Early Human Development

Regional cerebral and splanchnic tissue oxygen saturation in preterm infants – longitudinal normative measurements

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	NIRS; regional oxygen saturation; preterm infants; NEC
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Abstract:	Background: To investigate regional splanchnic and cerebral tissue oxygen saturation in preterm infants <30 weeks gestation. Methods: Cerebral (cTOI) and splanchnic (sTOI) Tissue Oxygenation Index were measured weekly in 5 minute epochs for a total period of 60 minutes using NIRS (NIRO-300) for the first 8 weeks of life, in 48 appropriately grown preterm infants born at <30 weeks gestation. Infants who developed HPI and/or NEC (n=12) and those that died (n=1) were excluded from our main outcome measure of regional gut and cerebral tissue oxygenation in healthy preterm infants <30weeks gestation. Results: Median birthweight 789g (460-1486), gestational age 25 +6 weeks (23 +0 - 29 +1) and 51.4% female. 217 NIRS measurements were completed across the first 8 weeks of life. Mean weekly cTOI ranged from 56.8-65.4% and sTOI ranged from 36.7-46.0%. Mean cTOI was significantly higher than mean sTOI (p<0.001) throughout the first 8 weeks of life. Mean cTOI decreased significantly with increasing postnatal age [-0.59% each week (-1.26% to -0.07%) p=0.04]. None of the examined confounding factors had a significant effect. Conclusions : This is the first report of regional cerebral and splanchnic tissue oxygen saturation ranges during the first 8 weeks of life for preterm infants born at <30 weeks gestation.
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13/10/21

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Dr E Maalouf (Editor-in-Chief)

Cover letter for submission of Manuscript

I am enclosing herewith a manuscript entitled "Regional cerebral and splanchnic tissue oxygen saturation in preterm infants – longitudinal normative measurements" for your consideration please. With increasing interest in the use of Near Infrared Spectroscopy (NIRS) in evaluating neonatal regional saturation and its potential clinical applications we feel this would be of interest to you and your readers.

The Corresponding author of this manuscript is Dr Claire Howarth, and the other authors are Dr Terence Leung, Dr Jayanta Banerjee, Professor Simon Eaton, Professor Joan Morris and Professor Narendra Aladangady as listed in the online manuscript submission.

With the submission of this manuscript, I would like to confirm that:

- All authors of this manuscript have contributed equally to the final version
- All authors of this manuscript have read and approved the final version submitted
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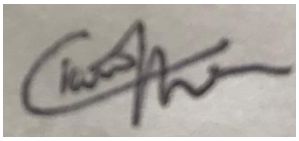
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- My Institute's (Homerton University Hospital NHS Foundation Trust) representative is fully aware of this submission
- None of the authors have any potential conflicts of interest
- This study was funded by the Barts Health Charity (Grant reference number MGU0388)
- The authors have no financial relationships relevant to this article to disclose. Dr Howarth was employed through the grant but none of the other authors had financial relationship with the charity or the fund. Dr Eaton acknowledges support from NIHR Biomedical Research Centre at Great Ormond Street Hospital and UCL Great Ormond Street Institute of Child Health.

Many thanks for taking the time to consider our work for publication.

Yours sincerely



Dr Claire Howarth

(Signature of corresponding author on behalf of all authors, author statement page submitted online with all the other signatures)

Highlights

- This is the first report of weekly measurements for both cerebral ***and*** splanchnic regional tissue oxygen saturation over the first 8 weeks of postnatal age for preterm infants <30 weeks gestation.
- Previous studies have examined more mature preterm infants and focused on early oxygenation measurements only
- Cerebral tissue oxygen saturation is higher than splanchnic throughout, and decreases over, the first 8 weeks.
- Awareness of this increases the clinical potential of NIRS to alert to deviations from the norm
- This potentially allows earlier identification of important neonatal conditions including NEC

Regional cerebral and splanchnic tissue oxygen saturation in preterm infants – longitudinal normative measurements

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Author contributions: Professor Aladangady jointly conceptualised the study, developed study design and consented parents, as well as reviewed and revised the manuscript. Dr Banerjee jointly conceptualised the study, jointly developed the study design and reviewed and revised the manuscript. Dr Howarth jointly developed the study design, consented parents, conducted all the measurements, data collection and wrote the first draft and reviewed and revised the manuscript. Professor Morris planned, supervised, and helped in executing the statistical analysis, as well as reviewed and revised the manuscript. Dr Leung helped with the NIRS analysis and reviewed and revised the manuscript. Dr Eaton jointly

developed the study design, reviewed, and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Statement of financial support: This study was funded by the Barts Health Charity (Grant reference number MGU0388).

Disclosure statement: The authors have no financial relationships relevant to this article to disclose. Dr Howarth was employed through the grant but none of the other authors had financial relationship with the charity or the fund. Dr Eaton acknowledges support from NIHR Biomedical Research Centre at Great Ormond Street Hospital and UCL Great Ormond Street Institute of Child Health.

Category of study: Clinical research article

Conflict of Interest: The authors have no conflicts of interest to disclose.

Ethical approval: The study received research ethics (HRA) approval (Ref number 16/LO/1353).

Consent Statement: Formal written consent was taken from parents for all the infants involved in this study.

