Is umbilical cord blood a reliable source for the evaluation of neonatal hemostasis at birth?

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Running title: Hemostatic profile of the umbilical cord blood

Wordcount: abstract 250, text 2931

Figure: 2

Tables: 2

Conflicts of interest: The authors have no conflict of interest to disclose relative to this manuscript

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43 **Abstract**

44 **Background** Phlebotomy is among the main determinants of anemia of prematurity. Blood sparing
45 policies endorsed umbilical cord blood (here called placental) as an alternative source for
46 laboratory testing. Little is known on the suitability of placental blood to evaluate neonatal
47 hemostasis of newborn infants. We aimed to compare the hemostatic profile of paired placental
48 and infant venous blood, by means of prothrombin time (PT), activated partial thromboplastin time
49 (APTT), fibrinogen, antithrombin, protein C, thromboelastography (TEG) and thrombin generation
50 assay (TGA).

51 **Study design** Observational single-centre study.

52 **Methods** We collected at birth venous citrated blood from both placental and infant venous source
53 and performed PT, APTT, fibrinogen, antithrombin, protein C, TEG (reaction time-R; kinetics-K
54 alpha angle-α, maximum amplitude-MA and lysis at 30 minutes-LY30) and TGA (endogenous
55 thrombin potential-ETP).

56 **Results** We enrolled 60 neonates with a median gestational age (range) of 37 weeks (28^{+1}-41)
57 and birth-weight 2417g (950-4170). Based on TEG and TGA, placental blood showed a
58 procoagulant imbalance as indicated by lower median R (4.0 vs 6.1 min; p<0.001) and K (1.3 vs
59 2.2 min; p<0.001); higher α-angle (69.7 vs 57.4°; p<0.001) and ETP (1260-vs-1078; p=0.002) than
60 those observed for infant venous blood. PT and APTT did not differ significantly between the two
61 groups.

62 **Conclusions** While placental and neonatal blood samples are equally suitable to measure the
63 standard coagulation tests PT and APTT, placental blood leads to a procoagulant imbalance when
64 testing is performed with TEG or TGA. These effects should be considered when interpreting
65 results stemming from investigation of neonatal hemostasis.

66 **Keywords** thromboelastography; thrombin generation assay; thrombosis; coagulation; neonate;
67 blood sparing; anemia of prematurity

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Introduction

Frequent blood sampling is a key contributor to anemia of prematurity, especially among Very Low Birth Weight (VLBW; <1500 grams) infants, who are exposed to iatrogenic blood loss up to 10%-15% of their estimated total blood volume.[1] Blood overdraw may lead to the need for multiple erythrocyte transfusions, which are common practice in the Neonatal Intensive Care Unit (NICU), reaching rates of 40% among VLBW and 90% among extremely low birth weight neonates.[2] However, there is increasing concern regarding the association of erythrocyte transfusions with complications of prematurity such as intraventricular hemorrhage, bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis.[3,4] Several strategies have been developed to reduce the number of erythrocyte transfusions. These strategies include placental transfusion, microsampling, and umbilical cord blood sampling (here called placental blood) for admission laboratory tests.[5-7] Indeed, the use of placental blood, which would be otherwise discarded immediately after placental delivery, resulted in higher haemoglobin levels, lower rate of red blood cell transfusion and use of vasopressors in the first week of life.[6] Moreover, the avoidance of neonatal phlebotomy contributes to the reduction of the procedural pain and neonatal stress, whose adverse impact on long-term developmental outcomes has been widely acknowledged.[8,9] Feasibility of placental blood collection has been confirmed even after delayed cord clamping or milking of the umbilical cord, thus appearing as a complementary practice to maximise neonatal blood volume.[6] Recently, placental blood collection has been suggested for complete blood count,[5-7,10] blood culture,[5,10,11] blood type,[5,6] antibody screen[5] and metabolic screen.[5,6] Currently, limited data are available on the comparison between placental and infant venous blood for the assessment of neonatal hemostasis, which is occasionally required for the initial diagnostic laboratory work up at NICU. Due to ethical and technical issues on neonatal peripheral blood drawing, previous studies on standard coagulation[12,13] and viscoelastic assays[14-16] have mainly relied on placental blood collected at the time of delivery, under the widely accepted (but unproven) assumption that the source of blood specimen would be interchangeable. Observations made, especially on investigation of platelets activity, raised concerns that the changes occurring during

labour and delivery might challenge the validity of the placental blood as a source of blood specimen to investigate neonatal hemostasis.[17,18]

