Regional tissue oxygenation and conventional indicators of red blood cell transfusion in anaemic preterm infants

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Summary

Background It is unresolved whether low haemoglobin (Hb) and symptoms of anaemia reflect oxygen delivery-consumption imbalances (fractional tissue oxygen extraction [FTOE]). Here, we test whether pre-transfusion Hb and symptoms of anaemia correlate with pre-transfusion cerebral and splanchnic FTOE.

Methods This prospective cohort study was carried out between Sept 1, 2014 and Nov 30, 2016 at Nepean Hospital, Sydney, Australia. The study enroled haemodynamically stable preterm infants: gestation <32 weeks; birth weight <1500 gs; postmenstrual age <37weeks, who received 15 mL/kg packed red blood cell transfusion (PRBCT) based on low Hb and symptoms of anaemia. FTOE was determined using simultaneous monitoring of near-infrared spectroscopy and pulse oximetry for 4 h before PRBCT.

Findings The study enroled 29 infants born with a median gestation of 26.4 weeks (IQR 25.4–28.1), birth weight 922 g (655–1064), at postmenstrual age 33.6 weeks (31.7–34.9), and weight 1487 g (1110–1785). There was no significant correlation between Hb (median 97 g/L, IQR 87–100) and cerebral FTOE (r=−0.12, 95% CI −0.47 to 0.27; p = 0.54, n = 29) as well as splanchnic FTOE (r=−0.09, 95% CI −0.45 to 0.29; p = 0.64, n = 29). Median cerebral FTOE (p = 0.67) and splanchnic FTOE (p = 0.53) did not differ between symptomatic and asymptomatic groups.

Interpretation Our preliminary findings suggest that pre-transfusion Hb and symptoms of anaemia might not accurately reflect oxygen delivery-consumption imbalances in both the brain and the gut. A lack of correlation with cerebral FTOE might be presumed to be due to the brain-sparing effect. However, the lack of correlation with splanchnic FTOE is more concerning. Hence, these results warrant larger studies incorporating FTOE along with the conventional criteria in the transfusion algorithm.

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Keywords: Packed red blood cell transfusions; Anaemia; Preterm; Fractional tissue oxygen extraction; Haemoglobin

Abbreviations: CPAP, Continuous positive airway pressure; Hb, Haemoglobin; **NIRS**, Near Infrared Spectroscopy; **HFNC**, High Flow Nasal Cannula; PDA, Patent Ductus Arteriosus; DO2', Oxygen delivery; VO2, Oxygen consumption; PRBCT, Packed Red Blood Cell Transfusion; StO2, Tissue oxygen saturation; FTOE, Fractional tissue oxygen extraction; NEC, Necrotising Enterocolitis

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Research in context

Evidence before this study

Existing guidelines use haemoglobin and symptoms of anaemia to determine packed red blood cell transfusion (PRBCT). Although haemoglobin determines oxygen delivery, the balance between oxygen delivery and consumption (fractional tissue oxygen extraction [FTOE]) may be a physiologically more important indicator of transfusion threshold than oxygen delivery per se. Our literature search included studies in premature neonates published from Jan 1, 2010 to Dec 31, 2021, using the following search terms: "near-infrared spectroscopy, preterm, newborn, fractional tissue oxygen extraction, regional tissue oxygen saturation, packed red blood cell transfusion, anaemia, correlation, and association".

Added value of this study

We demonstrated that the currently used tools to determine the transfusion threshold (haemoglobin and symptoms) might not accurately reflect tissue oxygen consumption-delivery balance in the brain and the gut.

Implications of all the available evidence

Our preliminary findings suggest that the haemoglobin threshold may not be the appropriate tool to perform PRBCT. This could be a reason for the conflicting reports of the outcome of liberal versus restrictive transfusion thresholds.