Manuscript (without Author Details) and references Click here to view linked References

Regional cerebral and splanchnic tissue oxygen saturation in preterm infants – longitudinal normative measurements

Abstract

Background: To investigate regional splanchnic and cerebral tissue oxygen saturation in preterm infants <30 weeks gestation.

Methods: Cerebral (cTOI) and splanchnic (sTOI) Tissue Oxygenation Index were measured weekly in 5 minute epochs for a total period of 60 minutes using NIRS (NIRO-300) for the first 8 weeks of life, in 48 appropriately grown preterm infants born at <30 weeks gestation. Infants who developed HPI and/or NEC (n=12) and those that died (n=1) were excluded from our main outcome measure of regional gut and cerebral tissue oxygenation in healthy preterm infants <30weeks gestation.

Results: Median birthweight 789g (460-1486), gestational age 25^{+6} weeks (23^{+0}-29^{+1}) and 51.4% female. 217 NIRS measurements were completed across the first 8 weeks of life. Mean weekly cTOI ranged from 56.8-65.4% and sTOI ranged from 36.7-46.0%. Mean cTOI was significantly higher than mean sTOI ($p<0.001$) throughout the first 8 weeks of life. Mean cTOI decreased significantly with increasing postnatal age [-0.59% each week (-1.26% to -0.07%) p=0.04]. None of the examined confounding factors had a significant effect.

Conclusions: This is the first report of regional cerebral and splanchnic tissue oxygen saturation ranges during the first 8 weeks of life for preterm infants born at <30 weeks gestation.

Introduction

Near Infrared Spectroscopy (NIRS) non-invasively measures regional tissue oxygen saturation at the bedside. Cerebral NIRS monitoring is a validated[1-3] and extensively studied tool as a measure of cerebral regional tissue oxygen saturation ($cRSO_2$). Splanchnic measurements are less well studied, predominately focusing on exploring the impact of bolus feeds, blood transfusions or necrotizing enterocolitis (NEC) on splanchnic regional tissue oxygenation, rather than establishing reference ranges[4].

Previous studies reporting reference ranges[5-10] (table 1) have only focused on early oxygenation measurements during birth, the acute transitional period, or over the first postnatal month. They have generally involved fewer infants and more mature preterm infants than those in our study. Additionally, the reported ranges dependent on the device used and population studied, and there are also inherent difficulties with repeatability due to probe placement[11]. The measurement accuracy of splanchnic regional tissue oxygenation saturation is affected by the amount of gaseous distension and faecal matter present in the abdomen and in very small preterm infants there is concern regarding whether it truly reflects splanchnic regional tissue oxygen saturation, or also stomach and liver[12,13].

The clinical use of NIRS in preterm infants is limited by this lack of normative data; in this study we sought to determine normal ranges of cerebral and splanchnic regional tissue oxygenation in preterm infants <30 weeks gestation for the first 8 weeks of life.

Methods

We performed a prospective observational study in appropriately grown preterm infants born at <30 weeks of gestational age (GA) admitted to a tertiary NICU at Homerton Hospital from October 2016 to May 2018. Eligible infants were recruited by day 7 of life and measurements performed weekly for the first 8 weeks of life, or until discharge to either home or their local hospital. The lowest age at enrolment for the first week's measurement was day 4 of life (i.e., after the first 72 hours of life thereby reducing the effect of the well-known cardiovascular transitions seen after birth). After the first recording the subsequent weekly follow up measurements were all performed at 7 day intervals. Infants with fetal growth restriction (birthweight $\leq 2^{nd}$ centile and abnormal antenatal Dopplers), major congenital anomalies and twin-to-twin transfusion syndrome were excluded.

Weekly cerebral (cTOI) and splanchnic (sTOI) tissue oxygenation indices were measured using a NIRS monitor (NIRO-300, Hamamatsu KK, Japan) along with concurrent measurement of post ductal peripheral arterial oxygen saturations using a pulse oximeter. The NIRO-300 machine allows simultaneous measurement of cerebral and splanchnic regional tissue oxygen saturation using a single device. The NIRS emitter and detector probes were placed simultaneously on the infant's forehead and abdomen for 60 minutes once a week by the same investigator (CH) to ensure uniform placement. The emitter and detector probes were placed in a protective rubber seal to ensure that the distance between them remained constant at 4cm for each infant thereby reducing the effect of sensor positioning on the NIRS readings. For measurement of cTOI the centre of this seal was placed in the centre of the forehead so that the emitter and detector probes were either side of the midline, and for sTOI placed in the hypogastric region, just below the umbilicus.

All infants were on bolus feeds and NIRS measurements were started at the onset of a feed for every infant. For each infant, the most recent pCO_2 was checked before starting any NIRS measurement as pCO_2 levels could impact cerebral perfusion; all infants had pCO_2 of between 4-8KPa. In addition, prior to commencing the NIRS measurements the device was calibrated; there were no infants where we were unable to obtain a reliable signal although there was one recruited infant in week one of life who we could not measure NIRS for week one as they did not tolerate the handling for positioning of the probes.

At our neonatal unit, all preterm infants have a targeted oxygen saturation range of 90-95% and regardless of whether they were breathing independently in air or ventilated needing supplementary oxygen, their supplementary oxygen was titrated to meet these saturation targets.