Standard coagulation tests such as prothrombin time (PT) and activated partial thromboplastin time (APTT) are not suited to represent the neonatal hemostatic balance, being mainly responsive to the procoagulant and much less to the anticoagulant drivers of coagulation.[19] Global tests such as thromboelastography (TEG) and thrombin generation assay (TGA) are emerging as candidates for the detection of both pro- and anti-coagulant determinants of clot formation.[20] In particular, TEG is an increasingly used bedside whole blood-based viscoelastic assay, carried out on small amounts of blood, which provides a dynamic evaluation of the entire clotting process, including fibrinolysis, within short turnaround times.[21] TGA is a more complex procedure, which evaluates thrombin generation (as a result of the plasma procoagulant activity) and decay (anticoagulant activity), thus providing an estimate of the endogenous thrombin potential (ETP), defined as the net amount of thrombin that can be generated by plasma under the driving forces of the pro- opposed by the anti-coagulants, under experimental conditions mimicking much more than any other conventional laboratory test the process operating in vivo.[19]

This study is aimed to assess the suitability of placental blood for the evaluation of hemostasis by comparing results of PT, APTT, fibrinogen, antithrombin, protein C, TEG and TGA in paired placental and infant venous blood, drawn from the same neonates.

Study design and methods

This observational study was performed at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milan, Italy. It was approved by the local ethics committee and written informed consent was obtained from the parents of all infants participating in the study.

All consecutive neonates born at our department between November 2017 and June 2018, who required blood sampling in the first day of life were screened at birth for study eligibility. Eligible neonates were born from either vaginal delivery or caesarean section and underwent routine perinatal care, which included delayed cord clamping for 60 seconds after birth, as suggested by current national recommendations[22] and prophylaxis with intramuscular vitamin K at birth.

Exclusion criteria included: (i) major chromosomopathy; (ii) congenital coagulopathy; (iii) exchange transfusion, administration of fresh frozen plasma (FFP) or platelets before blood collection; (iv) birth asphyxia. All eligible neonates were identified by a single operator (FM) throughout the study period. Laboratory tests included PT, APTT, fibrinogen, antithrombin, protein C, TEG and TGA.

Data collection

Each neonate underwent collection of paired placental blood at the time of delivery and peripheral blood venous samples within the first 24h of life. Placental blood was collected by direct sampling with a sterile needle, either from the umbilical vein or from a proximal vein on the fetal plate of the placenta. Venous blood was obtained either from a non-heparinized umbilical venous catheter or phlebotomy. In either case, blood was collected in test tubes containing 3.2% trisodium citrate as anticoagulant (9:1 whole blood to anticoagulant ratio) and stored at room temperature until testing, which was performed from 30 minutes to 2 hours from collection.[23,24] Samples were taken in conjunction with scheduled blood drawing. Samples were discarded if clotted or under-filled. Samples (whole blood or plasma) for TEG, TGA, PT, APTT, fibrinogen, antithrombin or protein C were used as appropriate.

We recorded demographic data (sex, gestational age, birth weight, mode of delivery, multiple birth, Apgar score, cord pH), antenatal data (maternal disease and pharmacological exposure) and clinical indications for neonatal blood sampling.

TEG assay

Citrated native TEG assay was performed using the two-channel TEG® 5000 Thromboelastograph Hemostasis Analyzer System (Haemonetics®, Niles, IL, USA), as previously described.[24] All TEG traces were carried out by a single operator, previously trained in specimen processing. After a gentle mixing of the blood sample, 340 µl of citrated blood were pipetted into a warmed (37°C) plastic TEG cup containing 20 µl of 0.2 M $CaCl_2$. We collected the following parameters:

- Reaction Time (R): time (in minutes) to reach the initial clot formation (2 mm).

