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Optimizing haemoglobin measurements in VLBW newborns: Insights from a comparative retrospective study

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ABSTRACT

Introduction: Haemoglobin levels assessment is a crucial part of neonatal intensive care practice, the painful experience of repeated heel pricks and venepunctures blood sampling may negatively affect neonatal clinical course. To date the reliability of haemoglobin levels obtained by point-of-care testing (POCT) analysis if compared to standard blood cell count remains controversial.

Materials and methods: Retrospective study conducted on all inborn premature infants (gestational age < 32 weeks) admitted to NICU of the IRCCS Giannina Gaslini Institute during the period May 2021–April 2023.

We considered blood samplings occurred within the first 28 days of life recording the laboratory haemoglobin levels (Hblab) (reference method), the point-of-care haemoglobin levels (HbPOCT) (alternative method) and the type of puncture (arterial, venous and capillary). A Bland-Altman analysis was performed to evaluate the Hb agreement, it determines the bias (mean difference between the reference and alternative methods) and limits of agreement (LOA; lower, l-LOA; upper, u-LOA) of measures. An acceptable limit of agreement was 1 g/dl according to the existing literature.

Results: We considered 845 blood samplings from 189 enrolled patients. The comparison between the reference and the alternative method showed a good agreement for the capillary sampling technique with l-LOA of −0.717 (−0.776; −0.659) and u-LOA of 0.549 (0.490; 0.607), these results were not achievable with the other techniques, with LOAs over ±1 g/dl threshold (venous<arterial).

Conclusions: The reliability of capillary POCT measured haemoglobin levels may reduce clinical-related costs and the number of painful experiences, with obvious positive effects on the daily neonatal life in the NICU and on the developing brain structures.

1. Introduction

Neonatal anaemia is defined by a blood haemoglobin (Hb) concentration below two standard deviations (SD) from the mean value reported for postnatal age [1]. All newborn babies undergo a physiological and dramatic fall in the rate of erythropoiesis and haemoglobin synthesis. In fact, since the first week of extra-uterine life Hb production sustained by bone marrow decreases, determining a nadir of Hb concentration in blood between 8 and 12 weeks of postnatal age [2].

In very preterm infants who were born very low birth weight (VLBW) and especially in those who were born extremely low birth weight (ELBW) the normal physiologic anaemia may manifest earlier. In those cohorts of preterm babies, the nadir of Hb concentration is reached between five and ten weeks of postnatal age and the levels of Hb can variably range from 8 g/dl to 10 g/dl [3].

Among the physiological and pathological causes of anaemia an important role is played by iatrogenic factors as most of the newborns admitted to the intensive care unit usually undergo a non-negligible number of blood draws especially during the first weeks of life [4]. Hb levels are repeatedly checked during admission, as they reflect the

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oxygen-carrying capacity of blood. Furthermore, they are necessary to diagnose anaemia and driving clinical decisions about red blood cell (RBC) transfusion, whose thresholds remain considerably variable because of the lack of consensus across studies [2].

The complete blood count (CBC) obtained through hemochromocytometric assay represents the gold standard method to determine the Hb value, but it requires a considerable interval of time to be obtained, as it is usually performed by the hospital analysis laboratory. It requires around 0.5–1 ml of blood to be processed (depending on the single laboratory equipment) potentially representing about 1 % of total neonatal blood volume, especially if considering preterm infants [5].

The Point-of-care (POCT) analysis is daily used in the NICUs to evaluate the acid-base balance, blood gases, electrolyte and other metabolite concentration with a little amount of blood to be performed [6]. The reason of POCT analysers diffusion is related to their capability to shorten the time of clinical decision-making, as their results are rapidly available at the point of care [7]. The same blood sample and the same instrument used to perform routine POCT blood-gas analysis can determine Hb levels. However, only general recommendations exist for POCT measurement of Hb, and no standards or guidelines have been yet developed [8,9].

The second important aspect related to blood sampling in the NICUs is the painful experience generated. Heel prick collection and venepunctures are the most common painful procedures which are performed to get blood samples commonly used for biochemical evaluation, blood gas analysis and neonatal screening tests. According to the literature, infants admitted to the NICUs may undergo up to 10 painful treatments per day of hospitalization, and pain relief is rarely considered during the clinical practice [10]. Many papers have followed the initial description of attenuated response to heel prick nociception by tasting sucrose prior by the procedure [11–14]. This aspect could be crucial since the repeated pain exposure during periods of rapid neurodevelopment has been shown to alter cortical and subcortical brain microstructure, particular regarding the thalamus [15–17]. Furthermore, pain might increase long-term adverse effects on emotional, behavioural, and cognitive development and the long-term value of the initial attenuation of pain response due to orally administered sucrose has been questioned [18–20]. For these reasons, neonatal care research has been targeted to limit painful procedures during the first days of life, especially in case of prematurity, as it seems to be a further very short window of cerebral vulnerability within the first three days of life [21,22].

