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Prenatal Diagnosis 2010; 30: 251-255.

Chapter 6

Hematological characteristics in neonates with twin anemia polycythemia sequence

Abstract

Objective:

To evaluate the neonatal hematological features of monochorionic twins with twin anemia-polycythemia sequence (TAPS) and to determine the additional diagnostic value of reticulocyte count measurement.

Methods:

A cohort of consecutive monochorionic twins with TAPS (n = 19) was included in the study and each twin pair was compared with two monochorionic twin pairs (n = 38) unaffected by TAPS or twin–twin transfusion syndrome (TTTS), matched for gestational age at birth. We measured full blood counts on day 1 and determined the incidence of anemia, polycythemia, reticulocytosis and thrombocytopenia.

Results:

Median inter-twin hemoglobin (Hb) difference in monochorionic twins with and without TAPS was 13.7 g/dL and 2.4 g/dL, respectively ($p < 0.01$). Median inter-twin reticulocyte count ratio in twins with and without TAPS was 3.1 and 1.0, respectively ($p < 0.01$). Thrombocytopenia (platelet count $<150 \times 10^9$ /L) occurred more often in the TAPS group than in the control group, 45% (17/38) versus 11% (11/38), respectively ($p < 0.01$). In the TAPS group, mean platelet count was significantly lower in recipients than in donors, 133×10^9/L versus 218×10^9/L, respectively ($p < 0.01$).

Conclusions:

TAPS twins have a large inter-twin Hb difference in combination with a large intertwin reticulocyte count ratio. Recipients are more often thrombocytopenic than donors, probably due to polycythemia.

Introduction

Twin anemia–polycythemia sequence (TAPS) is an atypical form of chronic feto-fetal blood transfusion which may occur in uncomplicated monochorionic twin pregnancies (spontaneous form) or after laser surgery for twin–twin transfusion syndrome (TTTS) (post-laser form) [1-3]. Both forms are characterized by the presence of large intertwin hemoglobin (Hb) difference without the degree of amniotic fluid discordance that is required for the diagnosis of TTTS [1-3]. The post-laser form of TAPS is reported to occur in up to 13% of TTTS patients treated with laser [1]. The spontaneous form of TAPS complicates approximately 3 to 5% of monochorionic twin pregnancies [4;5].

Both spontaneous and post-laser TAPS have a similar anatomic substrate which is based on the presence of only few minuscule arterio-venous anastomoses, allowing a very slow inter-twin blood transfusion [6]. The optimal management of TAPS (both for the spontaneous form and for the post-laser form) is not clear and may include expectant management, intrauterine blood transfusion or laser coagulation of the vascular anastomoses [7].

Diagnosis of TAPS can be reached after birth based on the presence of chronic anemia in one twin and polycythemia in the co-twin, in association with typical placental angioarchitecture following colored dye injection [5]. Diagnosis of TAPS can also be reached prenatally but requires different criteria based on Doppler ultrasound measurements [1-3]. Our knowledge of hematological features in TAPS is sparse and based on only a few casuistic reports [2;3]. Large cohort or case–control studies evaluating hematological aspects in TAPS are required to further develop accurate diagnostic criteria for TAPS.

The aim of this study was to report the hematological characteristics of neonates with TAPS and to determine the additional diagnostic value of reticulocyte count measurement in TAPS.

Methods

All consecutive monochorionic twin pairs with TAPS admitted to our neonatal nursery at the Leiden University Medical Centre (The Netherlands) between June 2002 and February 2009 were included in the study. The Leiden University Medical Centre is an academic referral center managing all types of complications of monochorionic pregnancies. Absence of prenatal signs of TTTS (i.e. absence of oligohydramnios–polyhydramnios sequence on ultrasound) is a conditio sine qua non for the diagnosis of TAPS.

For the purpose of this study, diagnosis of TAPS was based on postnatal criteria. Postnatal criteria were met if hematological investigations on day one showed chronic anemia in the donor (Hb <5th centile for gestational age) [8] and polycythemia in the recipient (venous hematocrit >65%) in combination with placental injection studies showing only small arterio-venous anastomoses (diameter <1 mm) and no superficial arterio-arterial anastomoses. Details on the technique used for placental injection and placental territory measurement have been described previously [6]. Part of the placental data reported in this study was included in a previous report on placental angioarchitecture in TAPS [6]. Two spontaneous TAPS cases and one TAPS case after laser surgery were previously published [2;9].

