6-7.1)[g]	7.6 (5.8-11.5)[d]	<0.001	5.1 (3.6-7.1)[h]	6.2 (4.4-7.2)[f]	0.178
UO day 1 < 1 ml/kg/h – n (%)	69 (40.6%)[i]	14 (60.9%)[j]	0.075	55 (42%)[k]	14 (37%)[l]	0.708
UO day 2 < 1 ml/kg/h – n (%)	8 (4.8%)[m]	9 (36.0%)[n]	<0.001	5 (4%)[e]	3 (8%)[l]	0.387
UO day 3 < 1 ml/kg/h – n (%)	4 (2.6%)[o]	2 (8.3%)[p]	0.192	2 (2%)[q]	2 (5%)[r]	0.252
MBP at birth – mmHg[b]	37 (33-43)[s]	39 (32-52)[t]	0.349	37 (33-42)[u]	39 (33-45)	0.247
Heartrate at birth – bpm[b]	154(143-166)[v]	146 (131-158)[t]	0.004	154 (142-166)[w]	156 (149-164)	0.298

[a]Refers to the number of neonates

[b]Value given as median (IQR)

[c]38.6% (106) missing, [d]23.7% (9) missing, [e]0.8% (1) missing, [f]10.8% (5) missing, [g]1.8% (5) missing, [h]43.9% (100) missing, [i]38.0% (104) missing, [j]39.5% (15) missing, [k]42.1% (96) missing, [l]17.4% (8) missing, [m]39.8% (109) missing, [n]34.2% (13) missing, [o]48.5% (133) missing, [p]36.8% (14) missing, [q]50% (114) missing, [r]19.6% (9) missing, [s]19.3% (53) missing, [t]13.2% (5) missing, [u]23.2% (53) missing, [v]13.1% (36) missing, [w]15.8% (36) missing.

Abbreviations: UO = urine output, MBP = mean blood pressure, bpm = beats per minute

Neonatal mortality and several perinatal and neonatal morbidities occurred less frequently in the laser group compared to the non-laser group, including hypotension and asphyxia, as shown in table 3. Renal function was only in assessed in 2 of the 4 neonates with asphyxia since 2 neonates died within 24 hours after birth.

TABLE 3 Clinical outcome in TTTS twins in the laser group and the non-laser group

	Laser group (n^a = 274)	Non-laser group (n^a = 38)	P-value
Hypotension – n (%)	12 (4.5%)[b]	8 (21.1%)	0.001
Cerebral injury – n (%)	8 (2.9%)	1 (2.7%)[c]	1.000
Necrotizing enterocolitis – n (%)	3 (1.1%)[g]	1 (2.6%)	0.408
Patent ductus arteriosus – n (%)	37 (13.5%)	10 (26.3%)	0.051
Sepsis – n (%)	25 (9.1%)	5 (13.5%)[c]	0.377
Asphyxia – n (%)	1 (0.4%)[d]	4 (11.1%)[e]	0.001
Mortality – n (%)	8 (2.9%)	5 (13.2%)	0.013

[a]Refers to the number of neonates

[b]2.2% (6) missing, [c]2.6% (1) missing, [d]0.4% (1) missing, [e]5.3% (2) missing.

Within the laser group, 228 had a successful laser surgery (complete laser group) and 46 twin pairs had incomplete laser surgery (post-laser TAPS, n = 36; recurrent or reversal TTTS, n = 10), as shown in table 2. The median creatinine level in the complete laser sub-group was significantly lower (69 µmol/L) compared to median creatinine level in the incomplete laser group (76 µmol/L) (p = 0.018). The number of neonates with a creatinine level >100 µmol/L and the incidence of oliguria was similar in both sub-groups.

In the non-laser group, donor twins had more often oliguria on day 1 (92.3% versus 20.0%, p = 0.001) and a lower mean blood pressure (34mmHg versus 47mmHg, p=0.031) compared to their recipient co-twin. We found no other differences between donors and recipients regarding other outcomes, including short-term renal dysfunction, creatinine levels and urea levels (data not shown).

A creatinine level of > 150 µmol/L was detected in 10 neonates and occurred less often in the laser group than in the non-laser group, respectively 1.1% (3/274) versus 18.4% (7/38) (p < 0.001) and more often in donors (7/10) than recipients (3/10). In the laser group, severely elevated creatinine levels were detected in 4.4% (2/46) in the incomplete laser group and in 0.4% (1/228) in the complete laser group. Neonatal mortality occurred in 3 of the 10 neonates and was due to multi-organ failure (n=2) and respiratory failure including recurrent bilateral tension pneumothorax and respiratory distress syndrome (n=1). The highest creatinine level (347 µmol/L) was detected in a donor twin in the non-laser group and was thought to be due to acute tubular necrosis after severe chronic renal hypoperfusion. Creatinine levels and renal function in this donor twin normalized within several weeks after hyperhydration. Renal ultrasound showed nephrocalcinosis. Urine calcium/creatinine ratio was not elevated and on the follow-up ultrasound at age 2 the nephrocalcinosis was barely visible.

Discussion

This is the first study on short-term renal function in a large cohort of TTTS twins treated with or without laser surgery, assessing also the impact of incomplete laser treatment. We found that the incidence of short-term renal failure in TTTS twins treated with laser surgery is low. In contrast, the incidence of renal failure and oliguria is increased in TTTS treated conservatively or after incomplete laser surgery. Our findings therefore suggest that laser surgery, when complete, may protect fetuses and neonates against renal injury. Although donor twins suffer from severe oliguria and oligohydramnios, these symptoms resolve after laser surgery (reflected by the reoccurrence of amniotic fluid in the donor's sac) antenatally and detection of normal renal function in the vast majority of survivors after birth. However, long-term evaluation of renal function in large TTTS cohorts is required to assess if the protective effect is also present after the initial neonatal period.

A few small studies reported on the short-term and long-term renal function in TTTS survivors after fetoscopic laser surgery. Halvorsen et al. found a slightly higher incidence of renal failure in a cohort of TTTS twins after laser surgery 10% (9/87) compared to our cohort, but criteria for the renal dysfunction were not provided.[11] Lenclen et al. also reported a similar risk of renal failure in TTTS twins after laser surgery (7.1%, 7/98), but again the definition of renal failure was not specified, preventing comparisons with our findings. In addition, only preterm neonates delivered before 30 weeks of gestation were included in this study.[10]

Although our data suggest that the vast majority of TTTS twins after laser surgery do not have short-term renal dysfunction, a few neonates still had severely increased creatinine levels. This may partly be explained by the incomplete laser surgery and persistence of inter-twin blood transfusion. Whether the risk of renal dysfunction is also low on the long-term is not well known as this was evaluated only in one small study from Beck et al. in 18 TTTS twins treated with laser surgery. They found no evidence of renal failure at a median age of 3 years, however the sample size was too small to reach firm conclusions.[9] Accurate renal follow-up should take much longer. Due to the large reserve capacity of the kidney, serum creatinine could be normal for a long time. When children grow older and particularly during the growth spurt, renal insufficiency might become evident.

In accordance with our findings, most studies in TTTS twins not treated with laser report an increased risk of short-term renal dysfunction, in particular in donor twins. In a pathology study evaluating the renal anatomy in 25 TTTS twin pairs after autopsy, De Paepe et al. found a loss of proximal convoluted tubules in 48% of donors and 48% of recipients. Although the glomerular density was higher in donor kidneys, the number of glomerular generations was similar in donors and recipients. The kidney weight of recipients was almost twice as large of that of donor twins.[5] By comparison, Oberg et al. reported in 8/9 donors varying degrees of tubular defiency[12], and in 11/21 donor kidneys (but in none of the 17 recipient twins) studied by Barr et al.[8] In a study by Chiang et al. in 22 neonates with TTTS, 9 (41%) had acute renal failure (defined as serum creatinine level above 1.5 mg/dL (133 μmol/L) regardless of urine amount), of which the vast majority (8/9) were donors.[4] In a study performed by our research group in 56 TTTS neonates treated conservatively, renal failure detected in 2 (4%) neonates, both donors, of which one died of terminal renal failure. Definition of renal failure was not described.[13] Cincotta et al. found an increased

risk of renal failure (defined as urine output < 1 ml/kg/h during the first three days of life and high creatinine levels) in a small group of TTTS twin pairs (n = 17) treated conservatively compared to uncomplicated monochorionic twins (48% versus 15%, p = 0.005), but no differences between donors and recipients were found.[14] Lastly, Lenclen et al. also found an increased incidence of renal failure in TTTS twins treated with amniodrainage (20.0%, 6/30). However, no differences between donors and recipients were found.
In these studies, renal dysfunction which was detected mainly in donors, is thought to result mainly from chronic poor renal perfusion and hypovolemia, in association of chronic hypoxia and anemia.[4;10;13;14]

In our study, we also found a high risk of oliguria in donors (>90%) in the non-laser group and a lower blood pressure at birth, both probably reflecting the hypovolemic state of these neonates at birth.