Some Authors have already tried to evaluate the precision of POCT Hb in comparison with the Hb measured by CBC or other methods. These studies obtained variable results depending on technique, type of sampling and considered population [8,23–27].

The aim of this study is to evaluate the diagnostic accuracy of point-of-care testing POCT in dosing blood Hb concentration versus the full blood count analyser in a population of VLBW infants. Findings might be helpful to contain the number of painful procedures that preterm infants experience daily in the NICUs around the world, and a reduction of costs in terms of materials and medical procedures performed.

2. Materials and methods

This is a retrospective study conducted on patients admitted to the Neonatal Intensive Care Unit (NICU) of the IRCCS Giannina Gaslini Institute, a tertiary level academic pediatric institution in Italy, during the period May 2021–April 2023.

2.1. Patients' inclusion/exclusion criteria

We considered all inborn very premature infants (gestational age < 32 weeks) diagnosed with VLBW (birth weight < 1500 g), without selecting them basing on the different complications presented.

A database was built to collect patients' sex, gestational age, birth weight and all the blood samplings occurred within the first 28 days of life in which a simultaneous (within 30′) laboratory hemochromocytometric test and a point of care haemoglobin test were performed. For each blood sampling we recorded the laboratory haemoglobin levels (Hblab) (assumed to be the reference method), the point-of-care haemoglobin levels (HbPOCT) (assumed to be the alternative method) and the type of puncture (arterial, venous – central or peripheral - and capillary). We excluded all patients who did not report a simultaneous blood sampling for Hb levels assessment during the selected period. We also excluded single samplings which were not simultaneously reported as a double technique testing.

2.2. Blood sampling, collection, and analysis

Among the venous samplings we differentiated between the venous umbilical cord line (VUCL) and the peripheral venous catheter (PVC) ones. Capillary samplings were obtained by the heel prick technique using a neonatal lancet (1 mm × 2.5 mm), after having wiped off the first two drops of blood (defining a volume of 0,05 ml per drop [28]), the subsequent 20 drops (total volume of 1 ml) were collected into a blood tube containing ethylenediaminetetraacetic acid for measuring Hblab while the subsequent drops of blood (0,1 ml) were collected into a blood gas syringe and immediately analysed to measure HbPOCT.

VUCL samplings were obtained through a 3.5/5.0Ch umbilical catheter (depending on neonatal birth weight); PVC samplings from a 24/26G cannula and arterial samplings were obtained by a 2.5Ch arterial umbilical cord line (AUCL). All the venous and arterial blood samplings were deposited into a blood tube containing ethylenediaminetetraacetic acid for measuring Hblab (1 ml) while a blood gas syringe was filled in with 0,1 ml of blood and immediately analysed to measure HbPOCT.

Hblab measurements were obtained using a haematology analyser (Siemens Advia 2120, Siemens, Munich, Germany, factory reported coefficient of variation for Hb 0.93) by transforming all haemoglobin forms into cyanmethemoglobin (HiCN), a stable-coloured product with a maximum absorbance at 540 nm, adhering strictly to Beer-Lambert's law [29]. The diluted sample's absorbance at 540 nm is compared with that of a standard HiCN solution with a known equivalent haemoglobin concentration. This method measures most haemoglobin derivatives, excluding sulfhemoglobin, without interference [30].

HbPOCT measurements were obtained using a point of care blood gas analyser (ABL 490 Radiometer, Copenhagen, Denmark; factory reported coefficient of variation for ctHb 1.41), which was calibrated several times per day. Its measurements rely on the absorbance spectrum of Hb. The optical system is based on a 128-wavelength spectrophotometer within a range of 478–672 nm connected to a hemolyzer and measuring chamber. In the hemolyzer, a 1 μl blood sample is ultrasonically hemolyzed at 37 °C, mixing red blood cell content with plasma to create an optically clear solution. A 4 W halogen lamp, regulated by a thermostatic photodiode, provides constant-intensity light to the cuvette. Light transmitted through the cuvette is directed to the spectrometer via optical fiber, passing through a slit, mirror, and concave grating. The grating separates light into all the different wavelengths, focused on a photodiode array. Each diode converts monochromatic signals to currents, forming the basis for the absorption spectrum. The spectrum is then sent to the analyser's computer, where desired parameter values are calculated [31,32].