Introduction

Anaemia of prematurity develops in a large proportion of premature newborns. Transfusion guidelines are based on blood haemoglobin (Hb) levels and symptoms suggestive of anaemia. There is significant variability in the thresholds used for packed red blood cell transfusions (PRBCT). Furthermore, restrictive versus liberal regimes deliver conflicting results with respect to long-term neurodevelopmental outcome.[1–4] While Hb is a major determinant of blood oxygen content, it is not the only factor that accounts for tissue oxygen delivery. Other factors include cardiac output,[5–7] regional blood flow,[8,9] arterial oxygen saturation, and the position of oxygen dissociation curve.[10] Hence, relying on Hb as the sole criteria for PRBCT proves to be inadequate. In addition, one must infer tissue oxygen delivery in the context of oxygen consumption. Oxygen consumption depends on the metabolic activity of the tissue. It varies with the nature of tissue (e.g., brain has higher oxygen consumption compared to gastrointestinal tract.[11]) and specific clinical conditions (e.g., oxygen consumption is increased in conditions such as chronic lung disease, sepsis, seizures, use of inotropes, etc.[12,13] The primary goal of maintaining aerobic cellular respiration is to deliver adequate oxygen to meet metabolic demands. Oxygen delivery to a particular tissue should be adequate to balance (satisfy) oxygen consumption of the tissue under specific condition. Thus, the balance between oxygen delivery and consumption is more important than absolute value of oxygen delivery. The balance between oxygen delivery and consumption is best reflected by the fractional tissue oxygen extraction (FTOE).[14–17] [FTOE = (oxygen consumption/oxygen delivery)]. There are no normative values for regional FTOE. Trends rather than absolute values for each tissue (cerebral or splanchnic) are more meaningful.[18] FTOE increases when oxygen consumption is out of proportion to oxygen delivery (inadequate oxygen delivery, increased oxygen consumption, or both).[13,19] Accordingly, it can be inferred that a declining Hb is associated with increasing FTOE. Low Hb levels are heuristically used as criteria for PRBCT threshold. Here we test, whether the conventional criteria for PRBCT correlate with brain oxygen delivery-consumption balance (cerebral FTOE). This question is especially relevant in the context of conflicting results of long-term neurodevelopmental outcomes applying restrictive versus liberal Hb thresholds.[1–4]

In addition, it is well known that during hypoxia there is a physiological brain sparing effect diverting blood away from the peripheral organs, best described for the splanchnic circulation.[20] Hence it can be conceptualised that the correlation of Hb with splanchnic FTOE (FTOEs) is likely stronger than cerebral FTOE (FTOEc). These associations have so far been poorly investigated.[21–23]

Besides low Hb levels, clinical symptoms such as cardiorespiratory instability and poor weight gain have been presumed to result from poor oxygen delivery. They have therefore been used as criteria for PRBCT.[24] Again, there is a lack of data correlating the symptoms of anaemia with FTOEc and FTOEs.

Our study aimed to determine the associations of pre-transfusion Hb and clinical symptoms of anaemia with FTOEc and FTOEs. It was hypothesised that FTOE is not associated with Hb and symptoms.

Methods

The current study is a post-hoc analysis of a prospective observational single centre study conducted in the neonatal intensive care unit (NICU), Nepean Hospital, Sydney, Australia, from Sept 1, 2014 to Nov 30, 2016. The primary study evaluated cerebral and splanchnic oxygenation associated with PRBCT and feeding.[25] The study protocol including parental consent was approved by the human research ethics committee, Nepean Blue Mountain Local Health Committee (Approval number: Study 12/67 - HREC/12/NEPEAN/148). The study was conducted after written, informed consent of parents, as

approved by the human research ethics committee, Nepean Blue Mountain Local Health Committee.

Study participants

Inclusion criteria: gestation <32 weeks; birth weight <1500 g; postmenstrual age <37 weeks; tolerating enteral feed volume at least 120 mL/Kg/day; hemodynamically stable and receiving elective PRBCT to treat anaemia of prematurity.

Exclusion criteria were infants with necrotising enterocolitis (NEC) (current or previous); feed intolerance (defined as the treating clinical team's decision to withhold feeds/withhold grading up of feeds for at least 12 h); sepsis (defined as the treating clinical team's decision to commence antibiotics); PRBCT in the previous 72 h; patent ductus arteriosus (PDA) or its treatment with Ibuprofen/Indomethacin/surgery in the previous 72 h and/or congenital gastrointestinal, complex cardiac/lethal anomalies.

Enrolment

An infant was enroled only once, even when receiving multiple transfusions. For pragmatic reasons, enrolment occurred when PRBCT was initiated during regular business hours (weekdays: 8 am to 5 pm) only. Consecutive babies satisfying the above criteria were enroled after written informed parental consent by the chief investigator/one of the designated officers.

Packed red blood cell transfusion (PRBCT)

No strict formal protocol existed regarding the Hb threshold for PRBCT. An independent clinical team not involved in the study decided on the indication to transfuse, based on a combination of low Hb and clinical symptoms perceived to be due to anaemia. In each case, the presence or absence of symptoms of anaemia was recorded. The symptoms included – increasing desaturations/bradycardia; increasing oxygen/ventilator requirement; failure to wean oxygen/breathing support, and failure to gain weight. As a general principle, PRBCT administration was considered when Hb was <120 g/L in critically unwell preterm infants on invasive ventilation/inotropes or when Hb was 80–100 g/L in infants on non-invasive ventilation, (continuous positive airway pressure (CPAP) and humidified-high flow nasal cannula (HFNC), or when Hb was <80 g/L for infants without respiratory support. Treating clinician teams were able to overrule these criteria based on their clinical acumen.