- Clot Kinetics (K): time (minutes) to reach a significant clot strength (20 mm).
- α (angle): slope of the tangent line between R and K, which indicates the speed of fibrin formation.
- Maximal Amplitude (MA): widest trace amplitude (mm), which indicates the maximum clot strength.
- Lysis at 30 minutes (LY30): rate of clot lysis (%) 30 minutes after MA attainment.

Blood samples remaining after the TEG procedure were immediately centrifuged at 3000g for 20 minutes (controlled room temperature) and the supernatant plasma was harvested, aliquoted in plastic capped tubes, snap frozen in liquid nitrogen and stored at -70°C until testing.

Standard coagulation assays

PT was measured by means of human recombinant thromboplastin (Recombiplastin 2G, Werfen, Bedford, MA, USA). A reagent composed by colloidal silica as activator and synthetic phospholipids was used to measure APTT (SynthasIL, Werfen). Both tests were performed on an automated coagulometer (ACLTOP, Werfen) and results were reported as clotting times (seconds).

Pro- and anti-coagulant factor assays

The determination of fibrinogen was based on the Clauss method (Fibrinogen-C, Werfen) by using excess thrombin to convert fibrinogen into fibrin and results were expressed in mg/dL. Protein C was measured by an assay based on a synthetic chromogenic substrate, after incubation of plasma with snake venom as activator (HemosIL protein C, Werfen). Antithrombin was measured by chromogenic assay containing bovine FXa and excess heparin (HemosIL liquid antithrombin, Werfen). Results of both tests were reported as percentage activity in comparison to a pooled normal plasma with an assigned arbitrary activity of 100%. All of the above tests were performed on automated coagulometer (ACLTOP, Werfen).

Thrombin Generation Assay (TGA)

TGA was performed according to Hemker[25] with a homemade method[26] based on the activation of coagulation by addition of recombinant human tissue factor (1pM final concentration) (Recombiplastin, Werfen) and synthetic phospholipids (1uM) (Avanti Polar, Alabaster, AL, USA). Thrombin generation curves were continuously recorded by means of an automated fluorometer (Fluoroskan Ascent, ThermoLabSystem, Helsinki, Finland) using a fluorogenic substrate (Z-Gly-Gly-Arg-AMC HCL, Bachem, Bubendorf, Switzerland). A dedicated software (Thrombinoscope, Thrombinoscope BV, Maastricht, the Netherlands) was used to obtain the thrombogram, which is defined by the following parameters:

- Peak: maximum thrombin concentration (nM)
- Endogenous Thrombin Potential (ETP): the area under the curve (nM x min) representing the ability of plasma to generate thrombin under the experimental conditions. ETP depends on the opposing pro- and anti-coagulant drivers operating in the test plasma.

Another plasma aliquot was used to test simultaneously for thrombin generation after the addition of rabbit thrombomodulin (4nM) (Haematolologic Technologies, Essex Junction, VT, USA). In vivo, thrombomodulin turns protein C into activated protein C, which in combination with its cofactor protein S inhibits factors Va and VIIIa. The ETP ratio with/without thrombomodulin was calculated and provides an in vitro measure of the resistance to the thrombomodulin anticoagulant activity.[27]

Statistical analysis

A sample size of 60 neonates was required to detect a 20% difference in the R parameter (5 minutes vs 6 minutes) with two-tailed Wilcoxon test with an $\alpha = 0.05$ and 90% power. Demographic characteristics are described as mean and standard deviation (SD), median and range or as number and percentage, as appropriate. Results of hemostatic parameters were expressed as median and range. Wilcoxon matched pair signed-rank test was used for comparison between placental and infant venous blood samples.

Data were stratified according to gestational age (preterm vs term), sex, mode of delivery, weight-for-age percentile (small for gestational age-SGA vs non-SGA), admission blood source

(phlebotomy vs umbilical vein catheter), and timing of neonatal blood sampling (<12h vs ≥ 12h after birth). Results from a two-way ANOVA model are presented. Statistical significance was set as p values <0.05. Data were analysed using R software version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

We enrolled 60 neonates, half of whom were born preterm. Median gestational age (range) was 37 weeks (28^{+1}-41) and birth weight 2417 grams (950-4170). Demographic characteristics of the study population are shown in Table 1. We collected 120 venous blood samples (60 pairs of placental and venous blood), which were all deemed suitable for TEG analysis. Half of samples were not adequate to complete the whole hemostatic panel by means of TGA and standard coagulation. Patients' recruitment and samples' collection are outlined in the study flowchart (Figure 1).