Antenatal and perinatal maternal and infant characteristics and weekly volume of enteral feeds, presence of patent ductus arteriosus (PDA), NEC (defined as ≥ Bell's stage 2), presence of Intraventricular Haemorrhage (IVH) or Haemorrhagic Parenchymal Infarct (HPI) and Haemoglobin (Hb) level at the time of the NIRS recording were documented.

Using Matlab R2019b software (Mathworks, Natick, Massachussets, US), raw NIRS data were extracted. Each NIRS reading was individually assessed for artefact; analysed in 5 minute epochs and noisy epochs (representing artefact) were removed. For each 60 minute recording, we produced a mean cTOI and sTOI and then cerebral (cFTOE) and splanchnic (sFTOE) fractional tissue oxygen extraction were calculated using the equation [SaO_2 –

TOI]/SaO_2[14]. The CSOR (cerebral splanchnic oxygenation ratio = sTOI/cTOI) was also calculated.

The study was approved by the London-Central Research Ethics Committee (Reference: 16/LO/1353) and informed written parental consent was obtained.

Statistics

Statistical analyses were performed using STATA/SE version 15.1 (STATA Corp LLC, Texas, US). cTOI and sTOI had Gaussian distributions. We used multi-level mixed effects linear regression models, nested within each baby because readings were taken over time and hence correlated within each baby. NIRS measurements were adjusted for confounding variables (PDA, gender, ethnicity, volume of enteral feeding, haemoglobin, and gestational age), which were included in the models as fixed effects. Enteral feeds and haemoglobin were analysed in categories at the closest time point to the NIRS measurement: Enteral Feed: 4 categories - 0 (group 1); 1-29ml/kg/day (group 2); 30-59 ml/kg/day (group 3) and ≥60ml/kg/day (group 4); Hb: 3 categories - <8g/dl (group 1); 8-11.9g/dl (group 2) and ≥12g/dl (group 3).

Results

Infant characteristics

We recruited 48 preterm infants born at <30 weeks GA to the study. To establish mean regional tissue oxygen saturation values for "healthy" infants, we excluded infants with HPI (n=5), due to the impact of venous stasis and damage to white matter on cTOI readings, and NEC (n=7), as there are reported differences in both cTOI and sTOI in infants with NEC compared to those infants without NEC[5,15-23]. One infant died the day after recruitment (26^{+3}, BW 590g) and did not have any NIRS measurements taken. This gave a total of 35 preterm infants <30 weeks GA and 217 NIRS measurements over the 8 week period. Infant characteristics and their relevant antenatal details are presented in table 2.

NIRS measurements

Figures 1 and 2, and Table 3 illustrate the mean cTOI, sTOI, cFTOE, sFTOE and CSOR per week for the remaining cohort of 35 infants over the first 8 weeks of life. Mean cTOI ranged from 56.8% to 65.4% and mean sTOI from 36.7% to 46.0%. Within each baby the mean cTOI was significantly higher than mean sTOI for each week in the first 8 weeks of life ($p<0.001$) with an average mean difference over the 8 weeks of 23%. The variability was much higher for splanchnic readings, although variability decreased over time.

Cerebral TOI significantly decreased by -0.59% each week with increasing postnatal age [(-1.26% to -0.07%) p = 0.04] and sTOI had no significant weekly change +0.56% [(-0.38% to 1.49%) p = 0.242]. Mean cFTOE ranged from 0.31 to 0.39 and mean sFTOE ranged from 0.49 to 0.59. Overall cFTOE (sd 0.11) had less variation than sFTOE (sd 0.17). Mean cFTOE was on average 0.26 lower than sFTOE and cFTOE significantly increased by +0.01 each week with postnatal age; [(0.001-0.02), p = 0.024] but sFTOE had no significant weekly

change [-0.01 (-0.02 to 0.04), p = 0.28]. CSOR varied from 0.58 to 0.77, was lowest in the first 2 weeks of life (0.63 and 0.58 respectively) and highest in week 4 (0.77).

Effects of confounders

Potential confounding factors including GA, ethnicity, birthweight, gender, Hb, enteral feed volume (ml/kg/day at the time of the NIRS measurement) and presence of PDA had no significant effect on NIRS measurements (all p values >0.05), except that Asian ethnicity infants had significantly higher sTOI (p=0.03) and consequently significantly lower sFTOE than white infants (p=0.01).

Discussion

We have reported mean values of cerebral and splanchnic tissue oxygen saturation for the first two months of postnatal age in appropriately grown preterm infants born at <30 weeks GA. We have shown that cerebral tissue oxygen saturation remains significantly higher than splanchnic tissue oxygen saturation throughout this period and that cerebral tissue oxygen saturation decreases significantly over time.

The cTOI range of 56.8% to 65.4% we report is in keeping with McNeill *et al*[6], albeit their study cohort had an older gestation (29 to 34 weeks), involved smaller numbers (n=12) and only reported values for the first 21 postnatal days. Similar to our study, McNeill[6] reported that regional cerebral oxygenation decreased over time using INVOS 5100 and our group[3] also previously demonstrated that with increasing postnatal age, the baseline pre-transfusion

cTOI decreases. This decrease may reflect the known nadir in haemoglobin[24,25] changes in cardiac output[10] or reflect brain growth and a resultant increase in cerebral oxygen consumption.