The main limitation of this study is its retrospective design and a selection bias between the conservative and the laser group. The two groups differed in term of presenting characteristics. The non-laser group was small and heterogeneous, as it contained a mixture of TTTS cases with lower Quintero stages and/or presentation at a later gestational age. Since TTTS in the non-laser group was less severe, the incidence of renal dysfunction in this subgroup was probably underestimated. In contrast, the laser group in this study is very large and homogeneous, limiting the risk of bias and supporting the reliability of our findings. However, we included only TTTS cases with double survivors (to compare the outcome within twin pairs), therefore renal function in survivors after single fetal demise requires further investigation. As recently shown, single fetal demise in TTTS twins not treated with laser can lead to severe renal ischemia and terminal renal failure due to acute exsanguination through the vascular anastomoses.[15] Since dichorionization of the placenta after complete laser surgery prevents acute exsanguination, this selection bias with double survivors may again have led to an underestimation of the risk of renal failure in the non-laser group. Finally, the assessment of short-term renal function in this study was based only on routine measurements at our neonatal nursery including urine production, creatinine and urea levels during the first week. More detailed information on renal function would require different assessments and a different study design.

In conclusion, our findings show that the risk of short-term renal dysfunction in TTTS treated with laser surgery is low, suggesting a protective effect of laser coagulation despite the presence of severe oliguria and oligohydramnios in donor twins during fetal life. Therefore, routine evaluation of renal function after complete laser surgery in all survivors does not seem warranted. In contrast, after incomplete laser surgery or in TTTS treated conservatively, the risk of renal dysfunction is increased and postnatal renal evaluation should be recommended. Future prospective research should focus on long-term renal outcome in TTTS treated with and without laser surgery to assess if the findings in the neonatal period persist through childhood and adulthood.

References

1 Baschat A, Chmait RH, Deprest J, Gratacos E, Hecher K, Kontopoulos E, Quintero R, Skupski DW, Valsky DV, Ville Y: Twin-to-twin transfusion syndrome (TTTS). J Perinat Med 2011;39:107-112.

2 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

3 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, Klumper FJ, DeKoninck P, Devlieger R, Kilby MD, Rustico MA, Deprest J, Favre R, Oepkes D: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144-2151.

4 Chiang MC, Lien R, Chao AS, Chou YH, En Chen YJ: Clinical consequences of twin-to-twin transfusion. Eur J Pediatr 2003;162:68-71.

5 De Paepe ME, Stopa E, Huang C, Hansen K, Luks FI: Renal tubular apoptosis in twin-to-twin transfusion syndrome. Pediatr Dev Pathol 2003;6:215-225.

6 Gubler MC: Renal tubular dysgenesis. Pediatr Nephrol 2014;29:51-59.

7 Christensen AM, Daouk GH, Norling LL, Catlin EA, Ingelfinger JR: Postnatal transient renal insufficiency in the feto-fetal transfusion syndrome. Pediatr Nephrol 1999;13:117-120.

8 Barr M, Jr., Sedman AB, Heidelberger KP: Renal tubular dysgenesis in twins. Pediatr Nephrol 1998;12:408-413.

9 Beck M, Graf C, Ellenrieder B, Bokenkamp A, Huber A, Hecher K, Bartmann P: Long-term outcome of kidney function after twin-twin transfusion syndrome treated by intrauterine laser coagulation. Pediatr Nephrol 2005;20:1657-1659.

10 Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, Ville Y: Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. Am J Obstet Gynecol 2007;196:450-457.

11 Halvorsen CP, Ek S, Dellgren A, Grunewald C, Kublickas M, Westgren M, Norman M: Survival and neonatal outcome after fetoscopic guided laser occlusion (FLOC) of twin-to-twin transfusion syndrome (TTTS) in Sweden. J Perinat Med 2012;40:533-538.

12 Oberg KC, Pestaner JP, Bielamowicz L, Hawkins EP: Renal tubular dysgenesis in twin-twin transfusion syndrome. Pediatr Dev Pathol 1999;2:25-32.

13 Lopriore E, Nagel HT, Vandenbussche FP, Walther FJ: Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1314-1319.

14 Cincotta RB, Gray PH, Phythian G, Rogers YM, Chan FY: Long term outcome of twin-twin transfusion syndrome. Arch Dis Child Fetal Neonatal Ed 2000;83:F171-F176.

15 Genova L, Sueters M, van SA, Oepkes D, Steggerda SJ, Lopriore E: Renal failure after single fetal demise in monochorionic twins: incidence and description of a case. Fetal Diagn Ther 2014;35:302-305.

Chapter 9
General discussion and future perspectives

General discussion and future perspectives

Twin pregnancies, either dichorionic or monochorionic, are associated with higher morbidity and mortality rates compared to singletons due to the higher rate of premature delivery.[1-3] Monochorionic twin pregnancies carry a higher risk compared to dichorionic twins due to their unique placenta architecture. All monochorionic placentas have vascular anastomoses which can lead to inter-twin blood transfusion. When the inter-twin transfusion is unbalanced, severe disorders such as twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) may occur.

The optimal treatment for TTTS is fetoscopic laser surgery to coagulate the vascular anastomoses.[4-6] Introduction of laser surgery in 1990 led to a strong decrease in perinatal mortality and neonatal morbidity. However, there is still room for improvement since incomplete laser treatment and residual anastomoses can lead to recurrent TTTS or post-laser TAPS.

The optimal treatment for TAPS is not yet found, since TAPS was only recently discovered in 2007. Different options have been described: expectant management, intrauterine transfusion in the donor twin with or without partial exchange transfusion in the recipient, fetoscopic laser coagulation of the vascular anastomoses or selective termination of pregnancy.[7-12] The only causal treatment is fetoscopic laser coagulation. A randomized controlled trial to determine the optimal treatment in TAPS is warranted. Given the rarity of TAPS, international collaboration is of paramount importance.

At birth, complicated monochorionic twins are at increased of neonatal morbidity, in particular cerebral injury such as severe intraventricular hemorrhage (IVH), cystic periventricular leukomalacia (PVL) and porencephalic cysts and cardiovascular morbidity such as right ventricular outflow tract obstruction (RVOTO) and persistent pulmonary hypertension (PPHN).[13-19] In this thesis we focused primarily on the risk of hematological and biochemical disorders during the neonatal period in the various subgroups of complicated monochorionic twins.

Hematological disorders

A. Red blood cell disorders:

A.1. In *chronic TTTS*, hemoglobin differences are already present antenatally[20;21], but may also be encountered at birth. The presence of hemoglobin differences after delivery depends on the type of antenatal treatment. In TTTS twins treated with fetoscopic laser coagulation, no significant differences in hemoglobin levels between donor twins and recipient twins are found (chapter 2 and 5). On the other hand, in TTTS twins treated conservatively, donor twins have lower hemoglobin levels at birth compared to recipients. Donors may be severely anemic whereas recipients may suffer from severe hyperviscosity-polycythemia.

A.2. In *TAPS*, large inter-twin hemoglobin differences at birth are found. TAPS is based on chronic unbalanced inter-twin transfusion. Donor twins have chronic anemia requiring a blood transfusion at birth. Recipient twins may be severely polycythemic and may require a partial exchange transfusion.[22;23] Due to hyperviscosity, recipients may suffer from vascular limb necrosis or severe cerebral injury.[24;25] Donor twins with TAPS always have a highly increased reticulocyte count and the ratio of reticulocyte counts between donor and recipient is >1.7. Reticulocyte count should always be measured to distinguish between TAPS (high reticulocyte count ratio >1.7) and acute peripartum TTTS (low reticulocyte count ratio < 1.7). Examination of the placenta is also crucial to discriminate between the two disorders. One of the diagnostic criteria for TAPS is based on the detection of small anastomoses after careful placenta injection. However, placenta injection is not always easy and is only performed in a few specialized centers. Alternatively, examination of the maternal side of the placenta can help perinatologists, since in TAPS the placenta share of the recipient is much darker compared to the pale placenta share of the donor. [26] In our experience, no color difference is detected between the two placenta shares in acute peripartum TTTS (unpublished data).

A.3. In *acute peripartum TTTS*, large inter-twin hemoglobin differences are always present at birth due to acute transfusion through large superficial anastomoses. The donor suffers from acute blood loss which may lead to hypovolemic shock requiring an acute blood transfusion. Recipient twins on the other hand may need partial exchange transfusion in case of severe polycythemia-hyperviscosity.

The diagnostic criteria for acute peripartum TTTS are based on a large difference in hemoglobin levels of > 8 g/dL at birth in the absence of chronic TTTS or TAPS. Presence of oligo-polyhydramnios antenatally (criterion for chronic TTTS) or large reticulocyte count ratio and minuscule anastomoses (criterion for TAPS) should therefore be ruled out before reaching the diagnosis of acute peripartum TTTS. However, in most case reports reticulocyte count or placenta injection studies were not performed and TAPS could not be ruled out. We suspect that the majority of these reported cases were wrongly diagnosed and were probably TAPS cases. In our experience, TAPS occurs more frequently than acute peripartum TTTS. Given the rarity of acute peripartum TTTS, international multicenter studies are needed to increase our knowledge on the true incidence, hematological consequences and risk factors of acute peripartum TTTS.

Importantly, hemoglobin levels are often measured directly after birth, but in case of acute blood loss or transfusion, equilibration of hemoglobin levels occurs only several hours after the event. In case of suspected acute peripartum TTTS, hemoglobin level measurements should therefore be repeated approximately 4-6 hours after birth to determine the true hemoglobin levels.

