Umbilical cord blood as an alternative to neonatal blood for complete

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Umbilical Cord Blood as an Alternative to Neonatal Blood for Complete Blood Count: A Comparison Study

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Objective To assess concordance between umbilical cord blood (UCB) and neonatal blood (NB) laboratory test results at birth.

Study design This retrospective study considered very preterm neonates (<32 weeks' gestational age) admitted to a tertiary neonatal intensive care unit from 2012 to 2023. Inclusion criteria required neonates with a complete blood count measured in both UCB and NB drawn within 2 hours after birth. Median hemoglobin (Hb) and hematocrit (Hct) concentrations were compared between UCB (venous samples) and NB (venous, arterial, or capillary samples).

Results A total of 432 neonates with paired UCB and NB values were included in the study. Hb concentration in UCB was 14.7 g/dL (IQR 13.5–16.1 g/dL) compared with 14.8 g/dL (IQR 12.6-19.3 g/dL) in venous NB samples, 13.9 g/dL (IQR 12.9-15.3 g/dL) in arterial NB and 18.7 g/dL (IQR 16.6-20.8 g/dL) in capillary NB. The regression equation showed a correction factor of 1.08 for converting Hb values from UCB to venous NB. Median Hct concentration in UCB was 0.45 L/L (IQR: 0.41-0.49 L/L) compared with 0.48 L/L (IQR 0.43-0.54 L/L) in venous NB, 0.42 L/L (IQR 0.38-0.45 L/L) in arterial NB and 0.57 L/L, (IQR 0.51-0.63 L/L) in capillary NB.

Conclusions Hb and Hct concentrations measured in UCB are similar to those measured in venous blood in very preterm infants and are valid alternatives for NB tests at birth. Hb and Hct concentrations in arterial and capillary NB are respectively lower and higher compared with UCB measurements. *(J Pediatr 2024;271:114059)*.

In the first days of life, very preterm infants undergo multiple phlebotomies to monitor their condition and optimize therapy[1,2] during their stay in the neonatal intensive care unit (NICU). Previous studies have shown that preterm infants can lose up to one third of their blood volume in the first month of life as a result of cumulative blood draws,[3] rendering iatrogenic blood loss, the leading cause of anemia in this vulnerable population. Laboratory testing must therefore be minimized to avoid blood loss and to reduce the need for subsequent red blood cell (RBC) transfusions.[4] Several strategies are currently used to reduce the risk of anemia including delayed cord clamping, using micro blood analyzers and transcutaneous measurements.[5,6] Umbilical cord blood (UCB) as an alternative blood source for diagnostic tests could also help prevent anemia.[7,8] Blood draws on the first day of life can total up to 10% of the circulating blood volume in the smallest neonates and partly be prevented by using UCB.[4,9] However, the hemoglobin (Hb) concentration in UCB might not be the same as in blood collected directly from the infant (hereafter referred to as neonatal blood (NB)). A few small studies comparing complete blood count (CBC) results from UCB to NB found lower hemoglobin and hematocrit (Hct) concentrations, lower erythrocyte and leukocyte counts, and higher platelet counts in UCB samples.[10-13] However, these studies results did not consider the type of vessel (arterial, venous, or capillary) from which the blood was drawn, leaving us with uncertainty about whether UCB is a good reflection of NB.

We aim to evaluate the concordance of complete blood count results between UCB and NB taking into account whether the blood sample was of arterial, venous, or capillary origin. In addition, we aim to derive a correction factor to enable adjustment of UCB complete blood count results to a predicted NB estimate.

CBC	complete blood count	NB	neonatal blood
GA	gestational age	NICU	neonatal intensive care unit
Hb	hemoglobin	RBC	red blood cell
Hct	hematocrit	SD	standard deviation
IQR	interquartile range	SGA	small for gestational age
IV	intravenous	UCB	umbilical cord blood

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Methods

Setting

We conducted a retrospective observational cohort study at the NICU of the Leiden University Medical Center, Leiden, the Netherlands. The Medical Ethics Committee Leiden Den Haag Delft approved this study and waived the need to ask for parental consent (no. 23-3011).