Notably, fetal haemoglobin (HbF), whose content is estimated to be around 50–80 % in newborns, with a transition towards the adult form (HbA) during the first years of life [33], differs in spectrum from this latest because of a slight molecular structure variation [34–36]. The presence of HbF in a sample may affect results if not corrected for, but as stated in the user manual, the ABL490 analyser automatically addresses this issue. The analyser detects HbF interference by analysing the difference spectrum between fetal and adult oxyhaemoglobin. The concentration of fetal oxyhaemoglobin (cO2HbF) is measured from the size

of the difference spectrum. When the amount of cO2HbF exceeds a certain level, it indicates HbF interference. The analyser corrects for this interference by subtracting the difference spectrum of fetal oxyhaemoglobin from the measured spectrum and performs additional calculations, utilizing cO2HbF to measure fetal haemoglobin concentration (FHbF) [31].

For other perturbators, according to the technical manual, the optical system of the POCT device addresses potential interfering substances by subtracting their spectra. This method is deployed, for example, for bilirubin determination, which exhibits its own distinct absorbance spectrum. Consequently, the analyser effectively mitigates interference using the previously mentioned approach [32].

2.3. Statistical analysis

Descriptive statistics were generated for the whole cohort; data were expressed as mean, standard deviation (SD), 95 % confidence interval of the mean, standard error of the mean (STDe) for continuous variables and absolute and relative frequencies for categorical variables.

Normality of distribution for all variables was assessed using the Shapiro-Wilk W test.

Significative differences between Hb levels of the reference method (Hblab) and the alternative method (HbPOCT) for each type of sampling were assessed using the Wilcoxon's rank test.

Gestational and birth weight effect on the agreement of the reference and alternative method measures on each sampling site was assessed calculating the adjusted Pearson's correlation coefficient, while the effect of sex was assessed using the Student's *t*-test.

To evaluate the agreement between the different Hb measurement methods, the method described by Bland and Altman for multiple observations per individual was used, which determines the bias (mean difference between the reference and alternative methods) and limits of agreement (LOA; lower, l-LOA; upper, u-LOA) of measures [37]. An acceptable limit of agreement was fixed at 1 g/dl according to the existing literature [8].

The correlation between Hblab values and Hblab-HbPOCT differences was studied to identify any interference performing a linear regression analysis. The paired samples Student's *t*-test was used to compare Hblab-HbPOCT differences between the VULC and the PVC samplings.

Neonatal anaemia in our cohort was defined according to Lundstrom et al. 1977 [38] considering an Hblab value of 11.7 g/dl (-2SD from the mean per birth weight and postnatal age) as a cut-off point, despite lower values of haemoglobin are usually considered to assess the need of transfusion according to different gestational ages. A predictivity model for the diagnosis of anaemia was built considering this single discriminating value.

A binomial logistic regression model (odds cut-off 0.5) was applied to assess the influence of birth weight, gestational age and sex on the anaemia diagnose, McFadden's r^2 and Odds Ratios were obtained for each factor.

A Receiver Operating Characteristic (ROC) analysis was then performed to the HbPOCT values in order to assess the predictivity and the optimal diagnostic cut-off: sensitivity, specificity, positive predictive value, negative predictive value and AUC (Area Under the Curve) were reported for each sampling category.

A *p*-value of <0.05 was considered statistically significant, and all *p*-values were based on two-tailed tests.

Statistical analysis was performed using the Jamovi project interface software, based on R language for statistical computing [39–43].

3. Results

3.1. Patients and overall characteristics

A total of 205 patients were eligible for this study according to the inclusion criteria reported above. Of them 16 patients were excluded (8.4 % of the total) because no simultaneous lab and POCT samplings were present within the clinical files. The 189 enrolled patients underwent 937 blood samplings within the first 28 days of life (either capillary, venous on arterial), among them 92 samplings (10.9 % of the total) were excluded according to the exclusion criterium of simultaneity and 845 samplings were considered eligible for the analysis. Specifically, 345 samplings were of capillary blood, 385 were of venous (213 from VULC, 172 from PVC), and 115 were arterial.