A.4. Hemoglobin differences in *uncomplicated monochorionic twins* and dichorionic twins are often found when delivered vaginally, but not after cesarean delivery. Second-born twins delivered vaginally have higher hemoglobin levels compared to first-born twins (chapter 3 and 4). Since dichorionic twins have their own separate placenta without vascular anastomoses, hemoglobin differences in dichorionic twins cannot only be explained by inter-twin transfusion. We hypothesize that the hemoglobin differences are related to the

timing of cord clamping. Delayed cord clamping is associated with higher hemoglobin levels at birth due to increased placento-fetal transfusion.[27;28] We suspect that the umbilical cord of first-born twins is clamped relatively earlier than the cord of second-born twins, and that this could lead to the difference in hemoglobin levels. However, in our retrospective studies timing of cord clamping was not recorded, making it difficult to confirm our hypothesis. A prospective study on the timing of cord clamping in twins after vaginal delivery could give new insights. An alternative explanation for hemoglobin differences in uncomplicated monochorionic twins is unbalanced intra-partum transfusion. Due to differences in pressure gradients and uterine contractions relatively more blood might flow from twin 1 to twin 2 resulting in a higher hemoglobin level in twin 2. Another hypothesis is that after birth of twin 1, twin 2 might receive blood from both placenta shares through the anastomoses, resulting in a higher hemoglobin level. More research is needed to study various hypothesis leading to hemoglobin differences in uncomplicated monochorionic twins.

A.5. Hemoglobin differences in *monochorionic twins with selective intrauterine growth restrictions* (sIUGR)

sIUGR occurs in 10-25% of monochorionic twins and is defined by the presence growth restriction in one fetus (estimated fetal weight < 10th percentile).[29;30] A recent study from Stagnati et al. describes that third-trimester inter-twin middle cerebral arterial peak systolic velocity (MCA-PSV) discrepancy in uncomplicated MC is associated with selective intrauterine growth restriction (sIUGR) at birth and an inter-twin birth weight discrepancy of > 25%. The optimal cut-off point for MCA-PSV discrepancy was 0.30 multiples of median (MoM) (sensitivity of 70% and specifity of 69% for sIUGR). Hemoglobin levels at birth were however not reported in this study.[31] Nevertheless, given the good correlation between MCA-PSV measurements and hemoglobin levels, twins with sIUGR may have differences in hemoglobin levels at birth.[32] Growth restriction due to placental insufficiency is associated with chronic hypoxia and increased erythropoiesis which could in turn lead to higher hemoglobin levels. Further research on hemoglobin levels in monochorionic twins with sIUGR in a large cohort is required to confirm these preliminary findings.

B. Thrombocytopenia or leucopenia:

Not much is known on the occurrence of other hematological complications such as thrombocytopenia or leucopenia in TTTS survivors. Polycythemia is known to be associated with thrombocytopenia.[33] Therefore, polycythemic recipient twins may be at risk for thrombocytopenia at birth. Research on white blood cell disorders is also sparse. A small study from Koenig et al. showed neutropenia in each of five donor twins. Since no evidence of infection was observed and no left shift was seen (as in neutropenia of pregnancy-induced hypertension) they postulated that reduced neutrophil production was related to accelerated erythropoiesis.[34] Further investigations on the occurrence and associated complications of thrombocytopenia and leucopenia is warranted, particularly in TTTS not treated with laser surgery or after incomplete laser.

In TAPS, thrombocytopenia is often found (in 45% of cases) and occurs more often recipient twins compared to donor twins (63% versus 26%).[23] Recipient twins are per definition polycythemic and therefore at risk for thrombocytopenia. In donor twins thrombocytopenia could be related to IUGR or chronic hypoxia.[35-37] The white blood cell line is not yet investigated in TAPS twins.

The our best knowledge no studies reported on thrombocytes or the white blood cell rihanabocytopenia.[35-37] Research on the occurrence of thrombocytopenia and white blood cell disorders in twins with sIUGR is warranted.

Biochemical disorders

In both TTTS and TAPS, knowledge on biochemical values is scarce. We hypothesize that inter-twin blood transfusion through the vascular anastomoses in TTTS and TAPS allows not only inter-twin flow of red blood cells, but also inter-twin flow of nutrients and proteins and can lead to biochemical disorders. In addition, inter-twin circulatory and hemodynamic imbalance can lead to renal hypo-or hyperperfusion and eventually to renal dysfunction.

A. Hypoalbuminemia

In TTTS twins treated conservatively, we found that donor twins had significantly lower albumin levels compared to recipient twins. In TTTS twins treated with fetoscopic laser surgery, no inter-twin differences in albumin were detected (chapter 5). Our findings suggest that vascular anastomoses not only allow significant loss of hemoglobin, but also loss of albumin. Hypothetically, hypoalbuminemia in donor twins may also result from a decreased production in the liver due to a subordinate physical condition of these donor twins. However, we did not analyze other proteins or other parameters for the function of the liver such as the coagulation. Furthermore, since renal dysfunction occurs more often in donor twins treated conservatively (chapter 8), hypoalbuminemia in donor twins might also result from proteinuria and renal loss.

We found no differences in neonatal morbidity and mortality between donors and recipients. However, our retrospective study was not powered to detect such differences. In the 4 conservatively treated TTTS donors, 1 donor had skin edema.

In TAPS twins, albumin levels were also lower in donor twins (chapter 6). Hypoalbuminemia occurred more often in donor twins. In the 5 TAPS donors with hypoalbuminemia (< 20 g/L), skin edema was present in 2 donors of which one had severe respiratory and circulatory failure. Another donor suffered from hydrops fetalis. Although no significant differences in neonatal morbidity between donors and recipients were found, these data could suggest that in a larger cohort differences in morbidity may be detected.

To be able to compare TTTS and TAPS twins with uncomplicated monochorionic twins, we evaluated albumin levels in a control group of uncomplicated monochorionic twins as well. We compared the larger twin to the smaller twin and found no differences in albumin levels. The overall incidence of hypoalbuminemia was 1% (1/100).

In adults hypoalbuminemia is an independent risk factor for poor clinical outcomes.[38] As for most diseases, hypoalbuminemia in children is less studied. However, several studies in critically ill children concluded that it is a risk factor for morbidity and mortality.[39-41] Prognostic value of hypoalbuminemia in neonates is less investigated. Three studies showed that hypoalbuminemia in term and preterm neonates is associated with increased risk of mortality.[42-44] In addition, several studies in term and preterm neonates showed

that hypoalbuminemia is associated with increased risk of morbidity, including hydrops fetalis, edema, necrotizing enterocolitis, sepsis, respiratory distress syndrome, chronic lung disease, mechanical ventilation and longer hospital stays.[45-48] Albumin levels are also known to be correlated with gestational age [44;49-51] and normal ranges in preterm neonates are not well known. In our studies we defined hypoalbuminemia as albumin < 25 g/l and albumin < 20 g/l, whereas median gestational age was 30 weeks in TTTS twins and 32 weeks in TAPS twins (chapter 5 and 6).

B. Renal function

During pregnancy, donor twins with TTTS suffer from severe renal hypoperfusion resulting in hypovolemia, oliguria and oligohydramnios. Several studies in TTTS twins treated conservatively reported various renal complications in TTTS twins after birth.[52-57] Renal function in TTTS twins treated with laser surgery is less investigated.[58;59] In our retrospective study we evaluated creatinine levels in the first week after birth and urine output in TTTS twins treated with laser surgery compared to TTTS twins treated conservatively (chapter 8). We found that renal dysfunction (defined as creatinine level > 100 μmol/L) occurs less often in twins treated with laser surgery compared to twins treated conservatively (7.1% versus 37.9%). In the laser group, creatinine levels were higher in twins treated with incomplete laser compared with twins treated with complete laser. No differences in creatinine levels between donors and recipients were found. In TTTS twins treated conservatively, we found a high risk of oliguria in donors (> 90%) and lower blood pressure at birth.

Higher creatinine levels may reflect a state of chronic hypovolemia due to chronic blood loss (pre-renal hypothesis). Circulatory imbalance could reduce renal perfusion and glomerular perfusion pressure. This theory is supported by the lower blood pressure and lower urine output in conservatively treated TTTS donors. An alternative hypothesis may be that elevated creatinine levels could result from chronic hypoxia and anemia (renal hypothesis). Alternatively, hormonal dysfunction may also affect TTTS twins. Besides the concept of unbalanced inter-twin blood transfusion, there is also evidence for an adaptive response of the renal, endocrine and cardiovascular system to alter blood volume and causing twin oligo-polyhydramnios sequence (TOPS). Several studies have reported on highly abnormal values of hormones. In donor fetuses, plasma and amniotic vasopressin levels are higher compared to recipients. Oligohydramnios and higher creatinine levels in donors may therefore occur as a consequence of vasopressin mediated reduction in fetal urine output.[60] In autopsy studies in TTTS twins, overexpression of the renin protein and transcript with evidence of renin synthesis was observed in donor kidneys. In recipients, renin expression was virtually absent and probably down-regulated.[61-63] Fetal atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and endothelin levels are higher in recipient twins compared to donor twins.[64;65] Hypervolemia causes stretch of the cardiac atria and release of ANP and BNP which have natriuretic and vasodilatory properties resulting in polyuria and polyhydramnios.