Study protocol

Once a clinical decision was made to transfuse an eligible infant, regional tissue oxygen saturation (StO_2) was determined using four-wavelength Near-infrared spectroscopy (NIRS) monitor (FORE-SIGHT® absolute cerebral oximeter, CASMED, Branford, Connecticut, 06,405 USA). A neonatal sensor was placed over the temporal region of the head to determine cerebral StO_2, and another neonatal sensor was placed on the lower quadrant of the abdomen just below the umbilicus to determine splanchnic StO_2.[26,27] A neonatal cap was placed over the cerebral sensor to hold it in place. The splanchnic sensor was held in place by the nappy. The sensors remained in place throughout the study period. The StO_2 measures oxygen saturation in the tissue bed located 1–2 cm beneath the sensor. Arterial oxygen saturation (SpO_2) was also monitored using the Masimo Radical-7 monitor (Radical-7® Pulse CO—Oximeter®, Masimo Corp. Irvine, CA). For babies needing supplemental oxygen, the fraction of inspired oxygen (FiO_2) was adjusted to a target range for SPO_2 between 91% and 95%. Simultaneously recorded cerebral StO_2, splanchnic StO_2, and SPO_2 were downloaded for a period of at least 4 h before the commencement of PRBCT. Enroled infants received 15 mL/kg PRBCT over 4 h, without Furosemide. RBCs were non-irradiated, leucodepleted, Cytomegalovirus (CMV) negative, and stored in SAG-M additive solution (Sodium chloride, 8.77 g/L; Adenine, 0.169 g/L; Glucose, 9 g/L; Mannitol, 5.25 g/L). Other demographic parameters (Table 1) were also collected.

Data processing

The analogue output StO_2 and SpO_2 data were downloaded in real-time and aligned along the time axis in LabChart reader format (.adicht files) using a PowerLab data acquisition system[28] (PowerLab, ADInstruments, Sydney, Australia). The .adicht files were converted into .mat file format using a simple Python script (Python version 3.7.3[29]) at a sample rate of hertz (1 Hz) and these signals were used for all further processing. Data that could not be physiologically explained (e.g., the absence of variability,[26] a thirty percent step change in StO_2 between two subsequent data points for StO_2[30]) were removed. Data during the period of 'cares' were presumed to be artefactual because of two reasons: NIRS sensors were lifted for inspection of underlying skin during this period; babies underwent a nappy change, oral care, change of position, etc. during this period that would have caused significant movement artifacts. The removed segments were replaced with 'NaN' or 'Not a Number' which is recognized by Matlab[31] (MATLAB 9.3, The MathWorks, Inc., Massachusetts, USA) and ignored for all subsequent processing.

FTOEs (%) and FTOEc (%) were derived from each baby's real-time StO_2 and SpO_2 data using the formula $FTOE = [(SpO_2 - StO_2) \times 100 / (SpO_2)]$. The real-time series of values over 4 h immediately prior to PRBCT commencement were used to determine the pre-transfusion FTOE.

Sample size

The sample size for this study was calculated based on previous study findings.[21] Specifically, the correlation coefficient (r=−0.462) between FTOEc and pre-transfusion Hb was used in the computation. For a power of 80%, a total of thirty four participants were needed for this study. The sample size of thirty participants was considered achieved with a sufficient power of 75% for this study. An alpha of less than 0.05 was considered statistically significant. The 'pwr' library package in R version 4.1.2; R Core Team 2021 was used for the sample size calculation.

Statistical analysis

The central tendency was represented by median (Interquartile range, IQR) for the data that were not normally distributed. Associations between regional tissue oxygenation and Hb/symptoms were determined using non-parametric tests due to the non-normal distribution of Hb and symptoms. Spearman rank correlation coefficient was employed to evaluate the correlations between Hb and region-specific tissue oxygenation (FTOE and StO_2). The difference between median FTOE/StO_2 in symptomatic vs. asymptomatic groups was computed using Wilcoxon Rank-Sum test. Scatterplots were created to compare pre-transfusion organ-specific FTOE vs. Hb and symptoms. Data were analysed using R Statistical Software" (V 4.1.2; R Core Team 2021). P values of <0.05 were considered statistically significant.

Role of the funding source

The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. All authors confirm that they had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Results

Demographic features

Characteristics of potentially eligible, excluded and analysed babies are depicted in Figure 1. Twenty-nine infants with median (IQR) gestation 26.4 (25.4–28.1) weeks, birth weight 922 (655–1064) g were enroled at postmenstrual age 33.6 (31.7–34.9) weeks. Other demographic parameters are represented in Table 1.