Although infants with HPI were excluded, infants with IVH were included; in keeping with the GA of the study population 48.6% (n=17) had some degree of IVH. Sorensen found a significant negative correlation between severity of IVH and $cRSO_2$[26]. In infants with a birthweight of <1500g the incidence of IVH is around 27 %[27]; as IVH is a common complication of prematurity and increasingly common in more immature infants, our results are representative of what would be considered normal.

Overall, cerebral tissue oxygen saturation in preterm infants has been reported as approximately 70%[10,28]. This is higher than our results, but this approximation includes all preterm infants up to 36^{+6} weeks GA, different NIRS devices, and predominantly only involves measurements in the first week of life. The repeated NIRS measures within subject standard deviation is 5-6%[11] and there is a methodological bias between sensors from INVOS-5100 and NIRO-300[29]. Schneider et al[30] looked at 4 NIRS devices and reported that the cerebral tissue oxygenation data yielded by different NIRS devices differed significantly from each other, ranging from a minimum difference of 2.93% to a maximum difference of 12.66%. Because authors report studies using different NIRS devices the emphasis has been on using NIRS to report trends[30] rather than absolute values. Our findings are therefore important as we are the first study to report normative values for preterm infants <30 weeks using the NIRO 300 device which is a commonly used device. The most used other device in the literature is the INVOS 5100; although the devices are different, both rSO_2 (INVOS 5100)

and TOI (NIRO 300) aim to measure the same biomarker. Thavasothy et al compared the INVOS 5100 to the NIRO 300 and reported that the INVOS 5100 under- reads cerebral oxygenation compared to the NIRO 300, with limits of agreement of ±14.7% [31].

Previous studies report that splanchnic tissue oxygen saturation is lower than cerebral, which is consistent with our study[32]. However mean sTOI in our study ranged from 36.7% to 46.0%, which is lower than previously reported, albeit in an older cohort of 29 to 34 weeks gestational age preterm infants[6]. This may represent either lower tissue perfusion or higher oxygen consumption; the preterm infant has higher metabolic demands and heart and brain perfusion would be prioritised over the gut in times of reduced oxygenation or hypotension. In addition, we examined splanchnic tissue oxygen saturation over the first few weeks of life, in more immature infants with a median GA of 25^{+6} weeks, rather than the first few days of life, in contrast to most published studies.

Our study reported that sTOI appeared to increase with increasing postnatal age but was not significant. This may be due to compensatory mechanisms occurring during vascular development of the splanchnic bed with maturation[33]. Previous authors have found associations between sTOI and postnatal age, but they have only been examining the first couple of weeks of life and with a different patient group of more mature preterm infants. McNeill *et al*[6] demonstrated that median splanchnic rSO_2 decreased over the first week and then started to increase. Cortez *et al*[5] showed similar findings with a decrease in splanchnic rSO_2 for the first 9 days then an increase from day 10 to 14 in infants <30 weeks GA. More recently van der Heide et al in their study of 220 infants <32 weeks GA, where they reported a daily 2 hour mean for the first week of life, (although due to missing data only 50 infants

had measurements on all 7 days), also reported a similar trend with regional splanchnic oxygenation decreasing with increasing postnatal age until day 4 after birth, and then increasing until day 7 after birth.

There are inherent difficulties in measuring splanchnic rSO_2 due to the varying bowel faecal and air content and its impact on NIRS measurements, which could account for the differences in reported splanchnic rSO_2. Variability was higher for splanchnic compared with cerebral and decreased over time which is expected given the maturation of the gut[33]. Studies have previously demonstrated high baseline abdominal variability[6,13]; in McNeill's study this exceeded 20% and they commented this could be associated with caregiving events or regional developmental maturation[6].

Asian ethnicity infants had significantly higher sTOI and consequently lower sFTOE than white infants. There are limited reports regarding the effect of ethnicity on NIRS readings although there are concerns that skin pigmentation seen in Afro-Caribbean ethnicity might affect NIRS values[34]. This observation may be a chance finding due to the number of variables tested in the multi-regression model rather than a true clinical significance. Importantly we found no effect of PDA or increasing feed volumes on sTOI.

FTOE reflects the balance between tissue oxygen supply and consumption and a high FTOE has been proposed as an early indicator of impaired tissue perfusion or ischaemia[7]. We observed the highest sFTOE in the first 3 weeks of life when the preterm infant's haemodynamics and physiology are most vulnerable and transitioning to extra-uterine life,

and it is also when enteral feeds are started. The CSOR reflects the relative cerebral and gut oxygenation; this varied from 0.58 to 0.77. Fortune *et al*[35] reported that a CSOR of <0.75 was highly predictive of intestinal ischaemia. In our study the CSOR was <0.75 for most of the time, suggesting preterm infants are at high risk of gut injury throughout the first 2 months of life.

Potential clinical applications

Although there is increasing interest in the use of splanchnic NIRS[4] the high variability limits its clinical use. Schat et al[21] found that splanchnic NIRS measurements were not helpful for the prediction and diagnosis of NEC in preterm infants, but this study involved only small numbers and another larger study suggested it could be used to predict NEC onset[15]. The validity of NIRS-derived $sRSO_2$ measurements for looking at splanchnic perfusion and oxygenation has been shown by strong correlation between $sRSO_2$ and gastric pH, serum lactate and systemic mixed venous saturation[36] and a recent small study by Gillam-Krakauer *et al*[37] using Doppler ultrasound scan confirmed the positive correlation between splanchnic NIRS and blood flow to the small intestine.