Although these hormonal reactions seem to be sufficient; increased release of renin and vasopressin in hypotensive/hypovolemic donors and increased release of ANP and BNP in hypertensive/hypervolemic recipients, the placental anastomoses facilitate exchange of these hormones and cause paradoxic effects.[63]

In TAPS, donor twins do not have oligohydramnios reflecting a different antenatal situation compared to TTTS twins. However, we found that donor twins with TAPS have higher creatinine levels compared to recipients and renal dysfunction occurred more often in donor twins compared to recipients twins (26.0% versus 6.3%). Besides, donors had a lower blood pressure at birth and lower urine production. Our data suggest that chronic blood loss in donor twins may not only lead to anemia and hypoalbuminemia, but may also affect the short-term renal function. Our hypothesis is that chronic anemia in donor twins causes hypoxia and results in an increased risk for renal dysfunction.

We also evaluated renal function in uncomplicated monochorionic twins and compared larger twins to smaller twins. No differences between these twins were found. The overall incidence of short-term renal dysfunction in uncomplicated monochorionic twins was 7.7% (8/104).

The assessment of short-term renal function in our studies was only based on routine measurements at our neonatal nursery including urine production, creatinine and urea levels during the first week. More detailed information on renal function would require different assessments and a different study design. Whether renal dysfunction may also have long-term consequences such as impaired renal function in adult age requires further investigation. Beck et al. found in a small group of 18 children (TTTS twins treated with laser surgery) at median age 3 year no renal dysfunction.[66] However, accurate renal follow-up should take much longer. Due to the large reserve capacity of the kidney, serum creatinine could be normal for a long time. When children grow older and particularly during the growth spurt, renal insufficiency might become evident.

Renal injury which may occur in donor twins may have permanent effects up to adult age, warranting further long-term investigations. This is in analogy with the 'Brenner hypothesis' which states that retardation of renal development may give rise to increased postnatal risk for systemic hypertension as well as enhanced risk of expression of renal disease.[67] To conclude, complicated monochorionic twins are at increased risk for hematological and biochemical disorders in the neonatal period. Future research should focus on the long-term outcome. In TTTS, treatment with fetoscopic laser surgery has a protective effect. Whether in TAPS fetoscopic laser surgery is the optimal treatment and also protective against hematological and biochemical disorders, must be determined by further trials. Our studies focused on TTTS and TAPS, however other complications in monochorionic twins such as monoamniotic twinning, sIUGR and twin reversed arterial perfusion sequence (TRAP) are probably also at risk for biochemical and hematological complications in the neonatal period, warranting further investigations. Given the rarity of these diseases, close collaboration between obstetricians and neonatologists and multicenter participation is of paramount importance to improve our understanding of neonatal morbidity in complicated monochorionic twins.

References

1 Kiely JL: The epidemiology of perinatal mortality in multiple births. Bull N Y Acad Med 1990;66:618-637.

2 McCarthy BJ, Sachs BP, Layde PM, Burton A, Terry JS, Rochat R: The epidemiology of neonatal death in twins. Am J Obstet Gynecol 1981;141:252-256.

3 Grothe W, Ruttgers H: Twin pregnancies: an 11-year review. Acta Genet Med Gemellol (Roma) 1985;34:49-58.

4 De Lia JE, Cruikshank DP, Keye WR, Jr.: Fetoscopic neodymium:YAG laser occlusion of placental vessels in severe twin-twin transfusion syndrome. Obstet Gynecol 1990;75:1046-1053.

5 Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y: Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136-144.

6 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, Klumper FJ, DeKoninck P, Devlieger R, Kilby MD, Rustico MA, Deprest J, Favre R, Oepkes D: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144-2151.

7 Sananes N, Veujoz M, Severac F, Barthoulot M, Meyer N, Weingertner AS, Kohler M, Guerra F, Gaudineau A, Nisand I, Favre R: Evaluation of the Utility of in utero Treatment of Twin Anemia-Polycythemia Sequence. Fetal Diagn Ther 2015;38:170-178.

8 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, Walther FJ, Vandenbussche FP, Lopriore E: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181-190.

9 Abdel-Sattar M, Platt LD, DeVore G, Porto M, Benirschke K, Chmait RH: Treatment of Complicated Spontaneous Twin Anemia-Polycythemia Sequence via Fetoscopic Laser Ablation of the Vascular Communications. Fetal Diagn Ther 2015;38:233-237.

10 Genova L, Slaghekke F, Klumper FJ, Middeldorp JM, Steggerda SJ, Oepkes D, Lopriore E: Management of twin anemia-polycythemia sequence using intrauterine blood transfusion for the donor and partial exchange transfusion for the recipient. Fetal Diagn Ther 2013;34:121-126.

11 Lopriore E, Hecher K, Vandenbussche FP, van den Wijngaard JP, Klumper FJ, Oepkes D: Fetoscopic laser treatment of twin-to-twin transfusion syndrome followed by severe twin anemia-polycythemia sequence with spontaneous resolution. Am J Obstet Gynecol 2008;198:e4-e7.

12 Guenot C, Robyr R, Jastrow N, Vial Y, Raio L, Baud D: Fetal Intra-Peritoneal Transfusion for the Management of Very Early Spontaneous Twin Anemia-Polycythemia Sequence in an Obese Patient With a Whole Anterior Placenta. Twin Res Hum Genet 2016;19:154-157.

13 Pruetz JD, Sklansky M, Detterich J, Korst LM, Llanes A, Chmait RH: Twin-twin transfusion syndrome treated with laser surgery: postnatal prevalence of congenital heart disease in surviving recipients and donors. Prenat Diagn 2011;31:973-977.

14 Michelfelder E, Tan X, Cnota J, Divanovic A, Statile C, Lim FY, Crombleholme T: Prevalence, Spectrum, and Outcome of Right Ventricular Outflow Tract Abnormalities in Twin-twin Transfusion Syndrome: A Large, Single-center Experience. Congenit Heart Dis 2015;10:209-218.

15 Eschbach SJ, Boons LS, van ZE, Middeldorp JM, Klumper FJ, Lopriore E, Teunissen AK, Rijlaarsdam ME, Oepkes D, Ten Harkel AD, Haak MC: Right ventricular outflow tract obstruction in complicated monochorionic twin pregnancies. Ultrasound Obstet Gynecol 2016.

16 Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ: Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006;194:1215-1220.

17 Delsing B, Lopriore E, Blom N, Te Pas AB, Vandenbussche FP, Walther FJ: Risk of persistent pulmonary hypertension of the neonate in twin-to-twin transfusion syndrome. Neonatology 2007;92:134-138.

18 Takahashi H, Takahashi S, Tsukamoto K, Ito Y, Nakamura T, Hayashi S, Sago H: Persistent pulmonary hypertension of the newborn in twin-twin transfusion syndrome following fetoscopic laser surgery. J Matern Fetal Neonatal Med 2012;25:543-545.
19 van Klink JM, Koopman HM, van Zwet EW, Oepkes D, Walther FJ, Lopriore E: Cerebral injury and neurodevelopmental impairment after amnioreduction versus laser surgery in twin-twin transfusion syndrome: a systematic review and meta-analysis. Fetal Diagn Ther 2013;33:81-89.
20 Berry SM, Puder KS, Bottoms SF, Uckele JE, Romero R, Cotton DB: Comparison of intrauterine hematologic and biochemical values between twin pairs with and without stuck twin syndrome. Am J Obstet Gynecol 1995;172:1403-1410.
21 Denbow M, Fogliani R, Kyle P, Letsky E, Nicolini U, Fisk N: Haematological indices at fetal blood sampling in monochorionic pregnancies complicated by feto-fetal transfusion syndrome. Prenat Diagn 1998;18:941-946.
22 Ashwal E, Yinon Y, Fishel-Bartal M, Tsur A, Chayen B, Weisz B, Lipitz S: Twin Anemia-Polycythemia Sequence: Perinatal Management and Outcome. Fetal Diagn Ther 2016;40:28-34.
23 Lopriore E, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, Walther FJ: Hematological characteristics in neonates with twin anemia-polycythemia sequence (TAPS). Prenat Diagn 2010;30:251-255.
24 Stranak Z, Korcek P, Hympanova L, Kyncl M, Krofta L: Prenatally Acquired Multiple Limb Ischemia in a Very Low Birth Weight Monochorionic Twin. Fetal Diagn Ther 2015.
25 Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard JP, Deprest J, Ville Y: Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194:796-803.
26 Tollenaar LS, Zhao DP, Middeldorp JM, Slaghekke F, Oepkes D, Lopriore E: Color Difference in Placentas with Twin Anemia-Polycythemia Sequence: An Additional Diagnostic Criterion? Fetal Diagn Ther 2016;40:123-127.
27 Rabe H, Diaz-Rossello JL, Duley L, Dowswell T: Effect of timing of umbilical cord clamping and other strategies to influence placental transfusion at preterm birth on maternal and infant outcomes. Cochrane Database Syst Rev 2012;CD003248.
28 McDonald SJ, Middleton P, Dowswell T, Morris PS: Effect of timing of umbilical cord clamping of term infants on maternal and neonatal outcomes. Evid Based Child Health 2014;9:303-397.
29 Lewi L, Gucciardo L, Huber A, Jani J, Van MT, Done E, Cannie M, Gratacos E, Diemert A, Hecher K, Lewi P, Deprest J: Clinical outcome and placental characteristics of monochorionic diamniotic twin pairs with early- and late-onset discordant growth. Am J Obstet Gynecol 2008;199:511-517.
30 Valsky DV, Eixarch E, Martinez JM, Crispi F, Gratacos E: Selective intrauterine growth restriction in monochorionic twins: pathophysiology, diagnostic approach and management dilemmas. Semin Fetal Neonatal Med 2010;15:342-348.
31 Stagnati V, Pagani G, Fichera A, Prefumo F: Intertwin discrepancy in middle cerebral artery peak systolic velocity and third-trimester fetal growth restriction in monochorionic-diamniotic twin pregnancy. Ultrasound Obstet Gynecol 2016;48:66-71.
32 Teixeira JM, Duncan K, Letsky E, Fisk NM: Middle cerebral artery peak systolic velocity in the prediction of fetal anemia. Ultrasound Obstet Gynecol 2000;15:205-208.
33 Sarkar S, Rosenkrantz TS: Neonatal polycythemia and hyperviscosity. Semin Fetal Neonatal Med 2008;13:248-255.
34 Koenig JM, Hunter DD, Christensen RD: Neutropenia in donor (anemic) twins involved in the twin-twin transfusion syndrome. J Perinatol 1991;11:355-358.
35 Eslami Z, Lookzadeh MH, Noorishadkam M, Hashemi A, Ghilian R, Pirdehghan A: Thrombocytopenia and Associated Factors in Neonates Admitted to NICU during Years 2010_2011. Iran J Ped Hematol Oncol 2013;3:205-215.