Correlations of pre-transfusion Hb with regional tissue oxygenation

Correlations of pre-transfusion Hb with pre-transfusion FTOEc and FTOEs are depicted in Figure 2 (Data shown in Supplemental Table 1). There was no significant correlation between Hb and both FTOEc [Spearman r = −0.12, 95% CI (−0.47 to 0.27), p = 0.54 two-tailed, n = 29] and FTOEs (Spearman r = −0.09, 95% CI (−0.45 to 0.29), p = 0.64 two-tailed, n = 29]. There was also no significant correlation between Hb and cerebral StO_2 [Spearman r = 0.31, 95% CI (−1 to 1), p = 0.11, two tailed, n = 29], and splanchnic StO_2 [Spearman r = 0.12, 95% CI (−1 to 1), p = 0.54, two tailed, n = 29]. (Supplemental Figs. 1 and 2. Data are shown in Supplemental Table 1).

Comparison of FTOE and Hb in infants with symptomatic vs. asymptomatic anaemia

Figure 3a and b show the comparison of FTOE in infants with symptomatic vs. asymptomatic anaemia. There were no significant differences between median FTOEc (p = 0.67) as well as FTOEs (p = 0.53) in the symptomatic versus asymptomatic groups. Further, there was no significant difference in the Hb (p = 0.64) between the two groups (Figure 3c). As illustrated in supplemental Figs. 3 and 4, there were no significant differences between median cerebral StO_2 (p = 0.38) as well as splanchnic StO_2 (p = 0.33) in the symptomatic versus asymptomatic groups. (All the data are shown in Supplemental Table 1.

Discussion

Our single-centre preliminary study indicated that there was no significant association between pre-transfusion Hb and pre-transfusion FTOEc suggesting that pre-transfusion Hb might not accurately reflect cerebral oxygen delivery-consumption balance. In addition, the association with FTOEs was also not significant. Furthermore, the median FTOE of symptomatic versus asymptomatic infants did not differ.

Previous reports have shown none to moderate correlations between cerebral oxygenation and pre-transfusion Hb in preterm infants.[21–23,32,33] Even in those studies where it was statistically significant,[21,33] the correlation was not strong.[34,35] Studies are also heterogeneous in terms of the correlated parameters, e.g., Hb[21,23,32] or haematocrit[22,33] versus StO_2[23,32] or FTOE.[21,22,33] These studies are summarised in Table 2.

Our study sought to determine the association between FTOEs and Hb, based on the well-accepted physiological principle of brain sparing that may result in more pronounced gut than brain oxygenation changes.[20] Our study showed no significant correlation between FTOEs and Hb. This is in alignment with other studies, as shown in Table 2.

Variability in the strengths of correlations can be explained by understanding the physiology of oxygen delivery (DO_2) and oxygen consumption (VO_2). FTOE, the ratio of VO_2/DO_2 represents the dynamic interplay between DO_2, and VO_2.[14] DO_2 is predominantly dependant on cardiac output, Hb, and the ability of Hb to unload oxygen (position of the oxy-Hb dissociation