Infant venous blood samples were obtained concurrently to routine blood tests from venipuncture (75%) or during the umbilical venous catheter placement (25%) at a median time of 11 hours of life. The main indications for neonatal blood sampling were low birth weight (53%), sepsis work-up (21%), pre-surgical screening (11%), respiratory distress (7%) and other conditions (10%).

With regard to the TEG profile, placental blood had lower median R and K times (p<0.001) with higher values of alpha angle (p<0.001) than infant venous blood (table 2, figure 2). MA and LY30 were not significantly different between the two groups of blood samples (table 2, figure 2). Compared to neonatal, placental blood had higher ETP levels without (p=0.002) or with thrombomodulin (p=0.03); higher peak-thrombin (p>0.05); higher ETP ratio (p=0.22); protein C activity (p<0.001) and lower levels of fibrinogen (p=0.030). PT, APTT and antithrombin were not significantly different between the two groups (table 2, figure 2). The overall difference between placental and infant venous blood did not vary after stratifying the results based on gestational age, sex, birthweight, mode of delivery, admission blood source, and timing of samples.

Discussion

The use of placental blood following placental delivery for admission laboratory testing has recently been endorsed to reduce iatrogenic blood loss and the subsequent need for blood transfusion, especially for preterm infants.[7,28,29] Previous studies have interchangeably relied on either placental or infant venous blood, as potential sources to assess neonatal coagulation at birth.[12-14] However, to the best of our knowledge, no studies have compared paired placental and venous blood from the same neonates to estimate their equivalence in terms of clotting parameters.

Our results showed that hemostasis, when assessed by means of global tests such as TEG and TGA, differs significantly according to whether placental or infant venous blood is used for testing. In contrast, no differences were recorded when testing was performed for the conventional coagulation tests PT and APTT. Specifically, if compared to infant venous blood, placental blood showed a thromboelastographic procoagulant profile characterized by an earlier onset (R parameter) and a faster rate (K and α parameters) of clot formation. These results were in line with those of the thrombin generation parameters, such as ETP with or without thrombomodulin, peak-thrombin and ETP ratio (with/without thrombomodulin), which were significantly higher in the placental than in the infant venous blood. Although we could not evaluate all the pro- and anti- coagulant factors, placental blood when compared to infant venous blood, showed lower levels of fibrinogen, higher levels of protein C and no differences for antithrombin. Three-quarters of the newborns enrolled were born by cesarean section and the delivery mode did not affect the hemostatic profile estimated by the two blood specimens. Similarly, prematurity did not show any impact on the hemostatic properties of placental vs infant venous blood.

Previous studies reported conflicting results in terms of between-pair (placental vs infant venous blood) differences for platelet count and/or function. In a flow cytometry-based study, the two blood specimens were equivalent in terms of platelet surface markers.[30] Similarly, platelet count was comparable between placental and infant venous blood samples collected at birth.[7] In contrast, there is evidence supporting the concept that the type of specimen has an impact on primary hemostasis. Closure times obtained by the platelet function analyzer PFA-100 were shorter in placental compared

to infant venous blood.[31] The above results are consistent with those observed for TEG in the present study.