36 Gupta A, Mathai SS, Kanitkar M: Incidence of thrombocytopenia in the neonatal intensive care unit. Med J Armed Forces India 2011;67:234-236.
37 Maruyama H, Shinozuka M, Kondoh Y, Akahori Y, Matsuda M, Inoue S, Sumida Y, Morishima T: Thrombocytopenia in preterm infants with intrauterine growth restriction. Acta Med Okayama 2008;62:313-317.
38 Vincent JL, Dubois MJ, Navickis RJ, Wilkes MM: Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. Ann Surg 2003;237:319-334.
39 Horowitz IN, Tai K: Hypoalbuminemia in critically ill children. Arch Pediatr Adolesc Med 2007;161:1048-1052.
40 Leite HP, Fisberg M, de Carvalho WB, de Camargo Carvalho AC: Serum albumin and clinical outcome in pediatric cardiac surgery. Nutrition 2005;21:553-558.
41 Wong CS, Hingorani S, Gillen DL, Sherrard DJ, Watkins SL, Brandt JR, Ball A, Stehman-Breen CO: Hypoalbuminemia and risk of death in pediatric patients with end-stage renal disease. Kidney Int 2002;61:630-637.
42 Kenny SE, Pierro A, Isherwood D, Donnell SC, Van Saene HK, Lloyd DA: Hypoalbuminaemia in surgical neonates receiving parenteral nutrition. J Pediatr Surg 1995;30:454-457.
43 Morris I, McCallion N, El-Khuffash A, Molloy EJ: Serum albumin and mortality in very low birth weight infants. Arch Dis Child Fetal Neonatal Ed 2008;93:F310-F312.
44 Torer B, Hanta D, Yapakci E, Gokmen Z, Parlakgumus A, Gulcan H, Tarcan A: Association of Serum Albumin Level and Mortality in Premature Infants. J Clin Lab Anal 2016;30:867-872.
45 Atkinson SD, Tuggle DW, Tunell WP: Hypoalbuminemia may predispose infants to necrotizing enterocolitis. J Pediatr Surg 1989;24:674-676.
46 Yang CY, Li BY, Xu P, Yang YJ, Yang QZ: Correlation of serum albumin with the clinical features and prognosis of preterm neonates in the neonatal intensive care unit. Clin Exp Obstet Gynecol 2016;43:149-153.
47 Yang C, Liu Z, Tian M, Xu P, Li B, Yang Q, Yang Y: Relationship Between Serum Albumin Levels and Infections in Newborn Late Preterm Infants. Med Sci Monit 2016;22:92-98.
48 Jardine LA, Jenkins-Manning S, Davies MW: Albumin infusion for low serum albumin in preterm newborn infants. Cochrane Database Syst Rev 2004;CD004208.
49 Cartlidge PH, Rutter N: Serum albumin concentrations and oedema in the newborn. Arch Dis Child 1986;61:657-660.
50 Reading RF, Ellis R, Fleetwood A: Plasma albumin and total protein in preterm babies from birth to eight weeks. Early Hum Dev 1990;22:81-87.
51 Kensara OA, Azzeh FS: Nutritional status of low birth weight infants in Makkah region: Evaluation of anthropometric and biochemical parameters. J Pak Med Assoc 2016;66:414-417.
52 Oberg KC, Pestaner JP, Bielamowicz L, Hawkins EP: Renal tubular dysgenesis in twin-twin transfusion syndrome. Pediatr Dev Pathol 1999;2:25-32.
53 Barr M, Jr., Sedman AB, Heidelberger KP: Renal tubular dysgenesis in twins. Pediatr Nephrol 1998;12:408-413.
54 Chiang MC, Lien R, Chao AS, Chou YH, En Chen YJ: Clinical consequences of twin-to-twin transfusion. Eur J Pediatr 2003;162:68-71.
55 De Paepe ME, Stopa E, Huang C, Hansen K, Luks FI: Renal tubular apoptosis in twin-to-twin transfusion syndrome. Pediatr Dev Pathol 2003;6:215-225.
56 Cincotta RB, Gray PH, Phythian G, Rogers YM, Chan FY: Long term outcome of twin-twin transfusion syndrome. Arch Dis Child Fetal Neonatal Ed 2000;83:F171-F176.
57 Lopriore E, Nagel HT, Vandenbussche FP, Walther FJ: Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2003;189:1314-1319.

58 Halvorsen CP, Ek S, Dellgren A, Grunewald C, Kublickas M, Westgren M, Norman M: Survival and neonatal outcome after fetoscopic guided laser occlusion (FLOC) of twin-to-twin transfusion syndrome (TTTS) in Sweden. J Perinat Med 2012;40:533-538.
59 Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, Ville Y: Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. Am J Obstet Gynecol 2007;196:450-457.
60 Bajoria R, Ward S, Sooranna SR: Influence of vasopressin in the pathogenesis of oligohydramnios-polyhydramnios in monochorionic twins. Eur J Obstet Gynecol Reprod Biol 2004;113:49-55.
61 Mahieu-Caputo D, Dommergues M, Delezoide AL, Lacoste M, Cai Y, Narcy F, Jolly D, Gonzales M, Dumez Y, Gubler MC: Twin-to-twin transfusion syndrome. Role of the fetal renin-angiotensin system. Am J Pathol 2000;156:629-636.
62 Kilby MD, Platt C, Whittle MJ, Oxley J, Lindop GB: Renin gene expression in fetal kidneys of pregnancies complicated by twin-twin transfusion syndrome. Pediatr Dev Pathol 2001;4:175-179.
63 Mahieu-Caputo D, Meulemans A, Martinovic J, Gubler MC, Delezoide AL, Muller F, Madelenat P, Fisk NM, Dommergues M: Paradoxic activation of the renin-angiotensin system in twin-twin transfusion syndrome: an explanation for cardiovascular disturbances in the recipient. Pediatr Res 2005;58:685-688.
64 Bajoria R, Ward S, Chatterjee R: Natriuretic peptides in the pathogenesis of cardiac dysfunction in the recipient fetus of twin-twin transfusion syndrome. Am J Obstet Gynecol 2002;186:121-127.
65 Bajoria R, Sullivan M, Fisk NM: Endothelin concentrations in monochorionic twins with severe twin-twin transfusion syndrome. Hum Reprod 1999;14:1614-1618.
66 Beck M, Graf C, Ellenrieder B, Bokenkamp A, Huber A, Hecher K, Bartmann P: Long-term outcome of kidney function after twin-twin transfusion syndrome treated by intrauterine laser coagulation. Pediatr Nephrol 2005;20:1657-1659.
67 Brenner BM: The etiology of adult hypertension and progressive renal injury: an hypothesis. Bull Mem Acad R Med Belg 1994;149:121-125.

Chapter 10
Summary/samenvatting

Summary

Twin-twin transfusion syndrome (TTTS) and twin anemia-polycythemia sequence (TAPS) are severe complications of monochorionic twin pregnancies associated with high perinatal mortality and morbidity if left untreated. The pathophysiology is based on the presence of vascular anastomoses in the monochorionic placenta connecting the blood circulations of both twins. In uncomplicated monochorionic twins inter-twin transfusion through the vascular anastomoses is balanced, but in TTTS and TAPS inter-twin transfusion it is unbalanced. In TTTS chronic unbalanced inter-twin blood transfusion leads to oligohydramnios in the donor twin and polyhydramnios in the recipient twin (so called twin oligo-polyhydramnios sequence; TOPS). In TAPS inter-twin transfusion is also unbalanced but the transfusion occurs more slowly through tiny anastomoses and leads to anemia in the donor twin and polycythemia in the recipient twin, without oliguria and polyuria as in TTTS.
Fetoscopic laser coagulation of the vascular anastomoses is the optimal treatment for TTTS. The optimal management of TAPS is not well known, and includes intrauterine blood transfusion with or without partial exchange transfusion, fetoscopic laser surgery or conservative management in mild cases. Improvement in the antenatal management of TTTS and TAPS in the past decades has led to an increased rate of perinatal survival. Nevertheless, survivors of TTTS and TAPS are often at risk of neonatal morbidity and mortality due to associated complications, in addition to the increased risk of premature delivery. Most studies on neonatal complications in TTTS or TAPS survivors focused on cerebral injury detected on neuroimaging or on long-term neurological complications. In this thesis we focused on biochemical and hematological complications during the neonatal period, and evaluated these disorders in the various subgroups of complicated monochorionic twins and uncomplicated monochorionic twins.