Study Sample

We included all neonates with a gestational age below 32 weeks admitted to our NICU between 01-01-2012 and 13-05-2023 in which UCB samples and NB samples were drawn within 2 hours of UCB collection. We chose 2 hours as a cut-off point to be able to compare UCB with NB directly after birth, before hemoconcentration occurs. This makes our study sample selected, as neonates with both samples were not randomly chosen or assigned. As this selection does not affect the association between UCB and NB, selection bias is unlikely. We excluded all neonates with a major bleeding at birth and those who received an RBC or platelet transfusion within the first 2 hours after birth, as this could affect their laboratory results. Major bleeding was defined as an intraventricular hemorrhage ≥ grade 3, intracranial bleeding with midline shift/compression, and pulmonary hemorrhage or gastrointestinal bleeding. Neonates with a spurious result, defined as a difference between UCB and NB value larger than 2 standard deviations, were excluded from the analysis. A posthoc power calculation was not indicated.[14,15]

Data Collection and Definitions

All data were collected from the electronic medical records. CBC results of blood samples taken at birth and during admission were collected. CBC results included Hb and Hct concentrations, platelet, leukocyte, reticulocyte, and erythrocyte counts. CBC measurements at birth are part of routine care of preterm neonates at our NICU. Cord blood is collected by needle puncture from the umbilical vein immediately after birth whereas neonatal blood is collected either through phlebotomy when collecting from peripheral veins, from an arterial line, or by performing a heel prick for capillary blood measurements. The Sysmex XN 9000 analyzer was used to evaluate both UCB and NB CBC samples.

The following data were collected: mode of delivery, birth weight, gestational age at birth, cord clamping time, small for gestational age (SGA) status (defined as an estimated birth weight below the 10th percentile[16]), and need of respiratory support on the first day of life. Date and time of RBC and platelet transfusions during the first 36 hours after birth were collected. If a major bleeding had occurred within this period, date and time of the event was recorded.

Statistical Analysis

Baseline characteristics were described using means and standard deviations or medians and IQRs, or numbers and percentages for categorical data. Normality was checked using histograms. Statistical analyses were performed using R Statistical Software version 4.2.1[17] and STATA Statistical Software version 16.1.[18]

Median and IQR were determined for each CBC result derived from the 4 different vascular origins, being venous UCB, and arterial, venous, or capillary NB. Values were displayed in a boxplot to evaluate the distribution. A linear regression model was used to model the associations of UCB results with NB results from the different vascular origins within 2 hours after birth. The resulting regression equation (consisting of an intercept and regression coefficient) can be used as a correction factor to predict an NB value from an UCB value. Additionally, a more practical correction factor was determined by performing a linear regression without an intercept. To evaluate the clinical significance of the variation observed between the UCB and NB samples, the defined thresholds for RBC transfusion (as used in the ETTNO[19] (Hct) and TOP[20] (Hb) trials) were incorporated into the scatter plots. A value below the threshold indicates "need for transfusion".

The concordance of Hb and Hct between UCB and NB was expressed as a scatterplot of all UCB and NB values, and the accompanying regression lines, with adjusted R^2 values. Adjusted R^2 is a corrected goodness-of-fit (model accuracy) measure for linear models. It identifies the percentage of variance in the target field that is explained by the input or inputs.[21]

Concordance of leukocyte, platelet, reticulocyte, and erythrocyte counts between UCB and NB were similarly evaluated. Reticulocyte counts were displayed in permille (‰). Additionally, to evaluate whether the correction factors can be used to predict neonatal CBC results throughout the entire first postnatal day, and thus potentially saving more blood draws, we performed Bland–Altman analyses for all values observed during the first 24 hours after birth as a secondary analysis. We defined 24 hours as the first 12-36 hours of life and used the sample closest to 24 hours in the analysis. Predicted values were determined by adjusting UCB values using the corresponding correction factor (with intercept). Limits of agreement were defined by the mean, and one SD of the mean for each blood parameter. We expected the correction factors to be unable to accurately predict laboratory NB results in this time period, as the clinical condition changes rapidly during the first 24 hours in these infants.