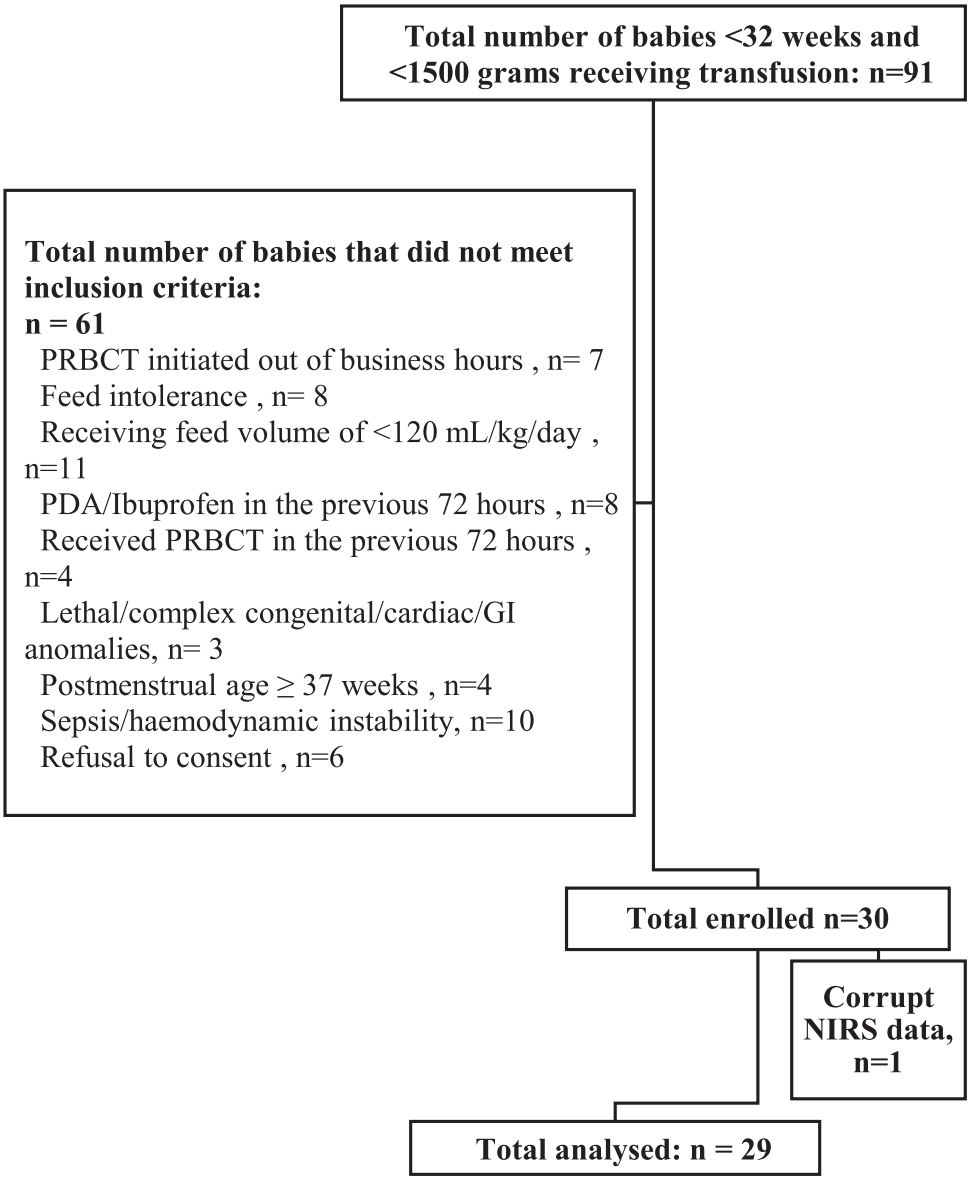

Figure 1. Participant flow diagram

PRBCT: Packed red blood cells; PDA: Patent ductus arteriosus; GI: Gastrointestinal tract; NIRS: Near-infrared spectroscopy; n: Number of patients; mL: Millilitres; kg: Kilograms.

curve).[10,24] With a delivery-consumption imbalance (decrease in DO_2 or increase in VO_2 or both), FTOE rises to maintain aerobic metabolism. Variability in the strengths of correlations in different studies most probably indicates variability in the compensatory measures such as cardiac output[5–7] and regional blood flow[8,9,36,37] that increases DO_2 or variability in the metabolic demands of tissues (VO_2) to match delivery. This underscores the fact that the thresholds of Hb required to maintain adequate delivery–consumption balances are highly variable. The lack of correlation in our study could also be contributed by our PRBCT practice conforming to the liberal transfusion threshold. At a relatively higher Hb level, oxygen delivery-consumption balance is more easily maintained by compensatory measures of oxygen delivery, potentially resulting in a lack of correlation. Other confounders include factors affecting the Hb-Oxygen dissociation curve, most notably pH and the proportion of adult Hb. Our cohort's median (IQR) pH was within the normal range of 7.36 (7.34–7.37). We did not study the concentration of adult Hb in our population. The Median (IQR) number of the previous PRBCT in our cohort was 2 (0–5), indicating a variable proportion of adult Hb in our population.

Characteristics	
Gestation, weeks, Median (IQR)	26.4 (25.4 - 28.1)
Birth weight, grams, Median (IQR)	922 (655 - 1064)
Male gender, N. (%)	13 (44.8%)
Female gender, N. (%)	16 (55.2%)
Small for Gestation, N. (%)	6 (20.7%)
Enrolment characteristics	
Postmenstrual age, weeks, Median (IQR)	33.6 (31.7 - 34.9)
Postnatal age, days, Median (IQR)	42 (27 - 58)
Weight, Grams, Median (IQR)	1487 (1110 - 1785)
Breathing support, N. (%)	
None	5 (17.2%)
Continuous positive airway pressure	14 (48.3%)
High flow nasal cannula	8 (27.6%)
Low flow oxygen	2 (6.9%)
Caffeine, N. (%)	25 (86.2%)
Patent ductus arteriosus, N. (%)	0 (0%)
Pre transfusion Hb, (g/L), Median (IQR)	97 (87 - 100)
No (%) of babies who received previous PRBCT	20 (72)
Number of previous PRBCT, Median (IQR)	2 (0 - 5)
pH, Median (IQR)	7.36 (7.34 - 7.37)
PCO_2, Median (IQR)	48 (44 - 50)

***Table 1*: Main clinical characteristics of studied patients (*n* = 29).**
Legends:.
Hb: Haemoglobin; PRBCT: Packed red blood cell transfusion; IQR: Inter quartile range; pH: Potential of Hydrogen; PCO_2: Partial pressure of carbon dioxide.