In *Chapter 2*, an overview of the literature on hematological complications in complicated monochorionic twins is presented. This review analyzes the disorders of red blood cell lines in monochorionic twin neonates with various forms of inter-twin blood transfusion such as TTTS, TAPS, acute peripartum TTTS, acute perimortem TTTS and uncomplicated monochorionic twins. The incidence, risk and management of anemia, polycythemia and thrombocytopenia is discussed. In addition, we evaluated the incidence and severity of these complications in monochorionic twins delivered at our center.

In *Chapter 3*, hemoglobin levels in uncomplicated monochorionic twins are evaluated. All consecutive uncomplicated monochorionic twins delivered in our center were included and hemoglobin levels at birth and on day 2 were analyzed in association with birth order, mode of delivery, and time interval between delivery of twin 1 and twin 2. We found that in twins delivered vaginally, mean hemoglobin levels at birth and on day 2 were significantly higher in second-born twins compared to first-born twins. Polycythemia occurred more often in second-born twins compared to first-born twins, but also only when delivered vaginally. No association between inter-twin delivery time intervals and hemoglobin differences were found. The causes of hemoglobin differences are not well known and may be due to the presence of placental vascular anastomoses allowing inter-twin intra-partum transfusion or increased placento-fetal transfusion in twin 2. However, other factors than vascular anastomoses may also lead to hemoglobin levels including differences in timing of umbilical cord clamping.

In *Chapter 4*, to test our hypothesis on hemoglobin differences could be due to differences in timing of umbilical cord clamping, we evaluated hemoglobin levels in dichorionic twins and compared these with uncomplicated monochorionic twins. We hypothesized that gynecologists may tend to clamp the cord of first-born twins relatively early in order to focus on the birth of the second twin. Early cord clamping results in less placenta-fetal transfusion and lower hemoglobin levels. Once the second twin is delivered, the gynecologist may experience less hurry to clamp the cord resulting in more time for placenta-fetal transfusion and a higher hemoglobin level. Unfortunately, timing of umbilical cord clamping is not recorded in our center. However, an alternative and indirect method to test our hypothesis is to evaluate hemoglobin differences in dichorionic twins since dichorionic twins do not have vascular anastomoses.

We found that also in dichorionic twins hemoglobin levels are higher in second-born twins compared to first-born twins when delivered vaginally. In twins delivered through cesarean section, no inter-twin differences in hemoglobin levels were detected. Given the absence of vascular anastomoses in dichorionic twins, other factor such as differences in timing of cord clamping, may lead to hemoglobin differences at birth. Targeted studies to evaluate the optimal timing of cord clamping in twins delivered vaginally are warranted.
In *Chapter 5*, we studied albumin levels at birth in neonates with TTTS. Several studies have reported significant inter-twin hematological differences, showing that the hemoglobin levels in donor twins are often significantly lower than in recipient twins when not treated with fetoscopic laser surgery. We hypothesized that placental anastomoses allow not only inter-twin flow of red blood cells, but also inter-twin flow of nutrients and proteins. Hypoalbuminemia in neonates is an independent risk factor for mortality and morbidity and has been associated with various adverse clinical conditions. We performed a matched case control study including twin pairs with TTTS treated conservatively or with fetoscopic laser surgery and analyzed the albumin levels at birth in donor and recipient twins. Median albumin levels in donor twins in the conservatively treated group were significantly lower than in recipients twins, 25.0 versus 33.0 g/l, respectively ($p = 0.001$). Hypoalbuminemia (albumin level < 20 g/l) occurred in 22% (4/18) donor twins in the conservative group. In TTTS twins treated with laser surgery, no differences between donor and recipient twins were found. Our findings suggest that placental anastomoses may lead to unbalanced inter-twin transfusion of proteins.

In *Chapter 6*, we analyzed albumin levels at birth in twins affected by TAPS and compared our results with a group of uncomplicated monochorionic twins, matched for gestational age. Since TAPS is also based on chronic unbalanced inter-twin transfusion, we hypothesized that donor twins have lower albumin levels at birth compared to recipients twins. In the 25 included TAPS twins pairs we found significant differences in albumin levels at birth. Median levels of albumin in donor twins were significantly lower than in recipient twins, i.e. 28.0 g/l versus 32.0 g/l ($p = 0.008$). The rate of hypoalbuminemia (albumin < 20 g/l) in donor twins with TAPS was 20% (5/25). In the control group, no differences were found between larger twins and smaller twins.

In *Chapter 7* we studied the short-term renal function in neonates with TAPS. Several studies reported on renal function in TTTS twins, but data on renal function in TAPS was not available. All monochorionic twins with TAPS with double survivors admitted between

August 2003 and December 2014 to the neonatal intensive care unit of three European centers were included in this retrospective study. TAPS twins were compared with uncomplicated monochorionic control twins pairs, matched for gestational age. In TAPS, donors had higher mean creatinine levels in the first week after birth compared to recipients, 85 versus 71 µmol/l, respectively (p = 0.001). Short-term renal dysfunction (defined as creatinine > 100 µmol/l in the first week after birth) was detected in 26.0% (13/50) of the donor twins versus 6.3% (3/48) of the recipient twins (p = 0.002). In addition, donors had a lower blood pressure at birth and lower urine production on day 1. In the control group the incidence of renal dysfunction was 7.7% (8/104). We suggest that chronic anemia in donor twins causes hypoxia and results in an increased risk for renal dysfunction.

Chapter 8 describes the short-term renal function in a large cohort of TTTS twins. TTTS twins treated with laser surgery were compared with TTTS twins treated conservatively. We also evaluated the differences in renal function in TTTS twins treated with complete laser surgery versus incomplete laser surgery. Creatinine levels in the first week after birth were significantly higher in the incomplete laser surgery subgroup compared to the group with twins with successful laser surgery (76 µmol/L versus 69 µmol/L, p=0.018). Median creatinine and urea levels in the first week after birth were lower in the laser group than in the conservatively treated group, 71 versus 82 µmol/L (p = 0.002). No differences were found between donors and recipients expect for a higher incidence of oliguria in donors in the conservatively treated group on day 1. During pregnancy, donor twins suffer from hypovolemia resulting in renal hypoperfusion, oliguria and oligohydramnios. Short-term renal dysfunction occurs less frequently in TTTS twins treated with fetoscopic laser coagulation, particularly after complete surgery, suggesting that laser surgery may have a protective effect on renal function.

In conclusion, twins affected by TTTS or TAPS are at increased risk for hematological and biochemical disorders in the neonatal period. Whether these complications also occur in other subgroups of complicated monochorionic twins such as selective intrauterine growth restriction (sIUGR) is not well known and requires further investigations. Lastly, the long-term implications of these neonatal disorders in TTTS and TAPS survivors are not well known. Long-term follow-up studies in these high risk groups should focus not only on neurodevelopmental outcome but also on long-term cardiovascular, renal and metabolic implications.

Samenvatting

Tweeling transfusie syndroom (TTS) en tweeling anemie polycythemie sequentie (TAPS) zijn ernstige complicaties die kunnen ontstaan tijdens de zwangerschap bij monochoriale tweelingen. Indien dit niet behandeld wordt is er een hoog risico op perinatale mortaliteit en morbiditeit. De pathofysiologie van TTS en TAPS is gebaseerd op vasculaire anastomosen in de placenta die de foetussen met elkaar delen. Hierdoor zijn de bloedcirculaties van beide foetussen met elkaar verbonden. Bij ongecompliceerde monochoriale tweelingen is de bloeduitwisseling door deze anastomosen in de placenta in balans. Bij TTS en TAPS is deze bloeduitwisseling uit balans waardoor de ene foetus relatief meer bloed doneert (de donor) aan de andere foetus (de recipiënt). Bij TTS leidt chronische transfusie tot een oligohydramnion bij de donor en een polyhydramnion bij de recipiënt, dit wordt tweeling oligo-polyhydramnion sequentie (TOPS) genoemd en is het diagnostische criterium voor TTS. Bij TAPS is er ook sprake van chronische transfusie, maar zijn de anastomosen minuscuul van formaat waardoor dit leidt tot anemie bij de donor en polycythemie bij de recipient, zonder dat er verschillen ontstaan in vruchtwater.

De beste behandeling voor TTS is intra-uteriene foetoscopische laserbehandeling, waarbij de anastomosen worden gecoaguleerd en er geen bloeduitwisseling tussen foetussen meer kan plaats vinden. De optimale behandeling voor TAPS is niet bekend. Huidige behandelingsmogelijkheden bestaan uit intra-uteriene bloedtransfusie aan de donor, eventueel in combinatie met partiële wisseltransfusie bij de recipiënt, foetoscopische laserbehandeling of een afwachtend beleid. De optimale therapie voor TAPS zal middels een internationale gerandomiseerde studie onderzocht moeten worden.
De verbeterde antenatale behandeling van TTTS en TAPS heeft geleid tot een afname van de perinatale sterfte. Echter hebben de neonaten een verhoogd risico op complicaties, naast het feit dat ze een verhoogd risico hebben op prematuriteit. De meeste studies naar de neonatale complicaties bij tweelingen met TTTS of TAPS zijn gericht op de neurologische en cardiale uitkomsten.

