Cerebral Oxygenation in Preterm Infants with Necrotizing Enterocolitis

Claire Howarth[1,2], BMBS, BMedSci, Jayanta Banerjee[3,4], MBBS, MD (Res) Terence Leung[5] PhD, Simon Eaton[6], PhD, Joan K Morris[7], PhD, Narendra Aladangady[1,2], MBBS, PhD

Affiliations: 1 = Homerton University Hospital NHS Foundation Trust, London, UK, 2 = Queen Mary University of London, London, UK, 3 = Imperial College Healthcare NHS Trust, London, UK, 4 = Imperial College London, UK, 5 = Department of Medical Physics and Biomedical Engineering, University College London, London, UK, 6 = UCL Great Ormond Street Institute of Child Health, London, UK, 7 = St George's, University of London, London, UK

Short title: Cerebral Oxygenation and Necrotizing Enterocolitis.

Corresponding author: Prof Narendra Aladangady, email: n.aladangady@nhs.net, Address: Neonatal Service, Homerton University Hospital NHS Foundation Trust, Homerton Row, London, E9 6SR, UK. Tel: 020 8510 7360 Fax: 0208 510 7035

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

Table of Contents Summary: This study demonstrates that infants with NEC have significantly lower cerebral oxygenation which may contribute to the worse neurodevelopmental outcome in these infants.

What is already known on this topic:

Near infra-red spectroscopy (NIRS) can non-invasively monitor regional oxygenation in preterm infants. Low cerebral oxygenation during the first week of life is associated with poor neurodevelopmental outcomes and Necrotising enterocolitis (NEC) is an independent risk factor for worse neurodevelopmental outcome.

What this study adds:

Infants with NEC have significantly lower cerebral oxygenation throughout their neonatal intensive care course which may contribute to their worse neurodevelopmental outcome. NIRS continuous monitoring could help identify infants with low cerebral oxygenation allowing earlier treatment and targeted neurodevelopmental interventions.

Contributors' Statement Page: 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. Professor 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.

Abstract

Background and Objectives: Preterm infants with NEC are known to have worse neurodevelopmental outcomes but there is no substantial evidence to support an underlying pathophysiology. We aimed to examine whether cerebral oxygenation differs in those infants who develop NEC compared with those who do not.

Methods: We examined 48 infants <30w gestation admitted to a tertiary level NICU from October 2016 to May 2018. Infants with birthweight ≤2nd centile, abnormal antenatal dopplers or Twin to Twin Transfusion Syndrome were excluded. Cerebral oximetry measurements were performed using a near infrared spectroscopy (NIRS) monitor weekly for 60 minutes allowing measurement of cerebral Tissue Oxygenation Index (cTOI) from first week of life to 36 weeks post conceptional age. Weekly clinical status was also recorded. NEC was defined as ≥Bells stage 2.

Results: Median birthweight was 884 (range 460-1600) grams, median gestational age 26^{+3} (23^{+0}-29^{+6}) weeks and 52% female. 276 NIRS measurements were completed and 7 infants developed NEC. NIRS measurements from 1 infant with NEC and 4 infants without NEC who developed Haemorrhagic Parenchymal Infarcts (HPI) were excluded from analysis. Infants who developed NEC had significantly lower cTOI than those that did not ($p = 0.011$), even when adjusted for confounders including gestational age, birthweight, Patent Ductus Arteriosus (PDA), enteral feeds, gender, ethnicity and Haemoglobin.

Conclusions: Infants with NEC have significantly lower cerebral tissue oxygenation throughout their neonatal intensive care stay in comparison to those who did not develop NEC. This is a novel finding and could explain their worse neurodevelopmental outcome.

Main body of article:

Introduction

With increasing survival of preterm infants, the population at risk of necrotising enterocolitis (NEC) is increasing. NEC is a recognised independent risk factor of worse neurodevelopmental outcomes in premature infants with low birth weight, and those who required surgery[1-3]. Although preterm infants with established NEC are known to have worse neurodevelopmental outcomes compared to infants of the same gestational age and birth weight, the underlying pathogenic mechanism has not been clearly established and it is likely to be multifactorial. It has been previously attributed to exposure to increased circulating cytokines in NEC resulting in white matter injury[4] and leading to periventricular leukomalacia[5,6]. However, the impaired cerebral autoregulation seen in preterm infants can cause fluctuations in cerebral blood flow; being a pressure passive circulation, it is directly determined by blood flow and both hypotension and hypertension can cause neuronal injury by cerebral ischaemia and cerebral haemorrhage respectively[7-9].