We excluded all twin pregnancies with single or double intrauterine death and patients with an incomplete placental injection. Patients with TAPS (spontaneous TAPS cases as well as post-laser TAPS cases) that were treated after diagnosis with fetoscopic laser surgery were excluded from the study.

Each twin pair with TAPS was compared with two control monochorionic twin pairs unaffected by TAPS or TTTS, matched by gestational age at birth (±1 week gestation). All monochorionic twins (with and without complications) delivered at our center are entered prospectively in a "monochorionic twins" database. The control population in this study was obtained from this dedicated database.

We recorded the gestational age at birth, birth weight and inter-twin birth weight discordance. Full blood count and reticulocyte count were measured routinely on day one in all monochorionic twins. Blood samplings were obtained primarily from umbilical cord blood. If cord blood was not available, samplings were routinely obtained on day one through heel stick or venous puncture. Anemia on day one was defined as Hb level <5th centile for gestational age [8]. Polycythemia on day one was defined as a venous hematocrit >65% [10]. Reticulocytosis was defined as a reticulocyte count >95th centile for gestational age [8].

We calculated the inter-twin Hb difference and intertwin reticulocyte count ratio. The inter-twin reticulocyte ratio was calculated by dividing the reticulocyte count of the infant with the lowest Hb by the reticulocyte count of its co-twin. Hematological characteristics were compared between the TAPS group and the control group. We also compared the hematological results between donors and recipients in the TAPS group and between the twin infant with the lower Hb level and its co-twin in the control group. In this study, the term "recipient" is used to define the twin infant with polycythemia, whereas the term "donor" is used to define the anemic twin infant.

Data are reported as means and standard deviations (SD) or as medians and interquartile range (IQR), as appropriate. The unpaired Student t-test was used for parametric and the Mann–Whitney U test for non-parametric comparisons for continuous variables. Results of categorical variables were compared using Fisher's exact test. For comparisons within twin pairs, the paired Student t-test was used for continuous variables, and the Mc Nemar test was used for the analysis of paired nominal variables. Sensitivity and specificity were calculated by standard formulas for a binominal proportion. The Spearman rank correlation was used to study the relationship between polycythemia and thrombocytopenia. A p-value <0.05 was considered to indicate statistical significance. Analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Results

A total of 22 consecutive TAPS patients were identified during the study period. Two spontaneous TAPS patients were treated with fetoscopic laser surgery after diagnosis and were thus excluded from the study. One post-laser TAPS patient was excluded because of the presence of a (small) superficial arterio-arterial anastomosis on placental injection (Lopriore et al., 2007c). Nineteen twin pairs thus fulfilled the inclusion criteria for this study. Five (26%) were spontaneous TAPS and 14 (74%) were post-laser TAPS cases. Doppler measurements of the middle cerebral artery-peak systolic flow velocity (MCA-PSV) from both fetuses were available from 79% (15/19) of patients in the TAPS group (MCA-PSV measurements were performed in two of the five spontaneous TAPS cases and in 13 of the 15 postlaser TAPS cases). Increased MCA-PSV (>1.5 multiples of the median) in the donor in combination with a decreased MCA-PSV (<0.8 multiples of the median) in the recipient was detected prenatally in 60% (9/15) of these patients (all in the post-laser TAPS cases). Two post-laser TAPS patients were treated prenatally with intrauterine blood transfusion(s), one was recently published as a case report [11].

	TAPS group (n=19 pregnancies; 38 neonates)	**Control group (n=38 pregnancies; 76 neonates)**
Gestational age at birth (weeks) [a]	32±2.8	32.2±2.7
Female n(%)	16 (42)	40 (53)
Vaginal delivery n (%)	10 (53)	18 (47)
Birth weight g [a]	1591±415	1780±521
Birth weight discordance (%) [a]	14±14	15±14
Small for gestational age n (%)	2 (5)	4 (5)

[a] Value given as mean (±SD), Birth weight discordance was calculated as follows: [(birth weight larger twin - birth weight smaller twin)/birth weight larger twin]×100%.