Symptoms commonly attributed to anaemia include cardiorespiratory instability and poor weight gain.[23,24] Our study showed no significant difference between the symptomatic and asymptomatic groups in both FTOEc and FTOEs. To the best of our knowledge, no other similar studies evaluated the association of symptoms with FTOEc and FTOEs. As the research aimed to study the pre-transfusion symptoms perceived to be due to anaemia, data on post-transfusion symptoms were not evaluated. Symptomatic anaemia was managed by other supportive measures along with PRBCT, making it difficult to ascertain if improvement in symptoms was solely due to PRBCT. On the other hand, the persistence of symptoms after PRBCT may indicate that symptoms were unrelated to anaemia. This would be a diagnosis in retrospect, and would not have influenced the transfusion practice.

The strength of our study is the comprehensive evaluation of cerebral and peripheral tissue oxygenation with both the Hb and the symptoms of anaemia. There are three other studies[22,23,33] where both cerebral and splanchnic tissue oxygenation were simultaneously correlated with Hb. However, no study has comprehensively monitored cerebral and splanchnic tissue oxygenation with both Hb and symptoms. Unlike other studies,[23,32] we measured FTOE that reflects oxygen consumption–delivery balance. A further strength of this study is the continuous four hours monitoring at a high sampling rate of 1 Hz, unlike shorter duration/episodic monitoring ranging from 15 to 60 min.[21,23,32] Longer duration and high-frequency sampling are especially important to monitor splanchnic tissue oxygenation to nullify the effect of tissue artifacts.

We acknowledge several limitations of the study. The study involved stable premature babies, and as such, caution must be exercised in inferring similar findings in extremely premature sick infants, those with cardiorespiratory instability, term infants, or adults. There were no strictly adhered guidelines for PRBCT, but as shown in the study, the criteria commonly used (e.g., Hb and symptoms) are unreliable in reflecting tissue oxygenation levels. Our PRBCT practice conformed to a liberal transfusion threshold and may have contributed to a lack of correlation. Although the sample size was similar to many other studies,[21,32,33] our study was slightly underpowered based on the published literature.[21] A larger study would also allow investigation of the impact of other confounders such as previous transfusion frequencies, weight, gestation, breathing support, acid-base balance.

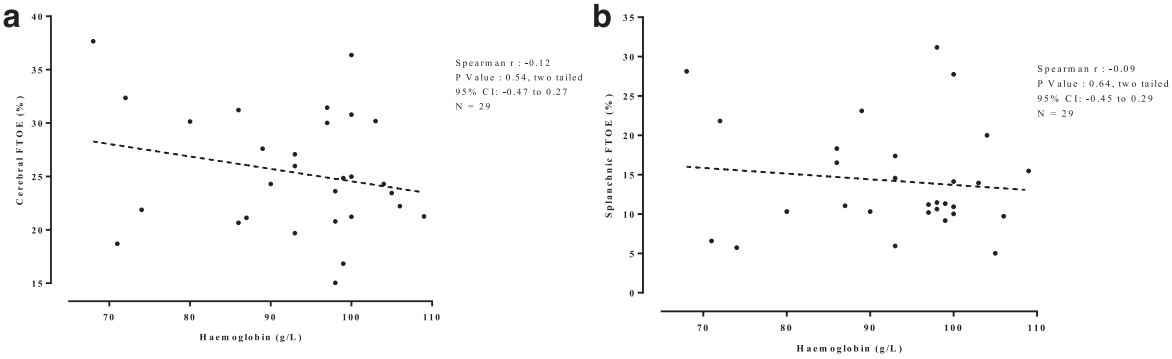

Figure 2. Correlation between cerebral (A) and splanchnic (B) FTOE and haemoglobin
FTOE: Fractional Tissue Oxygen Extraction; n: Number of patients; CI: Confidence interval; r: Correlation coefficient.