Near Infrared Spectroscopy (NIRS) is a non-invasive method of continuously measuring tissue oxygenation at the bedside. Cerebral NIRS monitoring is a validated tool[10-12] and has been extensively studied as a measure of cerebral regional oxygenation ($cRSO_2$). The normal $cRSO_2$ for preterm infants is reported to vary from 55% to 85%[13-16], however there are no clear reference ranges for preterm infants of varying gestations beyond the first month of life, and any reported ranges are dependent on the device used and the population studied.

We aimed to examine cerebral oxygenation in preterm infants throughout their neonatal unit stay to determine whether there were differences in cerebral oxygenation between those with NEC and those without NEC that persisted beyond the first week of life. We hypothesised that NEC would not alter cerebral oxygenation in preterm infants.

Methods

We performed a prospective cohort observational study in appropriately grown preterm infants born at less than 30 weeks of gestational age (GA) admitted to a tertiary Neonatal Intensive Care Unit (NICU) at Homerton University Hospital from October 2016 to May 2018. Eligible infants were recruited by day 7 of life and measurements were performed weekly from birth until 36 weeks post conceptional age or discharge from the neonatal unit to either home or their local hospital. Infants with abnormal antenatal dopplers, intrauterine growth restriction (defined as birthweight 2nd centile or less), major congenital anomalies and twin-to-twin transfusion syndrome were excluded. The study was approved by the Research Ethics Committee in the UK (REC reference: 16/LO/1353) and informed written parental consent was obtained.

Weekly cerebral tissue oxygenation index (cTOI) was measured using a NIRS monitor (NIRO-300, Hamamatsu KK, Japan) along with concurrent measurement of peripheral arterial oxygen saturations using a pulse oximeter.

The NIRO-300 monitor is CE marked for clinical use in the neonatal population, uses reusable probes and the sensors were cleaned thoroughly in between patients. The NIRS

emitter and detector probes were placed on the baby's forehead for 60 minutes once a week by the same investigator (CH) to ensure uniform placement every time. The probes were placed in a protective rubber seal to ensure the distance between them was constant for each infant. The centre of this seal was then placed in the centre of the forehead meaning the actual emitter and detector probes were paramedian. CH was present during the entire measurement and if any movement artefact developed this was addressed immediately. All infants were on bolus feeds during the study and NIRS measurements were always started at the onset of a feed for every infant.

Using Matlab R2019b software (Mathworks, Natick, Massachussets, US) raw NIRS data was extracted for each infant for each week. Each NIRS reading was individually assessed for artefact as each recording was analysed in 5 minute epochs and noisy epochs (representing artefact) were removed. For each 60 minute recording, we produced a mean cTOI and then calculated cerebral fractional tissue oxygen extraction (cFTOE) using the equation $[SaO_2 - TOI]/SaO_2)$[17].

Antenatal and perinatal maternal and infant characteristics and weekly clinical data were documented including volume of enteral feeds, sepsis (defined as culture positive or culture negative but meeting the European Medicines Agency definition[18]), NEC (defined as ≥ Bells stage 2), presence of Intraventricular Haemorrhage (IVH) or Haemorrhagic Parenchymal Infarct (HPI) and weekly blood results including maximum CRP and Haemoglobin (Hb) level at the time of the NIRS recording.

Statistics

All statistical analyses were performed using STATA/SE version 15.1 (STATA Corp LLC, Texas, US). We used multi-level mixed effects linear regression models, nested within each baby in order to allow for the fact that the readings were taken over time and hence were correlated within each baby. NIRS measurements were compared in babies with NEC with those without NEC after adjusting for confounding variables: presence of PDA, gender, ethnicity, volume of enteral feeding, haemoglobin and gestational age. Confounding variables were included in the models as fixed effects.

For two confounding factors – enteral feeds and haemoglobin, these were analysed in categories at the closest time point to the NIRS measurement: (1) 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); (2) Hb: 3 categories - <8g/dl (group 1); 8-11.9g/dl (group 2) and ≥12g/dl (group 3). Differences in characteristics between infants with NEC and those without NEC were analysed using Mann-Whitney U or Chi^2 tests.