In dit proefschrift hebben we gekeken naar de biochemische en hematologische waarden postpartum bij de verschillende subgroepen van gecompliceerde monochoriale tweelingen en ongecompliceerde monochoriale tweelingen.
In hoofdstuk 2 wordt een overzicht gegeven van de literatuur over hematologische problemen bij gecompliceerde monochoriale tweelingen. De incidentie, risico op en behandeling van anemie, polycythemie en trombocytopenie bij tweelingen met TTS, TAPS, acute peripartum TTS, acute perimortem TTS en ongecompliceerde monochoriale tweelingen wordt beschreven. Daarnaast hebben we de incidentie van deze complicaties geëvalueerd bij onze eigen populatie van monochoriale tweelingen. Zo kwam anemie waarvoor een erythrocytentransfusie nodig was voor bij 33% van de TTS donoren welke conservatief behandeld waren, vergeleken met 5% van de TTS donoren behandeld met complete lasertherapie. Indien de laserbehandeling incompleet was en er dus restanastomosen aanwezig waren, kreeg 55% van de donoren een erythrocytentransfusie. Bij de spontane TAPS donoren was in 83% een erythrocytentransfusie nodig in verband met anemie.

In *hoofdstuk 3* hebben we de hemoglobinewaarden postpartum bij ongecompliceerde monochoriale tweelingen bestudeerd. Hemoglobine (Hb) direct postpartum en op dag 2 zijn geanalyseerd in relatie tot geboortevolgorde, manier van geboorte en tijdinterval tussen geboorte van kind 1 en kind 2. Neonaten die als tweede zijn geboren bij een vaginale partus hadden een significant hoger Hb postpartum en op dag 2 vergeleken met neonaten die als eerste werden geboren. Polycythemie kwam significant vaker voor bij neonaten die als tweede werden geboren vergeleken met neonaten die als eerste werden geboren (12% tegenover 1%, $p<0.01$), maar ook alleen indien er sprake was van een vaginale partus. Er werd geen associatie gevonden tussen het tijdsinterval tussen geboorte van kind 1 en kind 2, en Hb verschillen. De oorzaak van het hogere Hb bij kind 2 indien vaginaal geboren is niet bekend. Mogelijk spelen de anastomosen een rol en doneert kind 1 meer bloed aan kind 2 tijdens de partus (intra-partum transfusie). Andere factoren, zoals een verschil in het afklemmen van de navelstreng, zouden ook een rol kunnen spelen.

Hoofdstuk 4 is een vervolg op hoofdstuk 3, gezien we hebben gekeken naar Hb waarden postpartum bij dichoriale tweelingen en deze hebben we vergeleken met de Hb waarden van ongecompliceerde monochoriale tweelingen. Een belangrijk verschil tussen dichoriale tweelingen en monochoriale tweelingen is de placenta. Dichoriale tweelingen hebben elk hun eigen placenta zonder anastomosen waardoor de bloedcirculaties van de foetussen niet met elkaar verbonden zijn, in tegenstelling tot monochoriale tweelingen die een placenta delen met daarin altijd anastomosen. Het analyseren van Hb waarden bij dichoriale tweelingen gaf hierdoor meer inzicht in de mogelijke pathofysiologie van Hb verschillen na een vaginale partus. Bij dichoriale tweelingen werd namelijk ook een significant hoger Hb gevonden bij neonaten die als tweede werden geboren vergeleken met neonaten die als eerste werden geboren (ook alleen bij een vaginale partus). Gezien dichoriale tweelingen geen anastomosen in de placenta hebben, zal bloedtransfusie van het ene kind naar het andere kind tijdens de partus niet de (enige) oorzaak zijn van de Hb verschillen. Onze hypothese was dat er mogelijk een verschil is in het tijdstip van het afklemmen van de navelstreng. Het zou kunnen dat de gynaecoloog de navelstreng van het eerste kind na geboorte relatief snel afklemt zodat het team zich vervolgens snel kan focussen op de geboorte van het tweede kind. Hierdoor is er relatief weinig tijd voor bloedtransfusie van de placenta naar het kind na geboorte (placenta-foetale transfusie). Indien het tweede kind is geboren, is er mogelijk minder haast voor het afklemmen van de navelstreng, resulterend in een hoger Hb door meer tijd voor bloedtransfusie vanuit de placenta naar het kind. Helaas wordt het tijdstip van het afklemmen van de navelstreng niet geregistreerd in ons centrum. Verdere studies naar het afklemmen van de navelstreng bij tweelingen zouden meer inzicht kunnen geven.

In *hoofdstuk 5* beschrijven we onze studie over albumine waarden postpartum bij neonaten met TTS. Verschillende studies hebben aangetoond dat donoren een significant lager Hb hebben vergeleken met recipiënten indien er geen behandeling middels laser heeft plaatsgevonden. Onze hypothese was dat door de anastomosen niet alleen Hb wordt uitgewisseld, maar ook eiwitten en voedingsstoffen. Een belangrijk eiwit is albumine. Hypoalbuminemie bij neonaten is een onafhankelijke risicofactor voor mortaliteit en morbiditeit. In onze retrospectieve studie hebben we gekeken naar albumine waarden postpartum bij tweelingen met TTS die conservatief zijn behandeld (amniodrainage of een afwachtend beleid, waardoor er dus nog anastomosen aanwezig waren) en deze groep

werd vergeleken met een controle groep van TTS tweelingen behandeld met laser (waardoor er in principe geen anastomosen meer aanwezig zijn). Donoren en recipiënten binnen deze twee groepen werden met elkaar vergeleken. Albumine waarden waren significant lager bij donoren vergeleken met recipiënten in de conservatief behandelde groep; 25.0 g/l tegenover 33.0 g/l (p=0.001). Hypoalbuminemie (albumine < 20 g/l) kwam voor bij 22% (4/18) van de donoren in de conservatief behandelde groep. In de TTS groep behandeld met laser werden geen verschillen tussen donoren en recipiënten gevonden. Onze bevindingen suggereren dat door de anastomosen ook uitwisseling van eiwitten plaatsvindt.

In *hoofdstuk 6* hebben we gekeken naar albumine waarden postpartum bij tweelingen met TAPS. Deze tweelingen hebben we vergeleken met een controle groep van ongecompliceerde monochoriale tweelingen van gelijke amenorroeduur. Gezien onze bevindingen bij TTS in hoofdstuk 5, en TAPS ook wordt veroorzaakt door chronische bloedtransfusie van de donor naar de recipiënt, was onze hypothese dat donoren een lager albumine zouden hebben. Onze retrospectieve studie toonde inderdaad een lager albumine bij donoren in de TAPS groep vergeleken met recipiënten; 28.0 g/l tegenover 32.0 g/l (p=0.008). Hypoalbuminemie kwam voor bij 20% (5/25) van de donoren. In de controle groep van ongecompliceerde monochoriale tweelingen werden geen verschillen in albumine gevonden tussen de grotere en de kleinere neonaat.

In *hoofdstuk 7* hebben we de nierfunctie in de eerste week postpartum bestudeerd bij neonaten met TAPS. Er zijn verschillende studies verricht naar de nierfunctie bij neonaten met TTS, maar bij tweelingen met TAPS is de nierfunctie nog niet eerder onderzocht. TAPS tweelingen uit drie Europese centra zijn geïncludeerd in deze retrospectieve studie. TAPS tweelingen werden vergeleken met ongecompliceerde tweelingen van gelijke amenorroeduur (de controle groep). In de TAPS groep bleken donoren een hoger creatinine te hebben vergeleken met recipiënten; 85 µmol/l tegenover 71 µmol/l (p=0.001). Nierfunctiestoornis in de eerste week postpartum (gedefinieerd als creatinine > 100 µmol/l) kwam voor bij 26.0% van de (13/50) donoren en bij 6.3% (3/48) van de recipiënten (p=0.002). Daarnaast hadden donoren een lagere bloeddruk bij geboorte en een lagere urineproductie op dag 1. In de controle groep was de incidentie van nierfunctiestoornis in de eerste week 7.7% (8/104). Onze hypothese is dat chronische anemie bij donoren hypoxie veroorzaakt wat resulteert in een verhoogde kans op nierfunctiestoornis de eerste week postpartum.

Hoofdstuk 8 is onze studie naar de nierfunctie in de eerste week postpartum in een groot cohort van TTS tweelingen. We hebben tweelingen die behandeld zijn met lasertherapie vergeleken met tweelingen die conservatief zijn behandeld (amniodrainage dan wel afwachtend beleid). Creatinine en ureum waarden waren significant lager in de lasergroep vergeleken met de conservatief behandelde groep; 71 µmol/l tegenover 82 µmol/l (p=0.002). Binnen de lasergroep hebben we incomplete laserbehandeling (waarbij restanastomosen aanwezig zijn) vergeleken met complete laserbehandeling. Tweelingen behandeld met complete lasertherapie hadden een significant lager creatinine vergeleken met tweelingen die incomplete laserbehandeling hebben gehad (76 µmol/l tegenover 69 µmol/l, p=0.018). In de groepen werden geen verschillen in creatinine waarden gevonden tussen donoren en recipiënten. Mogelijk is dit geassocieerd met de kleine aan-

tallen in de subgroepen. Wel hadden donoren uit de conservatief behandelde groep vaker oligurie op dag 1.