Table 1: Baseline characteristics in TAPS twin pairs and control twin pairs

	TAPS group (n=38)	Controls (n=76)	p value
Hemoglobin level (g/dL) [a]	15.3 (8.6–23.1)	16.4 (14.8–18.5)	0.86
Hematocrit (%) [a]	53 (29–74)	47 (42–52)	0.99
Inter-twin hemoglobin difference (g/dL) [a]	13.7 (12.2–16.9)	2.4 (0.6–3.9)	<0.01
Anemia n(%)	19 (50)	3 (4)	<0.01
Polycythemia n(%)	19 (50)	4 (5)	<0.01
Reticulocyte count (%) [a]	7.3 (3.6–14.5)	6.1 (5.2–7.0)	0.34
Reticulocytosis n/N (%) [b]	19/36 (53)	32/66 (48)	0.68
Inter-twin reticulocyte count difference (%) [a]	10.6 (5.7–14.4)	2.4 (0.6–3.9)	<0.01
Inter-twin reticulocyte count ratio [a,c]	3.1 (2.3–5.8)	1.0 (1.0–1.1)	<0.01
Platelet count (10^9/L) [a]	155 (112–231)	216 (174–259)	<0.01
Thrombocytopenia (<150) n (%)	17 (45)	11 (14)	<0.01
Thrombocytopenia (<100) n (%)	7 (18)	1 (1)	<0.01
Thrombocytopenia (<50) n (%)	1 (3)	0 (0)	0.33

[a] Value given as median (interquartile range).
[b] Reticulocytosis: reticulocyte count >95th centile.
[c] Reticulocyte count of infant with lower Hb level divided by the reticulocyte count of the co-twin.

Table 2: Hematological characteristics in TAPS neonates and controls at birth

Baseline characteristics in the groups with and without TAPS are presented in Table 1. Details on prenatal findings and neonatal morbidities in both groups are presented separately [12]. Other possible causes for neonatal anemia at birth, such as Rhesus incompatibility, partial abruption, infection and fetomaternal hemorrhage were ruled out with clinical, ultrasound and/or laboratory examinations. Similarly, chronic hypoxia as a possible cause of polycythemia at birth was ruled out in plethoric neonates. Anemia in one twin in combination with polycythemia in the co-twin occurred (by definition) in all TAPS twins, but also in one twin pair in the control group. In this control twin pair delivered at 35 weeks' gestation, the first-born twin infant was anemic (Hb 12.5 g/dL) but did not require a blood transfusion and the second-born twin was polycythemic (Hb 23.8 g/dL, venous hematocrit 73%) and needed a partial exchange transfusion. The anemic twin had no reticulocytosis (reticulocyte count value of 5.7%) and placental injection showed a large arterio-arterial anastomosis. Both factors precluded TAPS and were more suggestive of an acute form of peripartum feto-fetal transfusion.

Details on hematological characteristics in the TAPS group and control group are shown in Table 2. The median inter-twin Hb difference at birth in the group with and without TAPS was 13.7 g/dL (IQR 12.2–16.9; range 8.9–20.3 g/dL) and 2.4 g/dL (IQR 0.6–3.9; range 0–11.3 g/dL), respectively ($p < 0.01$). An inter-twin Hb difference <8 g/dL was found in all TAPS twin

pairs and in one control twin pair (3%, 1/38), leading to a sensitivity of 100% (19/19) and a specificity of 97% (37/38).

Reticulocyte count was measured in 95% (18/19) of twin pairs in the TAPS group and in 87% (33/38) of control twin pairs. The median inter-twin reticulocyte count ratio in twins with and without TAPS was 3.1 (IQR 2.3–5.8; range 1.8–10.0) and 1.0 (IQR 1.0–1.1; range 1–1.5), respectively ($p < 0.01$). All TAPS twins had an inter-twin reticulocyte count ratio >1.7, whereas this never occurred in the control twin pairs (sensitivity and specificity of 100%).