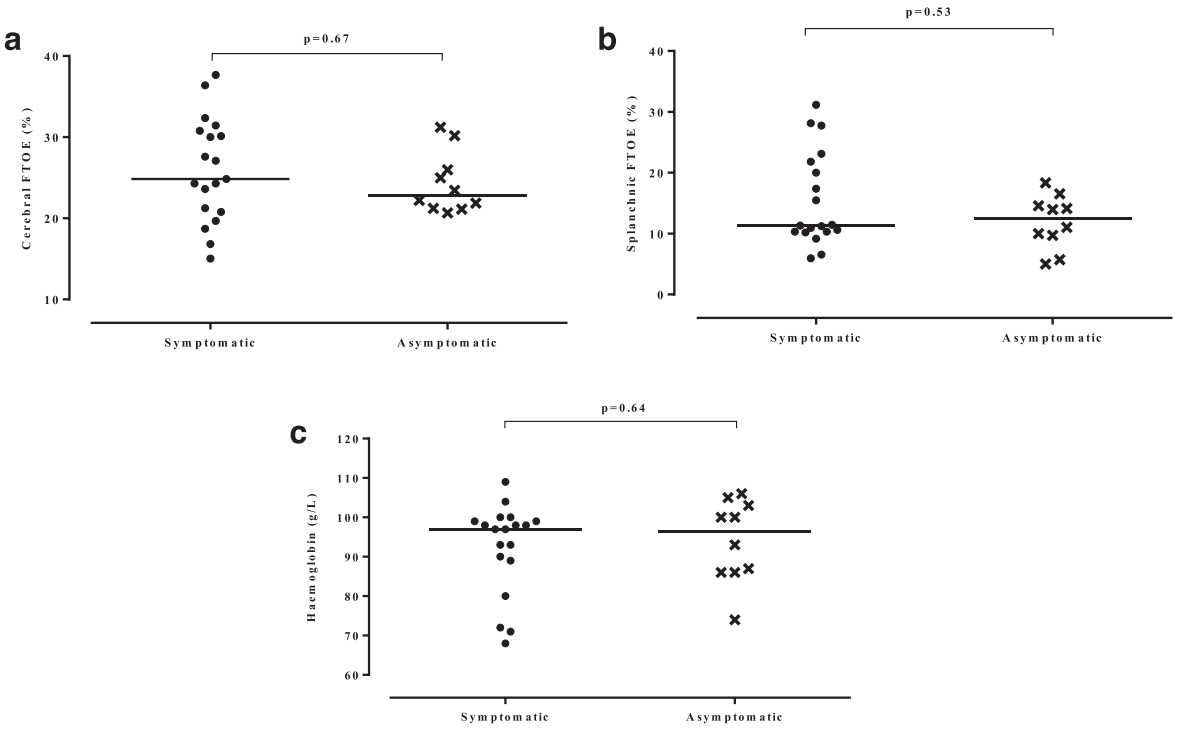

Figure 3. Comparison of cerebral (A) and splanchnic (B) FTOE and comparison of Haemoglobin (C) in infants with symptomatic versus asymptomatic anaemia

FTOE: Fractional Tissue Oxygen Extraction; Bars represent the median values.

The application of FTOE in routine clinical practice to determine PRBCT requirements is limited by the paucity of normative data that reflect the threshold for hypoxic damage. Currently, there are a limited number of small studies in preterm infants evaluating reference values of cerebral oxygenation[19,30,38–41] and splanchnic oxygenation.[26,42,43] Although splanchnic oxygenation may be affected earlier than cerebral oxygenation, the determination of robust normality data for splanchnic oxygenation is technically challenging. Available data shows large variability, likely because of variable segments of the intestine being interrogated by the NIRS probe as a result of peristalsis and false signals due to gas-fluid-faecal interfaces.[43–47] We have tried to ameliorate this variability by applying high frequency, longer duration, and continuous measurements of splanchnic oxygenation. Alternatively, a few investigators[48] have used muscle oxygenation to determine PRBCT requirement, although the normative data is again limited by sample size.[49] However, the gut has been recognized as a potential reserve for redistribution to the central nervous system, whereas the role of peripheral muscles in this physiological process remains unresolved. Bailey et al.[18] used splanchnic-cerebral oxygenation ratio as a marker of preterm infant blood transfusion needs, although normative values do not exit. Whitehead et al.[50] determined the Hb below which cerebral StO_2 critically decreases >2SD below the mean normative value (<55%) in preterm infants.

In summary, it is concerning that the conventional parameters used to determine the indication for PRBCT do not correlate with FTOEc and FTOEs. The findings must be seriously considered in the light of controversial outcomes of randomized controlled studies involving liberal versus restrictive Hb threshold for PRBCT. Our findings suggest that Hb threshold may not be the appropriate tool to perform such randomised studies. This brings up an important question of whether we can use FTOE as a guide to administering PRBCT. A recent systematic review[51] by our group indicated that regional oxygenation has a potential role to trigger PRBCT in anaemic preterm infants. However, there is a dearth of evidence that it can be used in its present form. Lack of normative values limits the usefulness of FTOE as the sole criteria to administer PRBCT. Our study warrants further investigations to establish normative data for FTOE for different gestation, weights, and postnatal age. This normative data could then be employed to create a composite algorithm including FTOEc and FTOEs along with low Hb and clinical features of anaemia to guide clinicians to determine the necessity for PRBCT.