Tijdens de zwangerschap is er sprake van hypovolemie bij de donor wat resulteert in verminderde nierperfusie, oligurie en oligohydramnion. Nierfunctiestoornis in de eerste week postpartum (gedefinieerd als creatinine > 100 µmol/l) kwam significant minder vaak voor bij gelaserde tweelingen, met name na complete laserbehandeling, wat suggereert dat laserbehandeling een beschermend effect heeft op de nierfunctie in de eerste week postpartum.

Concluderend hebben tweelingen met TTS of TAPS een verhoogd risico op hematologische en biochemische problemen in de neonatale periode. Of deze problemen zich ook voordoen bij andere subgroepen van gecompliceerde monochoriale tweelingen, zoals tweelingen met selectieve intra-uteriene groeiretardatie, is niet bekend en zal verder onderzocht moeten worden. Ten slotte zijn de lange termijn effecten van de hematologische en biochemische problemen in de neonatale periode niet bekend. Follow-up studies bij deze tweelingen zouden zich daarom niet alleen op de neurologische uitkomsten moeten focussen, maar ook op cardiovasculaire en renale uitkomsten op de langere termijn.

Chapter 11
Appendices

List of abbreviations
Authors and affiliations
Dankwoord (acknowledgements)
Publications
Curriculum vitae

List of abbreviations

AA	Arterio-arterial
AV	Arterio-venous
CS	Cesarean section
DC	Dichorionic
Hb	Hemoglobin
IUT	Intrauterine transfusion
IQR	Interquartile ranges
MC	Monochorionic
MCA-PSV	Middle Cerebral Artery Peak Systolic Velocity
MoM	Multiples of the Mean
PET	Partial Exchange Transfusion
sIUGR	Selective intrauterine growth restriction
TAPS	Twin anemia-polycythemia sequence
TOPS	Twin oligo-polyhydramnios sequence
TRAP	Twin Reversed Arterial Perfusion
TTTS	Twin-twin transfusion syndrome
VV	Veno-venous

Authors and affiliations

Leiden University Medical Center, Leiden, The Netherlands
Femke Slaghekke
Marieke Sueters
Johanna M. Middeldorp
Frans J. Klumper
Monique C. Haak
Dick Oepkes
Enrico Lopriore
Frans J. Walther
Depeng P. Zhao
Faiez Joemmanbaks
Jacoba M. Quak
Rám Sukhai
Lianne Verbeek

University Medical Center Groningen, Groningen, The Netherlands
Christian V. Hulzebos

Monash Institute of Medical Research, Melbourne, Australia
Stuart B. Hooper

Strasbourg Teaching Hospital, Strasbourg, France
Romain Favre
Marine Vieujoz

Vittore Buzzi Children's Hospital, Milan, Italy
Francesco Cavigioli
Gianluca Lista

Dankwoord (acknowledgements)

Alleen mijn naam staat op de voorkant van dit proefschrift, maar dit had niet tot stand gekomen zonder de (mentale) steun van anderen. Ik maak bij deze dan ook graag van de gelegenheid gebruik om iedereen te bedanken die mij heeft geholpen.

Allereerst mijn promotor en begeleider prof. dr. Enrico Lopriore. Beste Enrico, ik zou eigenlijk een hele pagina hieraan moeten besteden, maar gezien je zelf meer van 'kort en krachtig' bent zal ik het toch tot één alinea beperken. Dankzij jou heb ik mijn wetenschappelijke interesses verder kunnen ontwikkelen. Ik had dit niet kunnen combineren met mijn werk als arts-assistent als jij niet zo snel mijn vragen beantwoordde en werk corrigeerde. Je had steeds weer interessante nieuwe ideeën en jouw positieve instelling werkt stimulerend en motiverend. Ontzettend bedankt voor je begeleiding, enthousiasme en ideeën!

I would like to thank all co-authors: Dick Oepkes, Femke Slaghekke, Marieke Sueters, Annemieke Middeldorp, Frans Klumper, Monique Haak, Depeng Zhao, Arjan te Pas, Stuart Hooper, Christian Hulzebos, Frans Walther, Romain Favre, Marine Vieujoz, Francesco Cavigioli, Gianluca Lista, Faiez Joemmanbaks, Jacoba M. Quak and Rám Sukhai, thank you very much for improving our manuscripts. It was a pleasure working with you.

Many thanks to the thesis committee for evaluating my thesis.

Mijn collega's van het Westeinde, Juliana Kinderziekenhuis en LUMC wil ik bedanken voor hun flexibiliteit die soms nodig was om mijn werk als arts-assistent te combineren met mijn onderzoek. Bedankt voor jullie interesse in mijn onderzoek en tips over promoveren.

Ook wil ik mijn (schoon)familie, vrienden en vriendinnen bedanken voor hun steun en interesse in mij en mijn onderzoek. Tussen alle drukte door is het heel fijn om met jullie leuke dingen te doen, maar ook met een luisterend oor staan jullie altijd voor mij klaar. Dieneke, naast een goede vriendin ook een goede "Engelse spellingscheck", bedankt! In het speciaal bedank ik ook mijn paranimfen Anne en Lisan, super dat jullie mij bij staan tijdens mijn promotie.

Lieve pap en mam, jullie staan altijd voor me klaar en zijn altijd geïnteresseerd in mijn onderzoek. Van jullie heb ik geleerd om door te zetten en ergens voor te gaan. Lieve Riëlle, bedankt voor je steun op alle fronten, je bent een topzus!
Lieve Sander, jouw geduld, relativeringsvermogen, hulp bij laptop problemen, luisterend oor maar bovenal je liefde, hebben mij heel erg geholpen tijdens mijn promotietraject, maar vooral ook tijdens ons leven samen. Samen zijn met jou is een feestje!

Publications

Verbeek L, Joemmanbaks F, Quak JM, Sukhai R, Middeldorp JM, Oepkes D, Lopriore E. Renal function in neonates with twin-twin transfusion syndrome treated with or without fetoscopic laser surgery. *Submitted*.

Verbeek L, Slaghekke F, Sueters M, Middeldorp JM, Klumper FJ, Haak MC, Oepkes D, Lopriore E. Hematological disorders at birth in complicated monochorionic twins. In press, *Expert Rev Hematol*.

Verbeek L, Zhao DP, Middeldorp JM, Oepkes D, Hooper SB, te Pas AB, Lopriore E. Haemoglobin discordances in twins: due to differences in timing of cord clamping? *Arch Dis Child Fetal Neonatal Ed. 2016 Dec 9*.

Verbeek L, Zhao DP, te Pas AB, Middeldorp JM, Hooper SB, Oepkes D, Lopriore E. Hemoglobin differences in uncomplicated monochorionic twins in relation to birth order and mode of delivery. *Twin Res Hum Genet. 2016 Jun;19(3):241-5*.

Verbeek L, Slaghekke F, Favre R, Vieujoz M, Caviglioli, Lista G, Oepkes D, Lopriore E. Short-term postnatal renal function in twin anemia-polycythemia sequence. *Fetal Diagn Ther. 2016;39(3):192-7*.

Verbeek L, Slaghekke F, Hulzebos CV, Oepkes D, Walther FJ, Lopriore E: Hypoalbuminemia in donors with twin anemia-polycythemia sequence: a matched case-control study. *Fetal Diagn Ther. 2013;33(4):241-5*.

Verbeek L, Middeldorp JM, Hulzebos CV, Oepkes D, Walther FJ, Lopriore E. Hypo-albuminemia in donors with twin-twin transfusion syndrome. *Fetal Diagn Ther. 2013;33(2):98-102*.

Curriculum vitae

Lianne Verbeek werd geboren op 11 september 1990 in Rijswijk. Na enkele weken verhuisde zij samen met haar ouders naar Nootdorp, waar ze haar jeugd samen met haar zusje Riëlle heeft doorgebracht. In 2008 behaalde zij haar Atheneum diploma aan het Sint-Maartenscollege te Voorburg. Vervolgens begon zij aan haar studie geneeskunde aan de Universiteit Leiden. Tijdens haar studie heeft zij zich onder andere ingezet voor de Jaarvertegenwoordiging van geneeskunde jaar 2 en het Teddybear Hospital van de Medische Faculteit der Leidse Studenten. Daarnaast heeft zij gewerkt als doktersassistente bij de Trombosedienst en Scal medische diagnostiek. Naast studie-gerelateerde zaken vond zij ook ontspanning als lid van de studentenvereniging l.v.v.s. Augustinus en studententennisvereniging Qravel. Daarnaast heeft zij verschillende buitenlandse reizen gemaakt en een coschap in Suriname gevolgd. De basis voor haar wetenschappelijke carrière werd gelegd in het vierde jaar van de studie geneeskunde tijdens een college over tweeling-transfusiesyndroom. Na haar wetenschapsstage bij de afdeling Neonatologie, begeleid door prof. dr. Enrico Lopriore, heeft zij haar onderzoek voortgezet tijdens haar coschappen en werk als arts-assistent kindergeneeskunde, wat uitmondde in dit proefschrift. Eind 2014 studeerde zij af waarna zij tot oktober 2015 heeft gewerkt als arts-assistent kindergeneeskunde in het Haaglanden Medisch Centrum, locatie Westeinde en vervolgens tot juni 2016 in het Juliana Kinderziekenhuis (HagaZiekenhuis). Sinds juni 2016 werkt zij als arts-assistent neonatologie in het Leids Universitair Medisch Centrum en in maart 2017 is zij begonnen met de opleiding tot kinderarts (opleider dr. W.J.W. Kollen).