Results

Infant characteristics

We recruited 48 preterm infants born at <30 weeks GA. Parents of two infants withdrew their consent for the NIRS measurements after one week as their baby was unwell and they didn't want them having extra handling. Therefore, 46 infants had the complete weekly NIRS measurements until discharge or 36 weeks corrected gestational age. For the 7 infants that developed NEC the pre-NEC cerebral measurements were the weekly measurements taken up to and including 7 days before the onset of NEC, the measurements during NEC

were taken on day 2-3 of the diagnosis and the post NEC measurements were those taken weekly afterwards, starting 3 days after they finished treatment for NEC. The infant characteristics and their relevant antenatal details are presented in table 1. Two (4.2%) recruited infants died during the study (1 from tension pneumothorax and loss of cardiac output in the first week of life and 1 from NEC in week 9 of life).

Length of time in the study

For 46 infants with complete weekly NIRS measurements the median (range) weeks an infant stayed in the study was 7.3 (1-13) weeks. Seventeen (35.4%) infants completed the study up to 36 weeks gestational age, 29 (60.4%) did not complete the study as they were discharged to another hospital (n=25, 52%) or home (n = 4, 8.4%) before 36 weeks gestational age. Of these 29 infants the median (range) length of time in the study was 6 (1-8) weeks.

Infants diagnosed with NEC

Eight infants were treated for NEC with signs of abdominal distension, bilious aspirates and systemic features including metabolic acidosis and increased number of desaturations and bradycardias. The medical notes, X-rays and the radiologist's report of abdominal X-rays of these 8 infants were reviewed and only those with ≥Bells stage 2 were confirmed as NEC. After this there were 7 definite cases of NEC out of the 48 recruited infants giving an incidence of 14.6%. Only 1 out of the 7 infants required surgical treatment and there were no significant differences between infants with and without NEC, except for longer duration of ventilation in those infants with NEC, $p = 0.01$ (table 2). Of the 7 cases they presented with

NEC between week 3 and 9 of life and had chronological ages of two peaks; days 21-33 and days 59-60 with corrected gestational ages ranging from 28^{+6} to $34^{+3.}$

Sepsis episodes

Each infant was started on prophylactic antibiotics at birth after a blood culture was taken. Of these, 6 initial blood cultures were positive; 1 Coagulase Negative Staphylococcus, 1 Group B Streptococcus, 2 E. Coli, 1 Listeria and 1 Haemophilus Influenzae. There were 38 other instances of the infants being screened for sepsis, but after review and excluding contaminants there were 8 (21.1%) positive blood cultures, as well as 14 that met the EMA Expert Meeting on Neonatal and Paediatric Sepsis 2010 definition[18] (22 instances of sepsis in total).

Cranial ultrasound and echocardiography findings

Patent Ductus Arteriosus (PDA) was confirmed on echocardiogram in the first 2 weeks of life in 28 (58.3%) infants – all were haemodynamically significant (as judged clinically and/or on echocardiogram). Seven (14.6%) had no PDA and the remaining 13 (27.1%) did not have an echocardiogram but did have clinical features suggestive of a PDA. On cranial ultrasound scan 5 infants (10.4%) had HPI and 24 (50%) had IVH; of these 4 (16.7%) had Grade I IVH, 15 (62.5%) had grade II IVH and 5 (20.8%) had grade III IVH. There was no significant difference in grade of IVH or presence of PDA between those infants with NEC and those without (all $p>0.05$).

NIRS measurements

276 NIRS measurements were completed but 39 NIRS measurements from 5 babies who developed HPI were excluded from analysis (resulting in 237 measurements). Although 7 infants developed NEC, 1 of these infants also developed HPI, meaning there were 6 infants with NEC whose NIRS measurements could be used in the analysis. We excluded those infants with HPI because the venous stasis and damage to white matter would be expected to impact cTOI readings. The mean cerebral oxygenation measurements across the whole study of the NEC and non-NEC infants are presented in Table 3. Infants who developed NEC had significantly lower cTOI and a significantly higher cFTOE throughout their neonatal course, than those that did not develop NEC across the first 10 weeks of life (table 3, figures 1 and 2). This significance remained when adjusted for potential confounding factors including GA, ethnicity, birthweight, gender, Hb level, enteral feed volume and presence of PDA on the cerebral NIRS measurements.