Shapiro-Wilk test for normality showed a normal distribution for all the considered variables except for the gestational age.

Birth weight, gestational age and sex of the enrolled patients varied widely but did not affect the overall agreement of results of the two methods in each sampling site (AdjGA $r > 0.95$, AdjBW>0.95, $p < .001$ per each).

Moreover, standard error of the mean showed that capillary sampling was associated to a more accurate Hb measurement than the venous and arterial ones (Table 1).

Table 1
Patients' characteristics and Hb measurements comparison split by sampling technique.

$n = 189$ pts., 845 samplings							
Sex (M/F) (n)	104 M (55 %); 85F (45 %)						
	Mean	SD	STDe	CI95%	Min	Max	p-value
Gestational age (days)	220	9.52	0.69	118; 121	89	144	
Birth weight (g)	1152	253	18.4	1116; 1188	410	1500	
Capillary sampling	$n = 345$						
Hblab	12.9	3.2	0.17	12.6; 13.3	5.9	21.3	<0.001
HbPOCT	13	3.24	0.17	12.7; 13.3	6.6	21.8	
Total venous sampling	$n = 385$						
Hblab	13.5	3.02	0.15	13.2; 13.8	6.1	24.1	<0.001
HbPOCT	13.9	3.22	0.16	13.6; 14.2	6.0	25.5	
VULC sampling	$n = 213$						
Hblab	13.8	3.12	0.21	13.4; 14.1	7.4	24.1	0.001
HbPOCT	14.2	3.38	0.23	13.8; 14.7	6.5	25.5	
PVC sampling	$n = 172$						
Hblab	13.1	2.86	0.22	12.7; 13.6	6.1	19.9	<0.001
HbPOCT	13.5	2.97	0.23	13.0; 13.9	6.0	20.2	
AULC sampling	$n = 115$						
Hblab	12.5	2.57	0.24	12.0; 13.0	6.9	18.3	0.005
HbPOCT	12.6	2.79	0.26	12.1; 13.1	6.7	19.3	

Pts, patients; M, males; F, females; SD, standard deviation; STDe, standard error of the mean; CI95%, confidence interval 95 %; Min, minimum value; Max, maximum value; Hblab, Hb measured by laboratory emochromocytometric assay; HbPOCT, Hb measured by point-of-care analysis; VULC, venous umbilical line catheter; PVC, peripheral venous catheter; AULC, arterial umbilical line catheter.

3.2. Methods performance analysis

Comparison between the reference and the alternative method showed a good agreement for the capillary sampling technique with a lower LOA of −0.717 (−0.776; −0.659) and an upper LOA of 0.549 (0.490; 0.607), these results were not detectable for the other techniques, with LOAs significantly over the ±1 g/dl threshold (PVC < Total