Overall, our findings suggest that a local activation of the hemostasis leading to a procoagulant imbalance may occur in the feto-placental vessels. Typically, a procoagulant imbalance may be driven by reduced activity of anticoagulants, increased activity of procoagulants or both. PT and APTT, which are responsive to the pro- and much less to the anticoagulant factors, were similar in placental vs infant venous blood. This would point to a reduced activity of the anticoagulant pathway within the placental blood, as one of the possible mechanistic explanations for the procoagulant imbalance observed in placental blood. However, the occurrence of higher levels of protein C in placental blood, but similar antithrombin found in the two specimens, do not support this concept. Another possible explanation could rest on tissue factor, the primary initiator of blood coagulation. Indeed, Tay et al. showed significantly higher concentration of tissue factor in placental than in the maternal blood.[32] Trophoblasts, as well as amniotic fluid and myometrium, constitutively express high levels of tissue factor, thus creating a protective environment against haemorrhage, known as the "obstetrical hemostatic envelope".[33] A few stimuli can contribute to increase tissue factor expression, including reactive oxygen species, which are widely produced during labour and delivery.[34] Tissue factor can also be released in cord blood from damaged tissues, as a result of the handling and cutting of the umbilical cord.[32] However, according to this hypothesis, we would have expected a shorter PT in the placental blood, since tissue factor is a potent activator of the extrinsic pathway.[35] Finally, as postulated in the setting of placental vascular complications, extracellular membrane vesicles, known also as microvesicles, could provide a possible explanation for the procoagulant imbalance in placental blood.[36] Under physiological conditions, microvesicles are secreted by the endothelial cells and throphoblasts and subsequently released in the blood stream and other fluids. Microvesicles are classified based on their size and functional properties. Microparticles in particular are microvesicles smaller than 1 μm, which are disseminated into the blood circulation by the parent activated cells (e.g., platelets, monocytes and endothelial cells) following inflammation and hypoxia.[37,38] Their procoagulant activity can be attributed to anionic phospholipids or tissue factor carried on their surface. Indeed, higher numbers of microparticles were

observed in placental blood compared to maternal blood.[37] Tissue factor bearing microparticles have been commonly detected in placental blood.[39] Moreover, the numbers of tissue factor bearing microparticles were higher in placental blood of preterm than in term infants.[39]

Limitations of our study should be recognized. It would have been more informative if the complete panel of pro- and anti-coagulant drivers were assessed to identify the mediator(s) of the procoagulant imbalance in placental as compared to infant venous blood. However, this is a well-known issue when exploring neonatal hemostasis, requiring large and 'ethically not feasible' blood samples. Another limitation of our study is related to the inherent pre- and analytical variability of the TEG assay.[24] However, to reduce this variability we designed a single-centre study, with a single trained operator performing all TEG measurements. Moreover, it should also be realized that the procoagulant imbalance detected by TEG for the placental blood was substantiated by TGA, which is a procedure less prone to methodological variability.

In conclusion, this is the first study assessing hemostasis in placental and infant venous paired blood samples by standard coagulation tests in addition to TEG and TGA. While PT and APTT are not affected by the source of blood specimens, both TEG and TGA identify a procoagulant imbalance in the placental blood as compared to the infant venous counterpart. Placental blood cannot be considered as a suitable specimen for a reliable assessment of neonatal hemostasis at birth. Further studies are needed to unveil the feto-placental coagulation physiology, to identify the main drivers of the placental blood procoagulant imbalance and to evaluate the clinical implications.

Acknowledgments

We would like to thank NICU staff of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, especially Valeria Cortesi, Elena Di Francesco, Silvia Gulden and all the midwifes and nurses for their kind collaboration and support with samples collection; the Laboratory staff of Angelo Bianchi Bonomi Hemophilia and Thrombosis Center for their help with data analysis. We would especially like to thank the parents and babies who were involved in the study.

Preliminary results of this study were presented at the European Academy of Pediatric Societes EAPS 2018 and at the International Society on Thrombosis and Hemostasis ISTH 2019.

Authors' contribution

GR participated to the conceptualization and design of the work; she managed the enrolment of patients and acquisition of data; she gave substantial contributions to the analysis and interpretation of data. She co-wrote the first draft of the paper, gave final approval of the version published and ensured that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. AT participated to the conceptualization and design of the work; he gave essential contribution to the interpretation of data; he revised the work with important intellectual content and gave final approval of the version published. FM enrolled the patients and performed thromboelastographic tests, she gave substantial contributions to the analysis and interpretation of data, she co-wrote the first draft of the paper and gave final approval of the version published. ES participated to the acquisition, analysis and interpretation of data, she revised the work and gave final approval of the version published. NP managed the data analysis, he revised the work and gave final approval of the version published. IA participated to the acquisition, analysis and interpretation of data, she revised the work and gave final approval of the version published. GC participated to the conceptualization and design of the work; he gave substantial contribution to the interpretation of data; he critically

revised the work and gave final approval of the version published. EV participated to the conceptualization and design of the work; he gave substantial contribution to the interpretation of data; he critically revised the work and gave final approval of the version published. FP participated to the conceptualization and design of the work; she gave substantial contribution to the interpretation of data; she critically revised the work and gave final approval of the version published. FM participated to the conceptualization and design of the work; he gave substantial contribution to the interpretation of data; he critically revised the work and gave final approval of the version published. SG participated to the conceptualization and design of the work; he supervised the enrolment, obtained the ethical local permissions and gave essential contribution to the interpretation of data. He revised the draft giving important intellectual contribution and gave final approval of the version published and ensured that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Abbreviations