Results

Study Sample

During the study period, 1958 very preterm neonates were admitted to our NICU and eligible for inclusion. A total of 1318 neonates without UCB were excluded from the study. Of the remaining 449 neonates, 432 neonates had paired UCB and NB values without spurious samples or events and were thus included in the analysis. The flowchart displaying in-and exclusion is portrayed in **Figure 1**. Characteristics for all included neonates are provided in **Table**.

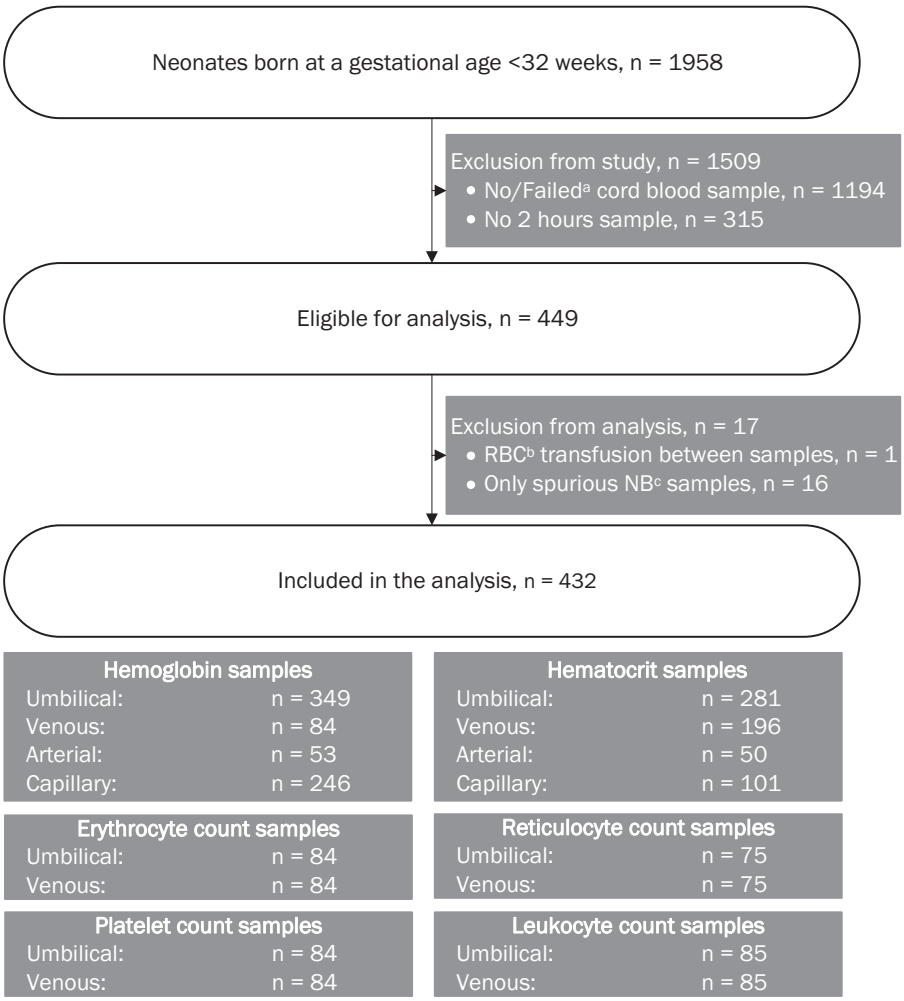

Figure 1. Flowchart showing in- and exclusion of neonates and laboratory samples in the analysis. a: No records available on exact number of failed attempts at collecting UCB. b: RBC transfusion = Red blood cell transfusion. c: NB = Neonatal blood, drawn within 2 hours after umbilical cord blood.