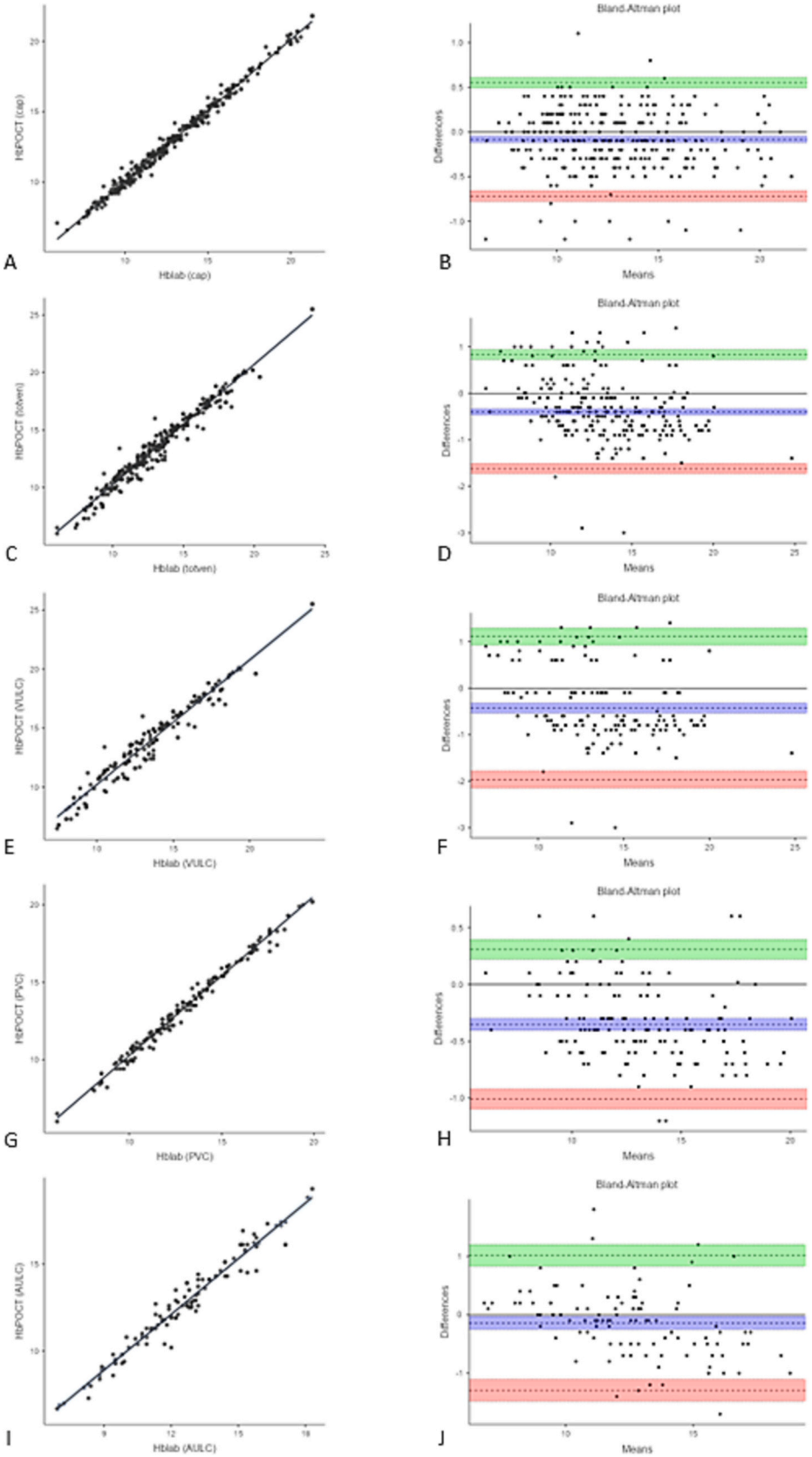

Fig. 1. Scattergram and Bland-Altman plot showing the distribution of values and differences of Hblab and HbPOCT values divided into capillary sampling (A, B), total venous sampling (C, D), VULC sampling (E, F), PVC sampling (G, H) and AULC (I, J). Acceptability threshold for concordance is set at ±1 g/dl.

Table 2
Comparisons between reference method (Hblab) and alternative method (HbPOCT) according to Bland-Altman methodology.

Blood sampling	Bias (CI95%)	l-LOA (CI95%)	u-LOA (CI95%)	Outliers |Hblab-HbPOCT| > 1 g/dl
Capillary	−0.008 (−0.118; −0.049)	−0.717 (−0.776; −0.659)	0.549 (0.490; 0.607)	10 (2.9 %)
Total venous	−0.394 (−0.457; −0.331)	−1.622 (−1.729; −1.515)	0.834 (0.727; 0.941)	56 (14.5 %)
VULC	−0.429 (−0.535; −0.323)	−1.969 (−2.151; −1.787)	1.111 (0.929; 1.293)	54 (25.4 %)
PVC	−0.350 (−0.401; −0.300)	−1.008 (−1.094; −0.922)	0.307 (0.221; 0.394)	2 (1.2 %)
AULC	−0.142 (−0.251; −0.033)	−1.296 (−1.482; −1.109)	1.012 (0.826; 1.198)	17 (14.8 %)

CI95%, confidence interval 95 %; l-LOA, lower level of agreement; u-LOA, upper level of agreement; Hblab, Hb measured by laboratory emochromocytometric assay; HbPOCT, Hb measured by point-of-care analysis; VULC, venous umbilical line catheter; PVC, peripheral venous catheter; AULC, arterial umbilical line catheter.

venous<AULC<VULC). The results obtained by the PVC sampling remain of interest as the obtained LOAs were on the borderline of acceptability [−1.008 (−1.094; −0.922); 0.307 (0.221; 0.394)] (Fig. 1).

Comparing the presence of outliers, we reported the highest level for the VULC samplings (25.4 %) and the lowest for the capillary and PVC ones (2.9 % and 1.2 % respectively), confirming the technical goodness of those measurements (Table 2).

The measures obtained comparing the reference and alternative method Hb results of each sampling technique were significantly different each other ($p = .002$) and we underline that VULC and PVC differences distribution were not similar ($p = .015$).