Thrombocytopenia (defined as a platelet count $< 150 \times 10^9$ /L) occurred more often in the TAPS group than in the control group, 45% (17/38) and 11% (11/38) ($p < 0.01$). In the TAPS group, platelet count was significantly lower in recipients than in donors, 133×10^9 /L and 218×10^9 /L, respectively ($p < 0.01$). One recipient twin had a clinically significant severe thrombocytopenia (24×10^9 /L) requiring a platelet transfusion at birth. Platelet count in recipient twins was negatively correlated to the Hb level at birth (Spearman correlation coefficient = −0.698, $p = 0.001$) (Figure 1).

Differences in hematological characteristics between donors and recipients in the TAPS group and between the twin infant with the lower Hb level and the co-twin in the control group are presented in Table 3. In the TAPS group, Hb values in the anemic donors ranged from 4.5 to 11.7 g/dL (mean value of 8.9 g/dL), whereas Hb values in the polycythemic recipient ranged from 19.0 to 26.6 (mean value of 23.0 g/dL). Mean reticulocyte count in donor twins in the TAPS group was 16.3% (range 7.1–27.9). Reticulocytosis (reticulocyte count <95th centile) was present in 94% (17/18) of donor twins, 11% (2/18) of recipient twins, 48% (16/33) of twin infants with the lowest Hb in the control group and 48% (16/33)

	TAPS group (N=38)			Control group (N=76)		
	Donor (n=19)	Recipient (n=19)	p value	Twin A (n=38)	Twin B (n=38)	p value
Hemoglobin level (g/dL) [a]	8.9±2.1	23.0±2.1	<0.01	15.3±2.2	18.0±2.5	<0.01
Hematocrit (%) [a]	29±6	74±7	<0.01	44±5	52±9	<0.01
Reticulocyte count (%) [a]	16.3±7.8	4.6±3.6	<0.01	6.4±1.6	6.2±1.4	0.09
Reticulocytosis n/N (%) [b]	17/18 (94)	2/18 (11)	<0.01	16/33 (48)	16/33 (48)	1.0
Platelet count (10^9/L) [a]	218±101	133±51	<0.01	213±64	221±53	0.20
Thrombocytopenia n(%)	5 (26)	12 (63)	0.02	6 (16)	5 (13)	0.51

[a] Value given as mean (±SD)
[b] Reticulocytosis: reticulocyte count >95th centile

Table 3: Hematological characteristics in TAPS neonates (donor vs recipients) and in controls [twin A is the infant with lower Hb (Hb) level and twin B is the co-twin with higher Hb level] at birth

of their co-twins, leading to a sensitivity of reticulocytosis in TAPS of 94% (17/18) and a specificity of 60% (50/84).

Discussion

This study reports detailed hematological features which are characteristic of TAPS, including a large intertwin Hb difference and a large inter-twin reticulocyte count difference. These hematological results can be used to establish clear criteria for postnatal diagnosis of TAPS. Since TAPS has just recently been described [1;2], uniform criteria for hematological values have not yet been established. Strict diagnostic criteria for TAPS are also required to allow comparison of outcome data between centers performing laser treatment in TTTS pregnancies. Occurrence of TAPS after laser treatment is often due to residual anastomoses [9;13] and is regarded as treatment failure [1;14-16].

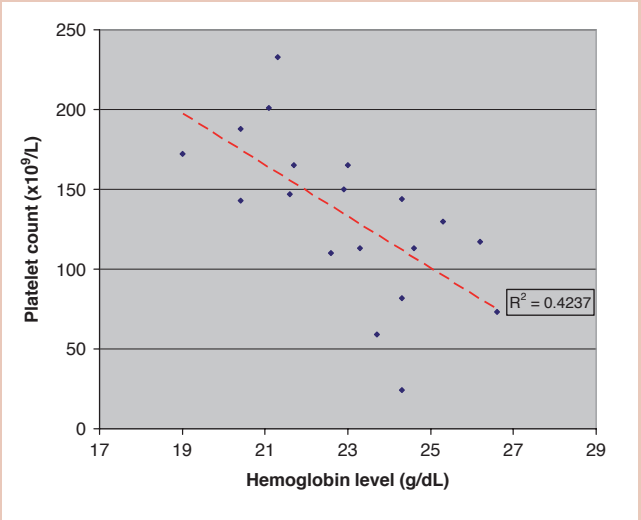

Figure 1. Negative correlation in recipient twins between hemoglobin level and platelet count at birth

Several different diagnostic criteria for TAPS have been proposed using different cut-off values. Specific cut-off levels for MCA-PSV measured with Doppler ultrasound have been used to define prenatal criteria for TAPS. Frequently reported criteria are an increased MCA-PSV >1.5 multiples of the median in one fetus (suggestive of fetal anemia) with a concurrent decrease in MCA-PSV <0.8 multiples of the median (suggestive of polycythemia) in the co-twin [1]. However, the sensitivity and specificity of these prenatal criteria have not yet been demonstrated.