To examine the impact of the 22 sepsis episodes, we compared the NIRS measurements at the time of the diagnosis of sepsis for those infants with sepsis with those infants who were born at the same GA but did not develop sepsis. We compared the NIRS measurements at the same postnatal week of life for the two groups. The mean cTOI for the infants who developed sepsis was 63.5±10.8 % and for those who did not have sepsis was 68.1±9.9%, and this difference was not significant ($p = 0.08$).

Discussion

NEC has been reported to occur in 14% infants <26 weeks gestation and 10% < 31 weeks gestation[19]. Our infants with NEC were all <28 weeks and our unit has a high incidence of ELBW infants, contributing to a higher than the national average rate of NEC. However, the incidence of NEC in our tertiary unit is similar to others in inner city South East England[19].

We have demonstrated that cerebral TOI was significantly lower and FTOE significantly higher in infants who developed NEC across the first 10 weeks of life. A multilevel model was constructed to eliminate the effect of confounding factors, but beyond week 10 it was difficult to analyse the effect as by this point there were only 2 infants left in the study as the others had been either discharged or reached 36 weeks corrected GA. NEC is more common in those infants born more prematurely and with a lower birthweight. We therefore wanted to look at any factors that could potentially affect the readings in NEC infants as confounders, including birthweight and gestational age. Because we found no significant effect of any of the potential confounding factors on the cerebral NIRS measurements we are confident that the lower cTOI seen in those infants with NEC was independent of these factors.

There is a dip in cTOI at week 7 (figure 1) with corresponding reciprocal changes in cFTOE (figure 2) in both NEC and non-NEC infants. This dip does not coincide with NEC development because no infant developed NEC during week 7 of life. We can speculate that this dip could be due to the known physiological drop in haemoglobin[20], however, there was only one infant with NEC who had this measurement therefore this apparent change at week 7 could be due to random error.

The current study adds to the existing report that not only low cerebral oxygenation in the first 48 hours of life is associated with an increased risk of NEC[21] but cerebral TOI was significantly lower and FTOE significantly higher in the cohort of infants with NEC across the first 10 weeks of life. It is interesting that the differences in cerebral oxygenation are present before the onset of NEC. This would suggest that there is an inherent effect of cerebral hypoxia in the transitional period and early extrauterine life which predisposes to NEC. One could hypothesise that cerebral hypoxia causes the body to adapt to prevent this by redirecting blood to the major organs such as the brain at the expense of other organs including the gut. These infants therefore have relative hypoxia of the gut during their first few days and weeks of life. During this time clinicians intervene starting enteral milk feeds and feeding in the context of a relatively hypoxic gut could lead to NEC.

It is well known that preterm infants are at risk of neurodevelopmental sequelae[22,23] and that cerebral ischaemia, hypoxia, and fluctuations in cerebral perfusion are involved in the aetiology of various cerebral pathology[24]. Neuronal injury can result from both ischaemia and haemorrhage, and the impaired cerebro-autoregulation seen in preterm infants causes fluctuations in cerebral blood flow as it is passively determined by blood pressure. Both hypotension and hypertension can cause neuronal injury by cerebral ischaemia and cerebral haemorrhage respectively[9,25]. Verhagen[26] and Ancora[27] described a strong association between worse motor outcome and cerebral ischaemia.

An observational cohort study in preterm babies born at less than 32 weeks GA, who had regional cerebral oxygenation measured continuously for first 72 hours after birth using

NIRS, found that low cerebral oxygenation was associated with an unfavorable cognitive outcome[28]. The authors of this study suggested that cerebral oxygenation should be maintained above a threshold of 65% when using the INVOS device in neonates. Although a similar cerebral oxygenation threshold for cTOI (NIRO-300) was not reported in the literature, a previous study showed that cTOI tended to be lower than crSO2 (INVOS 5100) with a mean difference of 10%[29].