Furthermore, the distribution of those differences was not affected by the Hblab levels only in the case of capillary sampling ($r = 0.05, p = .37$), while for all the other techniques a positive correlation was detected (Total venous $r = 0.22, p = .002$; VULC $r = 0.21, p = .001$; PVC $r = 0.29$, $p = .001$; AULC $r = 0.28$, $p = .002$), indicating a HbPOCT-Hblab gap much higher as Hblab increased.

3.3. Anaemia diagnoses and predictivity model

A binomial logistic regression analysis showed a correlation ($r^2 = 0.1$, $p = .002$) between anaemia diagnosis and birth weight distribution [OR = 0.72 (0.63; 0.77)], gestational age [OR = 0.91(0.89; 0.97)] and sex [OR = 0.38 (0.20; 0.72)].

ROC analysis for the HbPOCT values reported a varying cut-off ranging from 11.7 g/dl to 12.2 g/dl for the diagnosis of anaemia, with excellent sensitivity, specificity and predictive (positive/negative) profiles for each sampling technique (Table 3, Fig. 2).

The total blood volume that was necessary to perform Hblab and HbPOCT analysis (all sampling techniques) was equivalent to 964 ml (845 ml Hblab and 119 ml HbPOCT, considering the initial drops wiped out per each sampling). Performing only HbPOCT analysis in our cohort would have been resulted in a saving of 726 ml of blood, equivalent to the 85.9 % of the total.

4. Discussion

This study was addressed to a specific very preterm and VLBW infant population admitted to the NICU over a two-year period. The main outcome was to determine whether the use of POCT analysers for the Hb concentration assessment could represent a reliable and achievable alternative to standard laboratory workout.

A cut-off value of ±1 g/dl was enforced to define the measures obtained by the reference and by the alternative method as similar, according to Wittenmeier et al. 2019 [8]. The choice for this specific cue comes from the strict transfusion criteria for neonates, assuming that a higher gap between Hb concentration measures may consequentially lead to unnecessary or omitted transfusions.

According to the results obtained, we describe for the first time in the literature that POCT Hb concentration collected from a capillary blood sample appears superimposable on Hb concentration from a parallel CBC. On the other side, POCT Hb concentration from arterial, VULC and PCV blood samples wasn't similar enough to be considered interchangeable in this cohort.

This finding underlines the importance of considering the sample source when using hematologic reference ranges for healthy or sick neonates. When interpreting results, the term “peripheral” blood should be replaced with “capillary” or “venous”, so that an accurate assessment can be made [23].

This data are in accordance with other retrospective studies carried out on NICU patients, in which analysed samples were venous only or “peripheral”, meaning capillary, venous or arterial indistinctly [8,44], and also with studies performed on ICU adult population [45].

Even if it is demonstrated that a biological Hb concentration variability exists between different sampling sites [46], a reason for the important divergence in our data may be imputable to instrument and/or sampling method variability as well as human error [25,46] and this assumption is enforced by the consideration that VULC and AULC resulted in the worst agreement if compared to PVC. Following this hypothesis, we suggest that a great interference may have been caused by the intravenous (IV) fluid infusion that is usually required in these single lumen catheters. This infusion is carried out at higher velocities than in PVCs and often involves the use of unfractionated heparin for preventing arterial catheter occlusion (this latter influencing only AULC blood sample). Therefore, a variable degree of haemodilution among samples may occur, causing a higher gap between Hblab and HbPOCT since the latter is usually withdrawn later than the first one. To confirm this assumption, we found a significative difference between the VULC and the PVC results, underlying the presence of an external confounding factor probably related to the sampling technique.

Body weight, sex and gestational age did not affect the agreement of results, and it is consistent with the fact that the two blood samplings were taken in the same moment from the same patient. Furthermore,

Table 3
Predictivity model of HbPOCT values in the anaemia diagnosis.