Table. Baseline characteristics of 432 included neonates upon NICU admission

Characteristics	Total neonates (*n* = 432)
Male sex, *n* (%)	228 (52.8%)
Birth weight (grams)*	1264 (375)
Gestational age at birth (weeks and days)†	29 + 4 (27 + 6, 30 + 6)
Small for gestational age, *n* (%)	123 (28.5%)
Caesarian section, *n* (%)	204 (47.2%)
Multifetal, *n* (%)	167 (38.7%)
Delayed cord clamping‡	
Yes, *n* (%)	187 (43.3%)
No, *n* (%)	40 (9.3%)
Unknown, *n* (%)	205 (47.5%)
Cord clamping time (seconds)†	60 (30, 120)
Respiratory support on day 1, *n* (%)	425 (98.4%)

*Expressed as mean (SD).
†Expressed as median (IQR).
‡Defined as waiting to clamp the umbilical cord for at least 30 seconds.

Concurrence of UCB and NB for Hb and Hct

Median Hb concentration in UCB was 14.7 g/dL (IQR: 13.5–16.1 g/dL) and 14.8 g/dL (12.6-19.3 g/dL) in venous NB samples. In comparison, median arterial Hb concentration in NB was lower than UCB (13.9 g/dL, 12.9-15.3 g/dL) and median Hb concentration in capillary blood was higher (18.7 g/dL, 16.6-20.8 g/dL). Stratifying for delayed cord clamping showed a median Hb concentration in UCB of 14.3 (IQR: 13.7-16.3 g/dL), a venous Hb of 17.1 g/dL (IQR: 11.0-23.1 g/dL), an arterial Hb of 13.9 g/dL (IQR: 12.7-14.3 g/dL), and a median capillary Hb of 18.7 g/dL (IQR: 16.1-20.1 g/dL) if there was no delayed clamping applied. In neonates with delayed cord clamping, median Hb in UCB was 15.0 g/dL (IQR: 13.7-16.0 g/dL), median venous Hb was 15.5 g/dL (IQR: 14.3-19.5 g/dL), median arterial was Hb 14.5 g/dL (IQR: 13.9-15.3 g/dL), and median