Study type	Bailey et al.[23]	Sandal et al.[22]	Jain et al.[32]	Mintzer et al.[33]	Van Hoften et al.[21]
Nature of the study	Prospective observational cohort study	Case-control study	Prospective observational cohort study	Prospective observational cohort study	Prospective observational cohort study
Number of babies	30	23	30	27	33
Gestation (weeks)	Mean ± SD, 28.4 ± 3	Mean ± SD, 27.7 ± 1.8	Mean ± SD, 26.6 ± 2.03	Mean ± SD, 27 ± 2	Median, (Range), 27.3 (25−34)
Birth Weight (grams)	Mean ± SD, 1115 ± 426	Mean ± SD, 990 ± 285	Mean ± SD, 848 ± 270	Mean ± SD, 966 ± 181	Median, (Range) 1010, (605 - 2080)
Postmenstrual age at enrolment (weeks)	Mean ± SD 32.9 ± 3.4	Not available	Not available	Not available	Median (Range) 30.1 (25.9 - 39.0)
Weight at enrolment / transfusion(gram)	Mean ± SD 1415 ± 456	Mean ± SD 1416 ± 357	Mean ± SD 1008 ± 344	Not available	Not available
Postnatal age at enrolment (days)	Not available	Mean ± SD, 45 ± 14.3	Mean ± SD, 19 ± 12	first 10 days after birth	Median (Range), 17 days (1- 93)
PRBCT dose	15 mL/kg over 4 h	15 mL/kg over 2−4 h	15 mL/kg over 3 h	Did not involve correlation prior to PRBCT	15 mL/kg over 3 h
Hb (grams/dL) /Hct (%)	(Mean ± SD) Hb 9.3 ± 1.2	(Mean ± SD) Hb 8.7 ± 2.3	(Mean ± SD) Hb 9.8 ± 0.6	(Mean ± SD) Hct 39.7 ± 5.4%	Median (range) Hb 111 (60−128)
NIRS monitoring duration	20 min prior to PRBCT	10 −11 h prior to PRBCT	1 h prior to PRBCT	15 min prior to Hct determination	1 h prior to PRBCT
NIRS frequency	Every 30 s	Every one minute	Not available	Every 6 s	Not available
Correlation of Hb/Hct with cerebral NIRS	No significant correlation with StO_2 ($r = -0.01$, $r^2 = 0$, $n = 30$, $p = 0.98$, two tails)	Weak correlation with FTOE, ($r = -0.24$, $p = 0.041$).	No significant correlation with StO_2 ($R^2 = 0.018$. $p = 0.475$).	Moderate correlation with FTOE ($r = -0.527$, 95% CI (0.755 to −0.153) $p = 0.005$.	Significant correlation with StO_2: $r = 0.414$, $p = 0.004$. Significant correlation with FTOE: $r = -0.462$; $p = 0.001$
Correlation of Hb/Hct with splanchnic NIRS	No significant correlation with StO_2 ($r = -0.26$, $r^2 = 0.07$, $n = 30$, $p = 0.17$, two tails)	Weak correlation with FTOE ($r = -0.28$, $p = 0.045$)	Not available	Non-significant correlation with FTOE $r = -0.066$, 95% CI (- 0.433 to 0.122) p = not significant	Not available
Correlation between NIRS and symptoms	Not available	Not available	Not available	Not available	Not available

***Table 2*: Summary of studies demonstrating association of pre-transfusion Haemoglobin with regional tissue oxygenation.**
Legends.
Hb: Haemoglobin; Hct: Haematocrit; PRBCT: Packed Red Blood Cell Transfusion; NIRS: Near-infrared spectroscopy; FTOE: Fractional tissue oxygen extraction; StO_2: tissue oxygen saturation; SD: standard deviation; r: Correlation coefficient; CI: Confidence interval; g: Grams; kg: Kilo grams; mL: Millilitres.

Contributors

KKBV was responsible for conceptualisation, funding acquisition, investigation, methodology, project administration, resources, data acquisition, data interpretation, writing − original draft, and writing − review & editing, final approval of the version to be published, agreement to be accountable for all aspects of the work. GKKL was responsible for data analysis, revising the draft critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work. RKHN was responsible for conceptualisation, methodology, revising the draft critically for important intellectual content, final approval of the version to be published, agreement

to be accountable for all aspects of the work, overall supervision of the research. All authors have verified the underlying data. All authors confirm that they had full access to all the data in the study and accept responsibility for the decision to submit for publication.

Data sharing statement

Deidentified data generated and analysed during the current study, and the study protocol will be shared upon legitimate requests from the academic researchers for research purpose depending on the nature of the request, the merit of the proposed research, and the intended use. The usage proposal will be reviewed by the authors for the approval and will be made available with a signed data access agreement, by the corresponding author.

Declaration of interests

We declare no competing interests.

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