Anaemia HbPOCT “true” diagnosis (Hblab, yes/no)	P	Cutpoint	Se(%)	Sp(%)	PPV(%)	NPV(%)	Youden's index	AUC
Capillary	40.9	11.7	96.1	95.7	97	94.4	0.918	0.996
Total venous	27.3	12.2	94.6	99.1	99.6	87.4	0.937	0.990
VULC	22.5	12.2	93.3	97.9	99.4	81.3	0.913	0.984
PVC	33.1	12.0	99.1	98.3	99.1	98.2	0.974	0.990
AULC	38.3	12.1	97.2	93.2	95.8	95.4	0.904	0.984

Hblab, Hb measured by laboratory emochromocytometric assay; HbPOCT, Hb measured by point-of-care analysis; VULC, venous umbilical line catheter; PVC, peripheral venous catheter; AULC, arterial umbilical line catheter; Se, sensibility; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

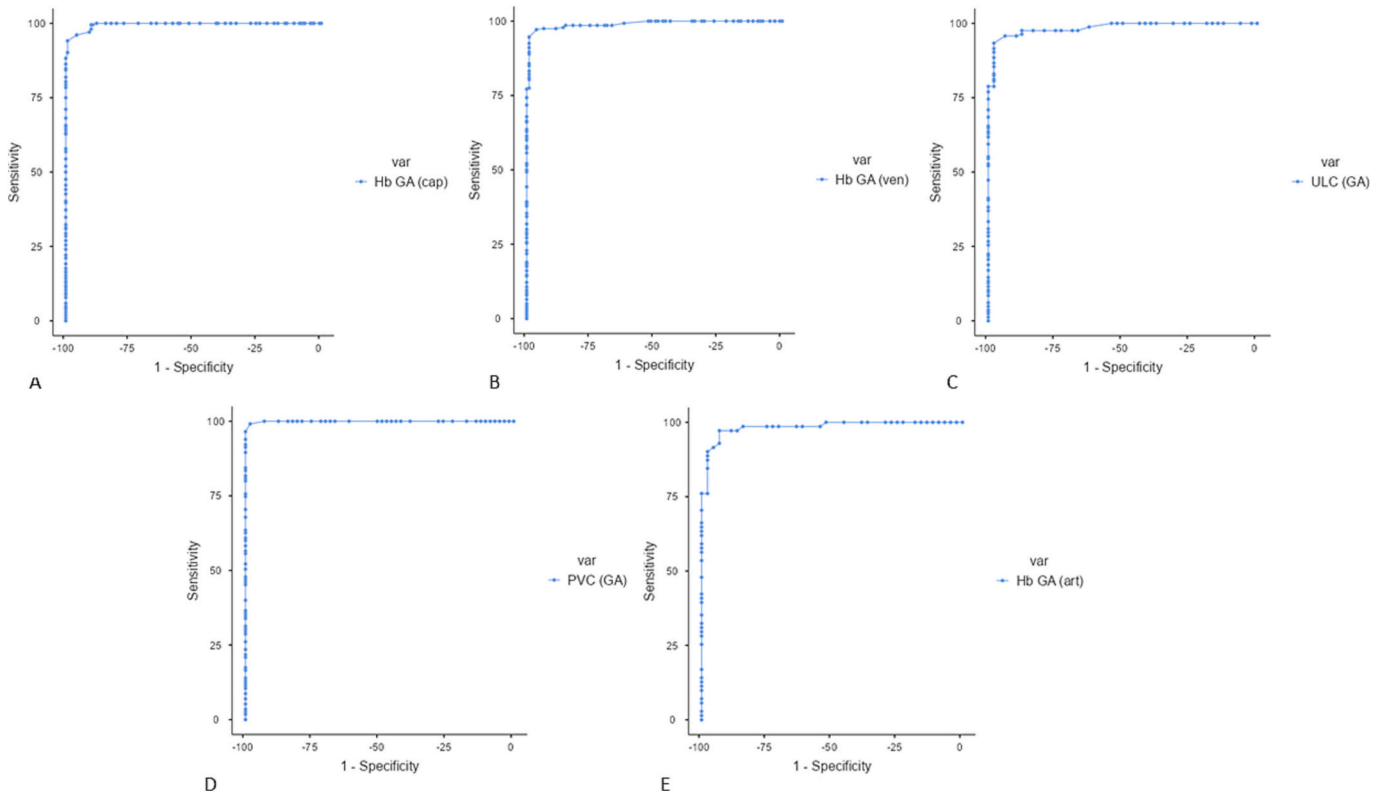

Fig. 2. Receiver Operating Characteristics (ROC) curve showing the profile of sensibility and specificity of HbPOCT values in the diagnosis of anaemia divided into capillary sampling (A), total venous sampling (B), VULC (C), PVC (D) and AULC (E).

statistical analysis describes a positive correlation between the Hblab – HbPOCT difference and the Hblab values in all the sampling techniques except for capillary samples. This finding may enforce the assumption that all the other samplings are exposed to an interference related to the technique itself, since this correlation is not explainable by any biological or analytical reason and it's not on the same page with existing literature [26,47]. However, as PVC sampling showed very close differences when compared to the predetermined limit, if a decision based on levels of Hb concentration must be taken and heel prick could not be performed, this technique is the preferable one.