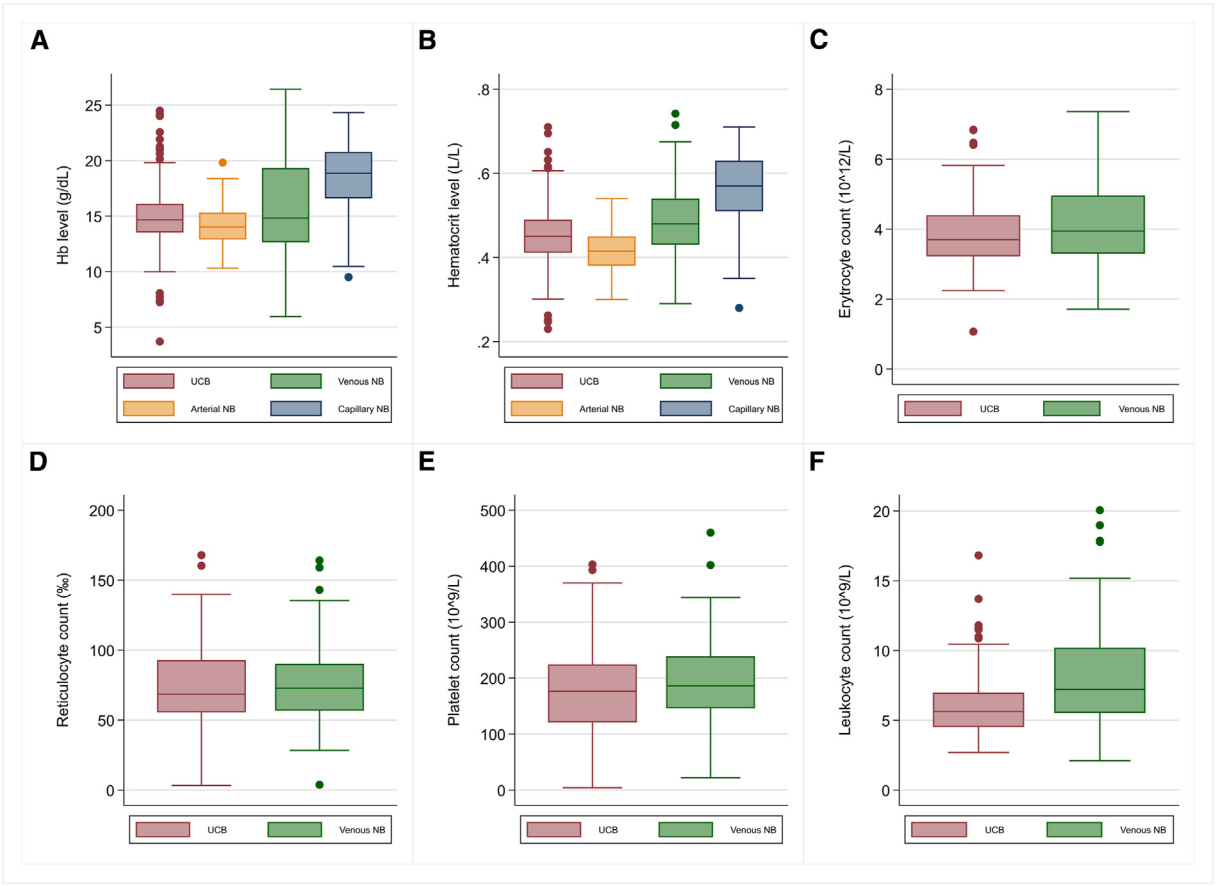

Figure 2. The distribution of the umbilical cord blood (UCB) and neonatal blood (NB) samples of 432 neonates with their concurrent samples. UCB= Umbilical Cord Blood, NB= Neonatal Blood **A:** Median hemoglobin concentration, UCB: 14.7 g/dL (349 samples), venous blood: 14.8 g/dL (84 samples), arterial blood: 14.0 g/dL (53 samples), and in capillary blood: 18.9 g/dL (246 samples). **B:** Median hematocrit concentration, UCB: 0.45 L/L (281 samples), venous blood: 0.48 L/L (196 samples), arterial blood: 0.41 L/L (50 samples), and in capillary blood: 0.57 L/L (101 samples). **C:** Median erythrocyte count, UCB: 3.7 x 10^12/L (84 samples) and venous blood: 3.9 x 10^12/L (84 samples). **D:** Median reticulocyte count, UCB: 69‰ (75 samples), and venous blood: 73‰ (75 samples). **E:** Median platelet count, UCB: 177 x 10^9/L (84 samples) and venous blood: 186 x 10^9/L (84 samples). **F:** Median leukocyte count, UCB: 5.6 x 10^9/L (85 samples) and venous blood: 7.2 x 10^9/L (85 samples).

capillary Hb was 19.0 g/dL (IQR: 16.8-20.3 g/dL). Median Hct concentration in UCB was 0.45 L/L (IQR: 0.41-0.49 L/L) compared with 0.48 L/L (0.43-0.54 L/L) in venous NB, 0.42 L/L (0.38-0.45 L/L) in arterial NB and 0.57 L/L (0.51-0.63 L/L) in capillary NB. **Figure 2** shows the distribution of UCB and NB values displayed in boxplots.