With regards to cerebral oxygenation there are more favourable results in terms of clinical use in preterm infants. In the same study by Schat et al[21] they found that cerebral oxygenation <71% in the first 8 hours after symptom onset could predict complicated NEC with sensitivity of 1 and specificity of 0.8, and in another study they reported that infants with a cerebral oxygenation <70% within first 48 hours after birth developed NEC significantly more often[22]. Our group, from the original cohort of infants in this study have recently

demonstrated that infants who developed NEC had significantly lower cTOI throughout their neonatal intensive care stay ($p = 0.011$)[23]. This finding suggests an underlying mechanistic relation between NEC and their worse neurodevelopmental outcome, therefore continuous NIRS monitoring would help identify those infants with lower cTOI who are at greater risk. In addition, previous authors have examined the use of NIRS to minimise fluctuations in cerebral perfusion[38] but there is inconsistent evidence regarding the significance of NIRS measurements and the development of IVH[26,39]. However, it would seem plausible to surmise that NIRS could non-invasively detect regional perfusion changes which are known to contribute to the development of IVH. Clearly further research is needed to establish the true relationship between NIRS and cerebral injury, therefore it is important to identify the normative values and trends in relation to cerebral tissue oxygen saturation in preterm infants.

There is a definite lack of awareness of normative values for splanchnic and cerebral regional tissue oxygen saturation levels at various weeks of life in preterm infants <30 weeks GA, as described in a recent review article summarising the clinical applicability of NIRS in preterm infants[40]. If NIRS is used as a routine clinical tool to measure tissue oxygenation, our results could potentially help with identifying deviations from the norm, alerting clinicians prior to symptom onset that there has been a change in regional oxygen saturation levels, or that there is cause for concern if they drop significantly below the norm. Of course, the clinical applicability of our study results is restricted to being used for comparison with NIRS measurements from the same NIRO-300 machine and therefore much larger prospective studies are needed using all the commonly used NIRS devices to help increase the clinical applicability of NIRS, particularly in predicting illnesses such as IVH and NEC.

Strengths of the study

Previous studies have only focused on early oxygenation measurements after birth during the acute transitional period, or over the first postnatal month. The numbers we report at each week of life is higher than most previous studies, we have measured more preterm infants of <30 weeks gestational age and we are the first to report weekly measurements for both cerebral and splanchnic regional tissue oxygen saturation over the first 8 weeks of postnatal age.

We completed 217 NIRS regional tissue oxygen saturation measurements in these infants demonstrating the feasibility of NIRS in the neonatal population. We successfully measured weekly sTOI in all recruited infants except one measurement in week one.

To increase the applicability of our data infants with possible abnormal cerebral and/or splanchnic oxygenation were excluded to establish most accurate normative regional oxygenation. CH was present during the entire measurement and if any movement artefact developed this was addressed immediately. Consistency in NIRS probe placement and fixation was maintained by having the same investigator (CH) performing all the measurements. In most NIRS studies around 10% of infants are excluded because of motion artefacts[3,41] but we excluded no infant because of motion artefact.

Complex multilevel modelling was used to investigate the effect of confounders and consider that in any study involving NIRS, each infant has several readings over the course of the study and that readings within infants are likely to be correlated.

Limitations of the study

We only conducted weekly cerebral and splanchnic NIRS measurements rather than daily or more frequently. However, other NIRS studies have used a similar approach and we remain the only study to have examined cTOI and sTOI in preterm infants for this length of time. There remains the inherent difficulties in measuring splanchnic oxygenation as discussed, but a recent systematic review would suggest that NIRS could be a useful additional bedside tool on the neonatal unit[4].

We also did not assess the repeatability of our measurements, but Menke et al[42] in their study where two observers repeated a total of 500 NIRS measurements in 25 neonates that inter-patient variance contributed most to the total variance, while the interobserver variance had the smallest effect. They demonstrated that $cRSO_2$ showed a good reproducibility, with an inter-measurement variance slightly but not significantly higher than the physiological baseline variation.

Although compared to most other published studies we have investigated a higher number of infants, we acknowledge that some were discharged home or transferred to their local hospital prior to 8 weeks of postnatal age, therefore, not all the 35 infants completed every weekly measurement for the first 8 weeks of life.

Conclusion

Cerebral tissue oxygen saturation is higher than splanchnic tissue oxygen saturation over the first 8 weeks of postnatal age, and decreases significantly over this time, in preterm infants <30 weeks GA, and we report values for each week of life using the NIRO-300 device. The long-term morbidities associated with prematurity are significant and can have devastating consequences. Many of these diseases, such as NEC and IVH, have been attributed to abnormalities of tissue oxygenation and perfusion, which NIRS could help identify. An awareness of normal values, such as those that we present, increases the clinical potential of NIRS on neonatal units to alert clinicians to deviations from the norm, potentially allowing identification of important neonatal conditions such as cerebral injury, sepsis, or NEC earlier.

Acknowledgements

With thanks to all the medical and nursing staff at Homerton University Hospital Neonatal Unit and to all the parents who consented for their infants to be involved in this study.

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Figures/tables (legends only)

Table 1: Summary of the previous studies looking at normal ranges of regional tissue oxygen saturation in preterm infants using NIRS.

Table 2: Infant characteristics and their relevant antenatal details (n=35).