Considering the predictivity power of HbPOCT in defining neonatal anaemia, we reported a brief variability in the best cut-off definition. In accordance with the other findings, the best concordance was found for capillary and PVC samplings, while VULC and AULC defined a higher cut-off for diagnosis, confirming the less accuracy of these measures.

The strength point of our study consists in having reported a considerable number of patients and samplings taken from a specific category of patients, that is, to the best of our knowledge, among the highest currently reported worldwide. The reason for this choice is related to the different needs which characterise this clinical setting, where avoiding unmotivated transfusions and containing painful experiences may greatly contribute to the outcome [22].

These results add to the already demonstrated reliability of for other minimally invasive techniques (classically called "micromethods") in measuring haemoglobin levels, concretising the effort in reducing invasiveness and nociceptive experience in such delicate critical patients [48].

A limitation of our analysis lies on its retrospective nature, which did not make possible to compare the different techniques used to obtain samples at the same time. In fact, according to Neufeld et al. 2002 [26], capillary Hb levels are normally higher than the venous ones and, if confirmed, it could have been corroborating evidence that Hb levels vary in relation to sample management [46].

We are aware of existing literature indicating that the measurement of total haemoglobin (Hb) levels might be compromised by the presence of fetal haemoglobin (HbF), especially when utilizing non-invasive or less invasive instruments distinct from those employed in our study [49,50]. Our analyses though were conducted using rigorously validated methodologies, as explicitly outlined in our Materials and methods section, resulting in a mitigation of this phenomenon to levels deemed inconsequential".

Lastly, further studies should be performed in order to assess the influence of IV fluids in the Hb concentration assessment and the differences related to samples handling and management [51].

In the end, clarifying the role of minimally or non-invasive sampling and techniques will be helpful to further reduce the invasiveness of treatments in the NICUs [24,52].

5. Conclusions

This study suggests that Hb concentration assessment in very preterm VLBW infants can be equally measured using a POCT or a haemochromocytometric assay, making a reliable opportunity of blood sparing, when a capillary sampling is obtained. This is a novel and unique finding which represents a great improvement not only for the amount of spared blood but also for the consequent reduction in nociceptive experiences in the NICU, known to be related to an adverse outcome [53–55]. Reducing phlebotomies and calf squeezes may represent a valid strategy especially to reduce impairments in the developing thalamus and later sensory problems [56]. Obviously, the act of saving blood will imply a reduction in the number of blood transfusions and potentially related adverse outcomes.

Our data confirm that VULC is not and ideal site of sampling for Hb measurements as differences between Hblab and HbPOCT are significant.

Ethics approval and consent to participate

The study was conducted in compliance with the terms of the Helsinki Declaration and written informed consent for the enrolment and for the publication of individual clinical details was obtained from parents. In our country, namely Italy, this type of clinical study does not require Institutional Review Board/Institutional Ethics Committee approval to publish the results.

Consent for publication

Written informed consent for publication of their clinical details was obtained from the parents of the patients. A copy of the consent form is available for review by the Editor of this journal.

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CRediT authorship contribution statement

Andrea Calandrino: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carolina Montobbio:** Data curation, Conceptualization. **Irene Bonato:** Investigation, Formal analysis, Data curation. **Gaia Cipresso:** Investigation, Data curation. **Francesco Vinci:** Writing – original draft, Investigation, Data curation. **Samuele Caruggi:** Methodology, Formal analysis, Data curation. **Marcella Battaglini:** Methodology, Formal analysis, Data curation, Conceptualization. **Chiara Andreato:** Writing – original draft, Methodology, Formal analysis, Data curation. **Federica Mongelli:** Supervision, Formal analysis, Conceptualization. **Paolo Massirio:** Supervision, Conceptualization. **Giorgia Brigati:** Supervision, Methodology, Formal analysis, Data curation. **Diego Minghetti:** Writing – original draft, Supervision, Conceptualization. **Luca Antonio Ramenghi:** Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors report no competing interests.

Data availability

All datasets generated and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request. Raw data were generated at IRCCS Istituto Giannina Gaslini. Individual patient data will be made available on request in agreement with data privacy statement signed by the parents.

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