Figure 3 shows scatterplots of Hb and Hct concentrations in UCB and corresponding NB, including linear regression models, and general thresholds for RBC transfusions. The linear regression line of venous NB vs UCB shows good fit with most observed values close to the regression line. The box indicates the area above the highest RBC transfusion threshold of 11 g/dL. Among the 84 infants with Hb both in UCB and venous NB, 83.3% (70/84) had both UCB and NB values above the transfusion threshold used in the TOP trial. In 8.3% (7/84) of these infants UCB was higher than 11 g/dL, and NB was below the threshold. In 4.7% (4/84) both UCB and NB were below the threshold, and 3.6% (3/84) of these infants had a UCB below, but NB above the threshold.

Equations predicting NB from UCB test results are shown in the linear regressions in **Figure 3** (and in **Supplementary Table 1**, online; available at www.jpeds.com). Two correction factors are presented. First, the full regression equation ($Y = \beta1^*x+\beta0$), where the predicted neonatal blood can be derived by the product of the cord blood x and regression coefficient $\beta1$ and adding the intercept $\beta0$. Secondly, the simpler regression model ($Y = \beta1^*x$); where the predicted neonatal value is the product of the regression coefficient $\beta1$, and the cord blood value x. The simpler Hb correction factor shows an eight percent increase of the venous NB Hb value compared with the UCB Hb value.