Several postnatal criteria for TAPS have been proposed. Lewi et al. defined TAPS as the presence of a Hb level <11 g/dL in the anemic twin and >20 g/dL in the polycythemic

co-twin[4]. Although the use of fixed cut-off levels for Hb values has clear practical advantages, it does not take the correlation of Hb levels with gestational age into consideration. Hb concentration is known to increase linearly with gestation [8;17]. We therefore chose to use gestational age-dependent cutoff levels for Hb concentration [2]. However, this definition has the practical disadvantage in that it requires the use of specific normograms for Hb in relation to gestational age. Several different normograms have been published [8;17]. Moreover, there is little consensus between neonatologists on the definition of neonatal anemia at birth (using different cut-off values for Hb levels) and international guidelines on when to transfuse anemic infants are also highly discordant [18]. Similarly, although most neonatologists define neonatal polycythemia as a hematocrit 65%, other definitions are also being used and consensus on the optimal management for polycythemia is highly controversial [10]. The criteria used in this study to define TAPS may therefore seem less practical compared to the fixed Hb levels proposed by Lewi et al. [4].

An alternative and more pragmatic method would be to use inter-twin Hb differences instead of specific cutoff levels for anemia and polycythemia. Since TAPS is a form of inter-fetal transfusion, the use of intertwin Hb difference seems a logical alternative. As shown in this study, all TAPS twins have a high intertwin Hb difference (>8 g/dL). However, large intertwin Hb difference may also occur in uncomplicated monochorionic twin pairs due to acute peripartum TTTS or due to acute placento-fetal transfusion after delivery of the first twin [19].

Differentiation between these two types of inter-twin transfusions is difficult to reach solely on the basis of physical examination. In both situations, neonatologists are confronted at birth with a pale anemic infant and a plethoric polycythemic co-twin. Although the absence of symptoms of shock in the pale anemic twin would argue in favor of TAPS, an additional objective criterion to guide the clinicians would be very useful. Since blood loss in acute peripartum TTTS or acute placento-fetal transfusion occurs rapidly, the reticulocyte count in the anemic co-twin is not increased. In contrast, the reticulocyte count in the anemic co-twin in TAPS is almost always increased, reflecting chronic blood loss. However, reticulocytosis (reticulocyte count >95th centile for gestational age) in the anemic twin was not highly discriminative and does not appear to be an adequate criterion for TAPS. Although the sensitivity of reticulocytosis in the anemic twin was 95%, its specificity was only 60%. The lack of specificity may also be explained by the fact that reticulocytosis reflects not only anemia but also chronic fetal distress. In addition, reference ranges for reticulocyte count may not be easily available. Moreover, in analogy to Hb concentration, the reticulocyte count is known to decrease linearly with advancing gestation [8]. Determination of

reticulocytosis thus requires appropriate graphs. We therefore propose, for practical reasons (see previous arguments for Hb levels), to use an inter-twin reticulocyte count ratio instead of a fixed reticulocyte count. As shown in this study, an increased inter-twin reticulocyte count ratio >1.7 appears to be pathognomonic for TAPS (sensitivity and specificity of 100%).

Another important finding of this study is the increased incidence of thrombocytopenia in the TAPS group, related principally to a decreased platelet count in recipient twins. Recipient twins with TAPS are per definition polycythemic. Polycythemia is known to be associated with thrombocytopenia [10]. Low platelet counts in infants with polycythemia may occur due to impaired production secondary to tissue hypoxia, slow spleen blood flow and decreased plasma fraction with normal concentrations [10]. Thrombocytopenia in recipient twins was mostly self-limiting, but one of the recipient twins required a platelet transfusion at birth due to severe thrombocytopenia.