APTT = activated partial thromboplastin time; α = alpha angle; CI = Coagulation Index; ETP = endogenous thrombin potential; K = kinetics; LY30 = lysis at 30 minutes; MA = maximum amplitude; PT = prothrombin time; R = reaction time; SGA = small for gestational age; TEG = Thromboelastography; TGA = Thrombin Generation Assay.

Conflicts of interest

The authors have no conflict of interest to disclose relative to this manuscript

Funding

None

Legend of tables and figures

Table 1. Demographic characteristics of the study population

Table 2. Hemostatic profile in paired placental and infant venous blood samples. Thromboelastographic profile (R, K, α, MA, Ly30), conventional tests (PT, APTT), antithrombin, protein C, fibrinogen, endogenous thrombin potential with/without thrombomodulin (ETP+TM, ETP) are described for placental and infant venous blood samples at birth. Values are expressed as median (range).

Figure 1. Study flowchart

Figure 2. Hemostatic profile in paired placental and infant venous blood samples. a) prothrombin time - PT, b) activated partial thromboplastin time - APTT, c) endogenous thrombin potential - ETP, d) endogenous thrombin potential + thrombomodulin - ETP+TM, e) reaction time - R, f) maximum amplitude - MA.

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Table 1. Demographic characteristics of the study population		
Variables	Preterm infants n=30	Term infants n=30
Gestational age (weeks$^{+\ days}$) median (range)	32 (28^{+1} – 36^{+6})	37^{+6} (37^{+0}– 41^{+0})
Birth weight (g), median (range)	2035 (950 – 3170)	2725 (1990 – 4170)
Male gender, n (%)	15 (50%)	16 (53%)
Caesarean delivery, n (%)	25 (83%)	21 (70%)
Multiple birth, n (%)	16 (53%)	7 (23%)
Small for Gestational Age (<10°pct), n (%)	5 (17%)	13 (43%)
Maternal-placental comorbidities, n (%): - Gestational diabetes - Pre-eclampsia - Maternal thrombophilia - Antenatal corticosteroids	 3 (10%) 1 (3%) 0 (0%) 19 (63%)	 1 (3%) 0 (0%) 1 (3%) 1 (3%)
Pct: percentile.		

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Table 2. Hemostatic profile in paired placental and infant venous blood samples.				
Test	**Number of pairs**	**Placental Blood** Median (range)	**Infant venous Blood** Median (range)	**p-value Wilcoxon**
PT (seconds)	27	13.5 (10.7-19)	13.6 (11.1-18.9)	0.665
APTT (seconds)	25	53.0 (30.7-68.1)	48.5 (38.6-74.3)	0.904
Antithrombin (%)	30	51 (25-75)	48 (24-76)	0.164
Protein C (%)	28	26 (13-60)	21 (11-61)	<0.001
Fibrinogen (mg/dL)	29	157 (46-333)	188 (89-349)	0.030
Thromboelastography				
R (min)	60	4.0 (1.5-8.0)	6.1 (2.4-15.5)	< 0.001
K (min)	60	1.3 (0.8-14.8)	2.2 (0.8-11.7)	< 0.001
α (°)	60	69.7 (27.2-79.1)	57.4 (20.5-77.5)	< 0.001
MA (mm)	60	59.9 (24.7-78.1)	58.4 (28.2-80.2)	0.052
LY30 (%)	60	1.2 (0.0 -13.8)	0.3 (0.0 -10.4)	0.108
Thrombin generation assay				
ETP (nM·min)	30	1260.5 (920.0-1689.5)	1078.0 (697.5-1612.0)	0.002
ETP+TM (nM·min)	28	1074.5 (256.0-1571.5)	845.0 (373.0-1503.0)	0.031
ETP ratio	28	0.9 (0.3-1.0)	0.8 (0.5-1.0)	0.227
PEAK (nM)	30	181.2 (106.2-248.3)	159.0 (102.0-242.8)	0.052

PT, prothrombin time. APTT, activated partial thromboplastin time. ETP: endogenous thrombin potential K: kinetics time; LY30: lysis at 30 minutes; MA: maximum amplitude; R: reaction time; TM: thrombomodulin; α: alpha angle.