Table 3: Mean cTOI, sTOI, cFTOE, sFTOE and CSOR per week for preterm infants <30 weeks GA over the first 8 weeks of life.

Figure 1: Cerebral and splanchnic tissue oxygenation index (TOI) for the study group across the first 8 weeks of life. For each week the median, mean (marked as X) and range of cTOI (black boxes) and sTOI (grey boxes) is presented.

Figure 2: Cerebral and splanchnic fractional tissue oxygen extraction (FTOE) for the study group across the first 8 weeks of life. For each week the median, mean (marked as X) and range of cFTOE (black boxes) and sFTOE (grey boxes) is presented.

Figure 1

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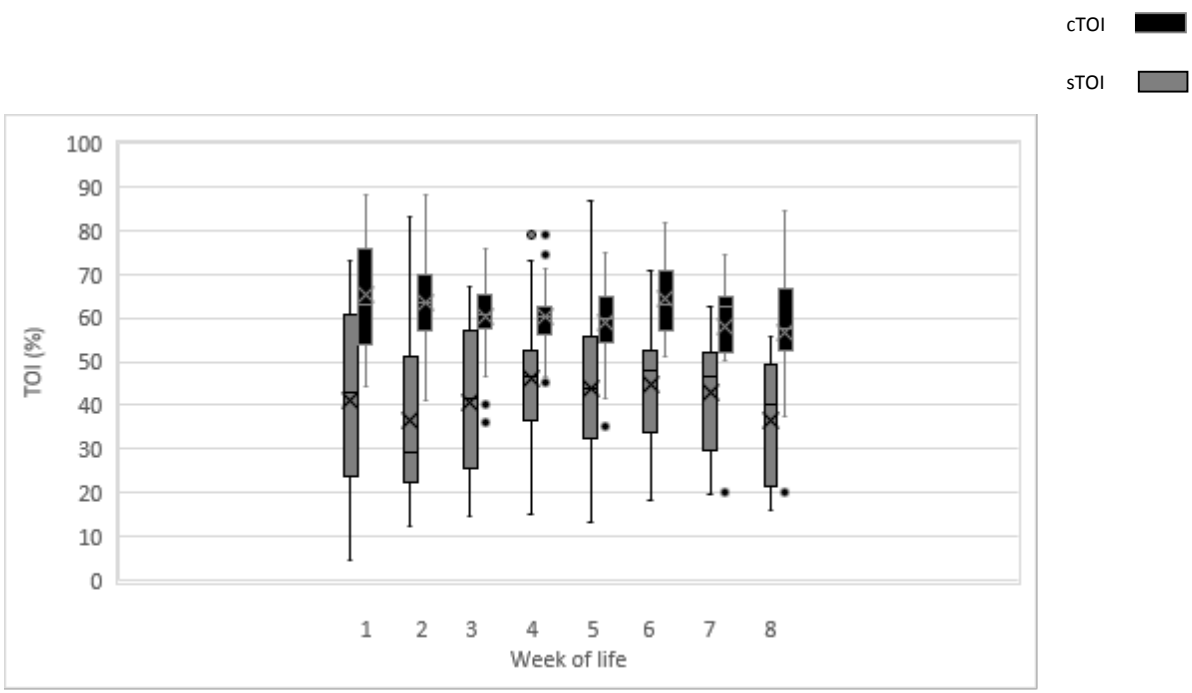


Figure 2

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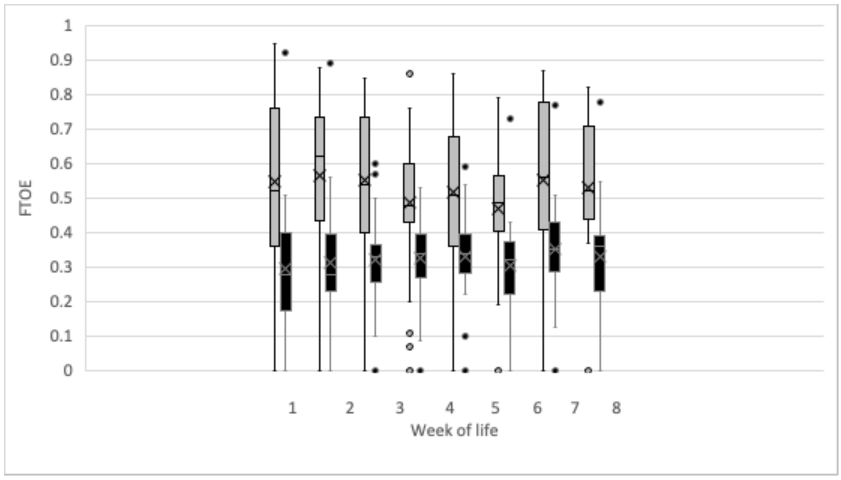


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Table 1: Summary of the previous studies looking at normal ranges of regional tissue oxygen saturation in preterm infants using NIRS.