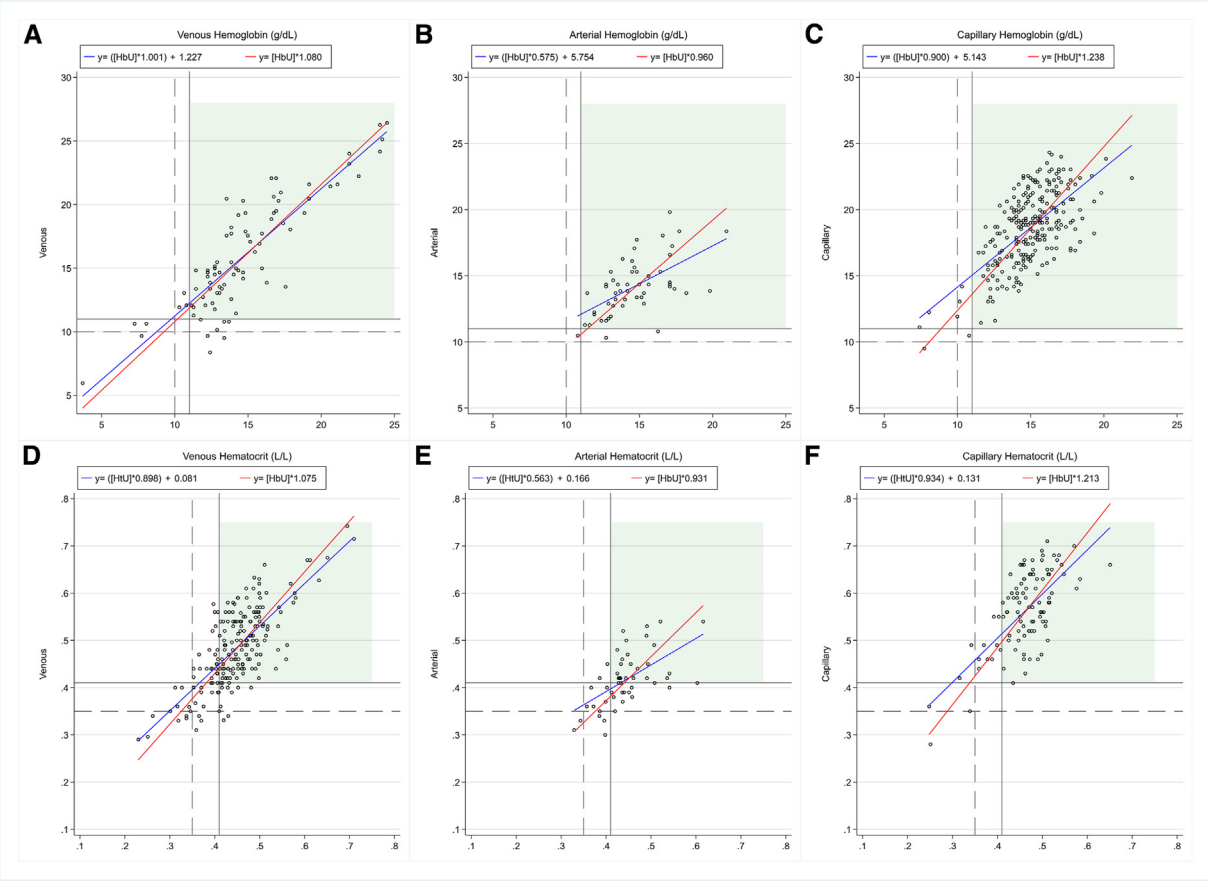

Figure 3. Linear regression umbilical cord blood (UCB, Y) and neonatal blood per vascular origin (NB, X) within 2 hours after birth. Correction factor determined from Y = $\beta 1$*x+$\beta 0$(blue/dotted line) and Y = $\beta 1$*x(red/dashed line). Reference lines for RBC transfusion are added to hemoglobin (TOP trial) and hematocrit (ETTNO trial) plots (solid line for respiratory support/clinically unstable condition, and dashed line for no respiratory support/clinically stable condition). UCB = Umbilical Cord Blood, NB = Neonatal Blood. **A:** Adjusted R^2 Venous Hemoglobin, *(Y = $\beta 1$*x+$\beta 0$) = 75% (84 samples).* **B:** Adjusted R2 Arterial Hemoglobin, (Y = $\beta 1$*x+$\beta 0$) = 34% (53 samples). **C:** Adjusted R2 Capillary Hemoglobin, (Y = $\beta 1$*x+$\beta 0$) = 40% (246 samples). **D:** Adjusted R2 Venous Hematocrit, (Y = $\beta 1$*x+$\beta 0$) = 55% (196 samples). **E:** Adjusted R2 Arterial Hematocrit, (Y = $\beta 1$*x+$\beta 0$) = 33% (50 samples). **F:** Adjusted R2 Capillary Hematocrit, (Y = $\beta 1$*x+$\beta 0$) = 45% (101 samples).

Concurrence of Platelet, Erythrocyte, Reticulocyte, and Leukocyte Counts

Scatter plots and linear regression lines for platelets, erythrocytes, reticulocytes, and leukocytes are displayed in **Supplementary Figure 1**, online; available at www.jpeds.com. Corresponding adjusted R^2 values are 58%, 76%, 97% and 58%, respectively.

Robustness of Regression Equations in the First Day of Life

To assess the accuracy of the correction factors in the first 24 hours, we performed Bland Altman analyses. Results are shown in **Supplementary Figure 2**, online; available at www.jpeds.com. Approximately one-third of the data are outside these limits of agreement for the CBC results. This indicates that in one-third of the neonates, the predicted NB value did not match the measured blood value.

Discussion

This study showed that CBC laboratory tests in very preterm neonates show good concordance between UCB and venous NB, suggesting that UCB measurements could be valid alternatives for hematological tests at birth. On average, Hb values in venous NB collected within 2 hours after birth were 8 percent higher than those observed in UCB.

Four studies have previously evaluated the differences in CBC measurements between UCB and NB at birth.[10-13] One study reported a strict window of 1 hour between UCB and NB sampling,[11] the others had a mean time window of less than 2 hours. In 3 of these studies, the study sample was older (mean GA at birth >32 weeks) than in our study.[10-12] Corroborating our findings, the 4 previous studies found lower UCB Hb concentrations compared with peripheral NB Hb at birth. Two of these studies performed a regression analysis, showing

correlation between UCB and NB for Hb values.[12,13] Two of these studies included Hct in their analyses. Both found a higher Hct in NB compared with UCB, similar to our study.[10,13] The higher observed Hb and Hct in NB compared with UCB has been attributed to a postpartum fluid shift concentrating blood immediately after birth.[22]

