Neonatal hematological and biochemical complications in TTTS and TAPS

Verbeek, L.I.

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

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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.

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