Author and year	Population studied and duration	Study design	Primary outcomes/findings
Chock et al[43]	-n =103 -very preterm infants with BW <1250 g -mean GA 26w -duration: up to 96 hours of postnatal age	-prospective, multicentre study (6 tertiary NICUs) -mean arterial blood pressure (BP) and cerebral oxygen saturation (Csat) were continuously monitored using a neonatal sensor -percentiles of correlation between Csat and mean BP were compared between infants with and without the adverse outcome of mortality or severe neuroradiographic abnormalities by early cranial ultrasound	-infants with adverse outcomes had a lower mean Csat (67 ± 9%) compared with those without adverse outcomes (72 ± 7%; p=0.02) -Csat of <50% was identified as a cut-point for identifying infants with adverse outcome (area under the curve, 0.76) -infants with adverse outcomes were more likely to have significant positive or negative correlations between Csat and mean arterial blood pressure, (p =0.006)
Cortez et al[5] (2011)	-n=21 -preterm infants <30w GA	-prospective observational cohort study -infants enrolled within 48 hours of birth -continuous NIRS probe placed on left paraumbilical region	-2 infants were excluded because of skin breakdown and missing data -demonstrated feasibility of using NIRS continuously for 14 days

	-duration: up to 14 days of postnatal age		-daily mean rSO_2 values decreased over the first 9 days ($p<0.0001$) followed by an increase from days 10–14 ($p=0.0061$) -infants with feed intolerance had lower splanchnic regional saturations as compared with those tolerating feeds($p=0.0043$)
McNeill et al[6] (2011)	-n=14 enrolled but 2 excluded from analysis meaning n=12 infants' data used for analysis -healthy preterm infants (29-34w GA) -duration: up to 21 days of postnatal age	-continuous monitoring of abdominal, cerebral and renal regional oxygenation using NIRS -six infants were between 29 and 30 weeks gestation and 6 were between 32 and 33 weeks gestation. Some of the analysis was done in these subgroups	-abdominal regional oxygenation (32-66%) was lower than cerebral (66-83%) and renal (64-87%) -cerebral and renal oxygenation decreased significantly over the first weeks of life ($p<0.01$) -abdominal oxygenation decreased over week 1 of life and then increased up to day 21. The median nadir was at day 7 (range 3–9) for those born at 29–30 weeks and day 4.5 (range 3–8) for those born at 32–33w GA -regional oxygenation variability was lowest for cerebral measurements and highest at the abdomen -abdominal variability decreased significantly over time ($p\leq0.05$)
Naulaers et al[7] (2002)	-n=15 -preterm infants with median GA 28w	-prospective observational cohort to look at normal ranges of cerebral oxygenation index (cTOI) using NIRS	-median cTOI was 57% (95% CI 54 to 65.7%) on day 1, 66.1% (95% CI 61.982.2%) on day 2, and 76.1% (95% CI 67.8 to 80.1%) on day 3 -cTOI increased significantly in the first 3 days of life $p < 0.05$

	-duration: up to 3 days of postnatal age	-used NIRO 300 NIRS device -30 minute samples	
Pichler et al[8] (2013)	-n=381 newborn infants -82 term infants after vaginal delivery -272 term infants after c-section delivery -27 preterm infants after c-section delivery -duration: up to first 15 minutes after birth	-prospective observational study -cerebral oxygenation ($crSO_2$) was measured using NIRS (INVOS 3100 device) for term and preterm infants -cFTOE subsequently calculated	-for the whole group median (10th-90th percentiles) $crSO_2$ was 41% (23-64) at 2 minutes, 68% (45-85) at 5 minutes, 79% (65-90) at 10 minutes, and 77% (63-89) at 15 minutes of age -for the whole group median (10th-90th percentiles) cFTOE was 33% (11-70) at 2 minutes, 21% (6-45) at 5 minutes, 15% (5-31) at 10 minutes, and 18% (7-34) at 15 minutes of age -no significant difference in $crSO_2$ between term and preterm infants
Roche-Labarbe et al[9] (2010)	-n=11 -preterm infants (28–34w GA)	-prospective observational cohort study -recorded quantitative frequency domain near infrared spectroscopy (FD- NIRS) measures of	-individual traces of StO_2 showed a consistent decrease during the first 6 weeks of life

	-duration: up to 6 weeks of postnatal age	cerebral tissue oxygenation (StO_2) and cerebral blood volume (CBV) with diffusion correlation spectroscopy (DCS) measures of a cerebral blood flow index (CBF_{ix}) -recorded once a week -in 9 patients, cerebral blood velocities from the middle cerebral artery were collected by transcranial Doppler (TCD) and compared with DCS values	-average StO_2 significantly decreases from 73 ± 4% to 57 ± 4% during this period (21% decrease, $R^2 = 0.98$, $p < 0.001$) -StO_2 seemed higher in neonates born after 32w GA compared with neonates born before 31w GA, for the first four weeks of life (but no statistics as too small sample size)
Takami et al[10] (2010)	-n=16 -ELBW infants -duration: up to first 72 hours of life	-prospective observational cohort study -measured cTOI using NIRS at 3 hours of life followed by samples every 6 hours up to 72 hours of life -cFTOE subsequently calculated -using echocardiography left ventricular end-systolic wall stress (ESWS), left ventricular ejection fraction (LVEF), left ventricular cardiac	-cTOI decreased until 12 hours of life and then an increased, which correlated with similar changes in SVC flow -cFTOE increased until 12hours of life and then decrease