Most importantly, in contrast to our study, none of the previous studies differentiated peripheral Hb into venous, arterial, or capillary origin.[10-13] We found lower arterial NB values compared with UCB, and higher venous and capillary concentrations compared with UCB. A study in adults comparing arterial blood to venous and capillary blood suggested that plasma in arterial blood exudes from the capillaries, forming tissue fluid, which may lead to an increase of Hb and Hct concentrations in capillary blood.[23]

Our study had several strengths and limitations. We included a very large number of neonates comparing UCB to NB, enhancing the generalizability of the results. We minimized bias by excluding neonates who experienced a major bleeding or received a blood transfusion between both samples. A major strength of our study was that we differentiated between venous, arterial, or capillary blood, allowing documentation of the difference between arterial and venous Hb and Hct concentrations in neonates.

Unfortunately, due to the retrospective nature of our study, it was not possible to identify the number of failed attempts at collecting UCB (or NB) for analysis. If the attempt failed, for example due to clotting, it was shown in our study as "no sample taken." In addition, we did not collect data on the clinical condition of the neonates prior to transfusion. We could therefore only assess the indications for transfusions based upon the hemoglobin thresholds.

Some might argue that infants may have received intravenous (IV) saline bolus injections between cord blood sampling and subsequently taken neonatal blood samples. As the injections dilute the blood, the NB sample results might have been lower after this event.[24] We expect this underestimation to be minimal, as these IV saline bolus injections are rarely given in the first 2 hours of life.

Improving the accuracy of the correction factors would be beneficial and strengthen the evidence for clinical use. Therefore, before implementation in clinical practice, the correction factors need to be validated in another NICU setting to confirm performance in a different patient population.

The vast majority (83.3%) of Hb values measured in UCB were above the transfusion threshold of 11 g/dL and concordant with venous measurement in NB. Repeating these Hb measurements in neonates at birth is therefore redundant and can be replaced by UCB measurements. In 4.7%, Hb values in UCB were lower than the transfusion threshold and were concordant with NB. By drawing blood from the umbilical cord rather than the neonate itself, iatrogenic blood loss in very preterm infants can be reduced.

However, in 8.3% of the neonates relying on UCB would withhold a transfusion as the UCB value was above the transfusion threshold while the NB value was below. In 3.6% of neonates, relying on UCB would give them a redundant transfusion as the UCB value was below the threshold and the NB value above. Based on our results, we therefore advise to repeat the Hb value in NB if the UCB value is below the transfusion threshold after applying the correction factor. Additionally, it is important to take the clinical condition of the neonates into account when deciding if a transfusion is appropriate. We acknowledge that, during a hectic clinical scenario, it might not be pragmatic to use a correction factor, which could be reserved for when there is a little more time and uncertainty regarding the UCB results. However, the regression equation for Venous Hb shows that NB is almost equal to NB +1.2 g/dL.

In addition to the Hb and Hct values, comparison for the CBC results indicate that UCB is a good replacement for NB when interpreting erythrocyte counts, and reticulocyte counts. Platelet counts and leukocyte counts show a positive correlation, although not as strongly.

As the NICU is a high impact department with many possible interventions, we compared the results of the correction factors over time. This analysis showed the correction factors are likely unsuitable for accurate prediction in the remaining 24-hour period following birth.

Because our study shows the Hb and Hct measured from UCB is concordant with values measured in venous blood in infants at birth, we suggest that UCB could be used as a valid alternative for venous NB at birth. Future research is needed to improve the accuracy of the correction factors in predicting NB, and to assess further the safety of their use in lower limits. Favoring the use of UCB would be prudent to reduce iatrogenic blood loss of preterm neonates at birth. ■

CRediT authorship contribution statement

Lisanne E. Heeger: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Myrthe I.J. Koster:** Writing – original draft, Formal analysis. **Camila Caram-Deelder:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Vincent Bekker:** Writing – review & editing, Data curation. **Johanna G. van der Bom:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Enrico Lopriore:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

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