References

1. Lin JC, Strauss RG, Kulhavy JC, et al. Phlebotomy overdraw in the neonatal intensive care nursery. Pediatrics 2000;106(2):e19.

2. Strauss RG, Widness JA. Is there a role for autologous/placental red blood cell transfusions in the anemia of prematurity? Transfus Med Rev 2010;24(2):125-129.

3. Howarth C, Banerjee J, Aladangady N. Red blood cell transfusion in preterm infants: current evidence and controversies. Neonatology 2018;114(1):7-16.

4. Ghirardello S, Dusi E, Cortinovis I, et al. Effects of Red Blood Cell Transfusions on the Risk of Developing Complications or Death: An Observational Study of a Cohort of Very Low Birth Weight Infants. Am J Perinatol 2017;34(01):88-95.

5. Christensen RD, Lambert DK, Baer VL, et al. Postponing or eliminating red blood cell transfusions of very low birth weight neonates by obtaining all baseline laboratory blood tests from otherwise discarded fetal blood in the placenta. Transfusion. 2011;51(2):253-258.

6. Baer V, Lambert D, Carroll P, Gerday E, Christensen R. Using umbilical cord blood for the initial blood tests of VLBW neonates results in higher hemoglobin and fewer RBC transfusions. J Perinatol 2013;33(5):363-5.

7. Carroll P, Nankervis C, Iams J, Kelleher K. Umbilical cord blood as a replacement source for admission complete blood count in premature infants. J Perinatol 2012;32(2):97-102.

8. Grunau RE, Holsti L, Peters JW. Long-term consequences of pain in human neonates. Semin Fetal Neonatal Med 2006 Aug;11(4):268-75.

9. Walker SM. Biological and neurodevelopmental implications of neonatal pain. Clin Perinatol 2013;40(3):471-491.

10. Hansen A, Forbes P, Buck R. Potential substitution of cord blood for infant blood in the neonatal sepsis evaluation. Biol Neonate 2005;88(1):12-8.

11. Beeram MR, Loughran C, Cipriani C, Govande V. Utilization of umbilical cord blood for the evaluation of group B streptococcal sepsis screening. Clin Pediatr 2012;51(5):447-453.

12. Neary E, McCallion N, Kevane B, et al. Coagulation indices in very preterm infants from cord blood and postnatal samples. J Thromb Haemost 2015 Nov;13(11):2021-30.

13. Christensen RD, Baer VL, Lambert DK, Henry E, Ilstrup SJ, Bennett ST. Reference intervals for common coagulation tests of preterm infants (CME). Transfusion. 2014;54(3):627-632.

14. Edwards RM, Naik-Mathuria BJ, Gay AN, Olutoye OO, Teruya J. Parameters of thromboelastography in healthy newborns. Am J Clin Pathol. 2008 Jul;130(1):99-102.

15. Sidlik R, Strauss T, Morag I, et al. Assessment of functional fibrinolysis in cord blood using modified thromboelastography. Pediatr Blood Cancer. 2016 May;63(5):839-43.

16. Schott NJ, Emery SP, Garbee C, Waters J. Thromboelastography in term neonates. J Matern Fetal Neonatal Med. 2018 Oct;31(19):2599-2604.

17. Israels SJ, Rand ML, Michelson AD. Neonatal platelet function. Semin Thromb Hemost. 2003 Aug;29(4):363-72.

18. Haley KM, Recht M, McCarty OJ. Neonatal platelets: mediators of primary hemostasis in the developing hemostatic system. Pediatr Res. 2014;76(3):230-237.

19. Tripodi A, Ramenghi LA, Chantarangkul V, et al. Normal thrombin generation in neonates in spite of prolonged conventional coagulation tests. Haematologica. 2008;93(8):1256-1259.