		output (LVCO), and superior vena cava (SVC) flow were also measured simultaneously	
van der Heide[44] (2021)	-n=220 -<32w GA and/or BW <1200g -excluded infants with NEC, sepsis or who died -duration: up to first 7 days of postnatal age	-prospective cohort study -for the first week after birth they measured a daily 2 hour mean of regional splanchnic oxygen saturation (r_sSO_2) to assess its associations with sex, GA, postnatal age (PNA), small-for-gestational age (SGA) status, patent ductus arteriosus, haemoglobin, nutrition, and head circumference at birth -they then used this to create a prediction model	-note due to missing data only 50 infants had measurements on all 7 days on day 1, the mean ± SD r_sSO_2 value was 48.2% ± 16.6 - reported that r_sSO_2 is lower in infants with a lower GA and those who are SGA -r_sSO_2 decreases with increasing postnatal age until day 4 after birth and then increases until day 7 after birth -created a model of the reference values of r_sSO_2 which was: $r_sSO_2 = 3.2 - 7.0 \times PNA + 0.8 \times PNA^2 - 4.0 \times SGA + 1.8 \times GA.$

Table 2: Infant characteristics and their relevant antenatal details (n=35)

Characteristic	Studied infants (n = 35)
Birthweight (g) [median (range)]	789 (460 to 1486)
Gestational age (wks) [median (range)]	25^{+6} (23^{+0} to 29^{+1})
Gender [number (%)]	
Male	17 (48.6)
Female	18 (51.4)
Ethnicity [number (%)]	
White (white British /white other)	22 (62.9)
Black (Afro Caribbean/ Black other)	7 (20.0)
Asian (Indian Asian/Pakistani /Asian other)	6 (17.1)
Singletons [number (%)]	29 (82.9)
Twins [number (%)]	6 (17.1)
Antenatal steroids [number (%)]	
Complete course	22 (62.9)

Incomplete course	10 (28.5)
No steroids	3 (8.6)
Antenatal Magnesium Sulphate	28 (80)
Placental histology	
Chorioamnionitis	26 (60)
Normal	11 (31.4)
Unknown	3 (8.6)

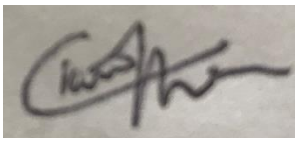
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Table 3: Mean cTOI, sTOI, cFTOE, sFTOE and CSOR per week for preterm infants <30 weeks GA over the first 8 weeks of life

	Week of life							
NIRS measurements	**1 (n=35)**	**2 (n=34)**	**3 (n=33)**	**4 (n=31)**	**5 (n=26)**	**6 (n=19)**	**7 (n=19)**	**8 (n=17)**
cTOI % (sd)	65.4 (12.9)	63.5 (11.0)	60.5 (8.9)	60.1 (7.6)	59.1 (9.5)	64.4 (9.3)	58.0 (12.3)	56.8 (16.3)
sTOI % (sd)	40.9 (20.7)	36.7 (18.9)	40.7 (16.4)	46.0 (14.7)	44.1 (16.0)	44.7 (14.0)	43.2 (14.1)	36.7 (14.3)
cFTOE (sd)	0.31 (0.19)	0.33 (0.16)	0.34 (0.10)	0.34 (0.09)	0.35 (0.10)	0.33 (0.14)	0.39 (0.13)	0.37 (0.18)
sFTOE (sd)	0.57 (0.24)	0.58 (0.20)	0.57 (0.18)	0.50 (0.17)	0.53 (0.18)	0.49 (0.14)	0.59 (0.17)	0.57 (0.14)
CSOR (sd)	0.61 (0.28)	0.58 (0.23)	0.67 (0.25)	0.77 (0.21)	0.75 (0.21)	0.69 (0.22)	0.74 (0.19)	0.65 (0.18)

Author statement

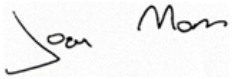
"I declare that I participated in the design, execution, and analysis of the paper by C Howarth and colleagues entitled “Regional cerebral and splanchnic tissue oxygen saturation in preterm infants – longitudinal normative measurements” that I have seen and approved the final version and that it has neither been published nor submitted elsewhere. I also declare that I have no conflict of interest, other than any noted in the covering letter to the editor"

Claire N Howarth________

Terence S Leung__________

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Author contributions: Professor Aladangady jointly conceptualised the study, developed study design and consented parents, as well as reviewed and revised the manuscript. Dr Banerjee jointly conceptualised the study, jointly developed the study design and reviewed and revised the manuscript. Dr Howarth jointly developed the study design, consented

parents, conducted all the measurements, data collection and wrote the first draft and reviewed and revised the manuscript. Professor Morris planned, supervised, and helped in executing the statistical analysis, as well as reviewed and revised the manuscript. Dr Leung helped with the NIRS analysis and reviewed and revised the manuscript. Dr Eaton jointly developed the study design, reviewed, and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Statement of financial support: This study was funded by the Barts Health Charity (Grant reference number MGU0388).

Disclosure statement: The authors have no financial relationships relevant to this article to disclose. Dr Howarth was employed through the Barts Heath Charity Grant but none of the other authors had financial relationship with the charity or the fund. Dr Eaton acknowledges support from NIHR Biomedical Research Centre at Great Ormond Street Hospital and UCL Great Ormond Street Institute of Child Health.

Category of study: Clinical research article

Conflict of Interest: The authors have no conflicts of interest to disclose.

Ethical approval: The study received research ethics (HRA) approval (Ref number 16/LO/1353).

Consent Statement: Formal written consent was taken from parents for all the infants involved in this study.

Figure legends

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