In conclusion, TAPS twins have a large inter-twin Hb difference in combination with a large inter-twin reticulocyte count ratio. Determination of reticulocyte count in monochorionic twins with discordant Hb levels at birth is important to differentiate between chronic fetofetal transfusion (TAPS) and acute feto-fetal transfusion (such as peripartum TTTS) or placento-fetal transfusion at birth. A high inter-twin reticulocyte count ratio (>1.7) appears to be pathognomonic for TAPS. We propose that postnatal hematological criteria for TAPS should include an inter-twin Hb difference (>8 g/dL) in association with a high inter-twin reticulocyte count ratio.

References

1. Robyr R, Lewi L, Salomon LJ, et al. 2006. Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twinto-twin transfusion syndrome. Am J Obstet Gynecol 194: 796–803.
2. Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP. 2007. Twin anemia–polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 28: 47–51.
3. Lopriore E, van den Wijngaard JP, Middeldorp JM, et al. 2007. Assessment of feto-fetal transfusion flow through placental arteriovenous anastomoses in a unique case of twin-to-twin transfusion syndrome. Placenta 28: 209–211.
4. Lewi L, Jani J, Blickstein I, et al. 2008. The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 199: 514–518.
5. Lopriore E, Oepkes D. 2008. Fetal and neonatal haematological complications in monochorionic twins. Semin Fetal Neonatal Med 13: 231–238.
6. Lopriore E, Deprest J, Slaghekke F, et al. 2008. Placental characteristics in monochorionic twins with and without twin anemia–polycythemia sequence. Obstet Gynecol 112: 753–758.
7. Lopriore E, Hecher K, Vandenbussche FP, van den Wijngaard JP, Klumper FJ, Oepkes D. 2008. Fetoscopic laser treatment of twin-to-twin transfusion syndrome followed by severe twin anemia–polycythemia sequence with spontaneous resolution. Am J Obstet Gynecol 198: e4–e7.
8. Nicolaides KH, Thilaganathan B, Mibashan RS. 1989. Cordocentesis in the investigation of fetal erythropoiesis. Am J Obstet Gynecol 161: 1197–1200.
9. Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ, Vandenbussche FP. 2007. Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome. Placenta 28: 204–208.
10. Sarkar S, Rosenkrantz TS. 2008. Neonatal polycythemia and hyperviscosity. Semin Fetal Neonatal Med 13: 248–255.
11. Lopriore E, van den Wijngaard JP, Pasman SA, et al. 2009. Quantification of feto-fetal transfusion rate through a single placental arterio-venous anastomosis in a monochorionic twin pregnancy. Placenta 30: 223–225.
12. Lopriore, E, Slaghekke, F, Oepkes, D, Middeldorp, JM, Vandenbussche, FP, Walther, FJ. 2010. Clinical outcome in neonates with twin anemia–polycythemia sequence. Am J Obstet Gynecol (in press).
13. Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. 2009. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 201: e1–4.
14. Robyr, R, Lewi, L, Salomon, LJ, et al. 2005. Recurrence of twin–twin transfusion syndrome (TTTS) and feto-fetal hemorrhage: two complications of laser treatment with distinct ultrasound features. Ultrasound Obstet Gynecol 26: 433–434.
15. Yamamoto M, El Murr L, Robyr R, Leleu F, Takahashi Y, Ville Y. 2005. Incidence and impact of perioperative
16. Lewi L, Jani J, Cannie M, et al. 2006. Intertwin anastomoses in monochorionic placentas after fetoscopic laser coagulation for twinto-twin transfusion syndrome: is there more than meets the eye? Am J Obstet Gynecol 194: 790–795.
17. Jopling J, Henry E, Wiedmeier SE, Christensen RD. 2009. Reference ranges for hematocrit and blood hemoglobin concentration during the neonatal period: data from a multihospital health care system. Pediatrics 123: e333–e337.
18. New HV, Stanworth SJ, Engelfriet CP, et al. 2009. Neonatal transfusions. Vox Sang 96: 62–85.
19. Lopriore E, Sueters M, Middeldorp JM, Vandenbussche FP, Walther FJ. 2005. Haemoglobin differences at birth in monochorionic twins without chronic twin-to-twin transfusion syndrome. Prenat Diagn 25: 844–850.