20. Sewell EK, Forman KR, Wong EC, Gallagher M, Luban NL, Massaro AN. Thromboelastography in term neonates: an alternative approach to evaluating coagulopathy. Arch Dis Child Fetal Neonatal Ed. 2017 Jan;102(1):F79-F84.

21. Whiting D, DiNardo JA. TEG and ROTEM: technology and clinical applications. Am J Hematol. 2014 Feb;89(2):228-32.

22. Ghirardello S, Locatelli A, Perrone B, Pratesi S, Saracco P. Italian recommendations for placental transfusion strategies. Front Pediatr 2018;6:372.

23. Zambruni A, Thalheimer U, Leandro G, Perry D, Burroughs AK. Thromboelastography with citrated blood: comparability with native blood, stability of citrate storage and effect of repeated sampling. Blood Coagul Fibrinolysis 2004 Jan;15(1):103-7.

24. Ghirardello S, Raffaeli G, Scalambrino E, et al. The intra-assay reproducibility of thromboelastography in very low birth weight infants. Early Hum Dev 2018;127:48-52.

25. Hemker H, Giesen P, AlDieri R, et al. The calibrated automated thrombogram (CAT): a universal routine test for hyper-and hypocoagulability. Pathophysiol Haemost Thromb 2002 Sep-Dec;32(5-6):249-53.

26. Chantarangkul V, Clerici M, Bressi C, Giesen P, Tripodi A. Thrombin generation assessed as endogenous thrombin potential in patients with hyper-or hypo-coagulability. Haematologica 2003;88(5):547-554.

27. Tripodi A. Detection of procoagulant imbalance. Modified endogenous thrombin potential with results expressed as ratio of values with-to-without thrombomodulin. Thromb Haemost 2017 May 3;117(5):830-836.

28. Lemyre B, Sample M, Lacaze-Masmonteil T. Minimizing blood loss and the need for transfusions in very premature infants. Paediatr Child Health 2015;20(8):451-62.

29. Carroll PD. Umbilical cord blood-an untapped resource: strategies to decrease early red blood cell transfusions and improve neonatal outcomes. Clin Perinatol 2015;42(3):541-556.

30. Sitaru A, Holzhauer S, Speer C, et al. Neonatal platelets from cord blood and peripheral blood. Platelets 2005;16(3-4):203-210.

31. Saxonhouse MA, Garner R, Mammel L, et al. Closure times measured by the platelet function analyzer PFA-100® are longer in neonatal blood compared to cord blood samples. Neonatology 2010;97(3):242-249.

32. Tay S-P, Cheong S-K, Boo N-Y. Circulating tissue factor, tissue factor pathway inhibitor and D-dimer in umbilical cord blood of normal term neonates and adult plasma. Blood Coagul Fibrinolysis 2003;14(2):125-129.

33. Kuczyński J, Uszyński W, Żekanowska E, Soszka T, Uszyński M. Tissue factor (TF) and tissue factor pathway inhibitor (TFPI) in the placenta and myometrium. Eur J Obstet Gynecol Reprod Biol 2002;105(1):15-9.

34. Fogel I, Pinchuk I, Kupferminc MJ, Lichtenberg D, Fainaru O. Oxidative stress in the fetal circulation does not depend on mode of delivery. Am J Obstet Gynecol 2005;193(1):241-246.

35. Dahlbäck B. Blood coagulation. The Lancet 2000;355(9215):1627-1632.

36. Aharon A. The role of extracellular vesicles in placental vascular complications. Thromb Res 2015;135:S23-S25.

37. Uszyński M, Żekanowska E, Uszyński W, Kuczyński J, Żyliński A. Microparticles (MPs), tissue factor (TF) and tissue factor inhibitor (TFPI) in cord blood plasma. A preliminary study and literature survey of procoagulant properties of MPs. Eur J Obstet Gynecol Reprod Biol 2011;158(1):37-41.

38. Owens III AP, Mackman N. Microparticles in hemostasis and thrombosis. Circ Res 2011;108(10):1284-1297.

39. Korbal P, Słomka A, Sadowska-Krawczenko I, Żekanowska E. Evaluation of tissue factor bearing microparticles in the cord blood of preterm and term newborns. Thromb Res 2017;153:95-96.