Those infants who developed NEC had a mean cTOI of 56.9% (95%CI 53.2 – 60.6%) and those who did not of 63.9% (95% CI 62.2 – 65.5%). The relationship between cerebral oxygenation and brain damage has been assessed in piglet studies, suggesting that regional cerebral oxygenation of <40-50% could be dangerous for the newborn brain, and both the magnitude and duration of deviation from the baseline are important[30,31]. Data on the clinical significance of cerebral oxygenation in preterm infants is lacking but in adults Heringlake et al. found that a pre-cardiac surgery cerebral oxygenation <50% was an independent risk factor for 30-day and 1-year mortality[32] and that intraoperative desaturation during cardiac surgery is associated with post-operative cognitive dysfunction[33]. However, not every individual will be affected to the same extent meaning it is difficult to predict whether, and to what degree, an individual infant will develop neurodevelopmental sequelae from a drop in cTOI[34], particularly as the relationship between Hb level and perfusion is unclear[35].

The precise safe optimal range for cerebral oxygenation in preterm infants has not been established. Like other studies, we noted a large variance between, and within, the studied infants. Due to these large variations there is insufficient data in this study to accurately determine specific “safe” cut-offs for cerebral oxygenation levels to distinguish between

those infants who developed NEC and those who did not. One limitation of NIRS is that repeated measures within subject standard deviation is about 5% to 6%[36] and there is a methodological bias between sensors from INVOS-5100 and NIRO-300[37]. Because authors report studies on cerebral oxygenation using different NIRS devices the emphasis should be on using NIRS to report trends in regional oxygenation[38].

Rallis et al[39] reported that septic neonates had significantly decreased cerebral oxygenation (62.7 ± 7 vs. $71.4 \pm 4.4\%$, $p<0.001$) on the seventh day of sepsis. We saw no effect of sepsis, however, our NIRS measurements were completed weekly meaning some were early in the sepsis episode (<72 hours) and some late (5-6 days), and in Rallis's study they too observed no difference in cerebral oxygenation in those infants with sepsis in the first three days. Furthermore, our study population and definition of sepsis were different and our infants were treated with appropriate antibiotics at the earliest sign of suspected sepsis which may have prevented changes in brain oxygenation.

A Cochrane review[40] looked at the evidence for cerebral NIRS monitoring for prevention of brain injury in very preterm infants but it involved one RCT which was only powered to explore differences in cerebral oxygenation rather than clinical outcomes. It did demonstrate a significant reduction in cerebral oxygenation out of normal range in the experimental group, but no consistent differences were reported in neonatal morbidities or mortality at term equivalent. The SafeBoosC-III trial is currently ongoing exploring whether treatment based on NIRS monitoring for extreme preterm infants during the first 72 hours of life will result in reduction in severe brain injury or death at 36 weeks postmenstrual age[41].

Strengths of the study

We studied 48 extreme preterm infants which is representative of a tertiary neonatal unit and completed 276 NIRS cerebral oxygenation measurements which is a large number of NIRS measurements compared with previous studies and demonstrates the feasibility of using NIRS in the neonatal population.

Consistency in NIRS probe placement and fixation was maintained by having the same investigator (CH) performing all the measurements. The NIRS analysis was completed in 5 minute epochs and any areas within the 60 minute recording deemed to be "noisy" were removed from the analysis under the guidance of a medical physicist in NIRS (TSL). In most NIRS studies around 10% of infants are excluded because of motion artefacts[12,42], but no baby was excluded because of motion artefact from the present study.

We also applied a strict NEC definition (≥Bells stage 2) and all the abdominal x-rays were reviewed by an independent paediatric radiologist to confirm the cases of NEC.

Finally, complex multilevel modelling was used to investigate the effect confounders and also take into account 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

We were unable to look at the difference in cTOI in infants with NEC requiring surgery versus those treated medically as only 1 infant required surgery. Infants with surgically managed NEC have a higher incidence of neurodevelopmental dysfunction compared with those treated medically[43,44]. Surgery and anaesthesia cause haemodynamic instability and can cause a cytokine surge, potentially causing increased white matter damage which could cause the worse neurodevelopmental outcome seen in infants requiring surgery for NEC, or it could be secondary to disease severity[45,46].

We only conducted weekly cerebral NIRS measurements which may have introduced bias. However, other studies using NIRS have used a similar approach and we remain the only study to have examined cTOI in preterm infants for this length of time.

We also did not record infant pCO_2 levels and pCO_2 levels could impact cerebral perfusion. However, no infant had a pCO_2 of <4 or >8 kPA during the NIRS recordings.

Finally, we have not followed up recruited infants' long term to see if those infants who developed NEC, and had lower cTOI, had worse neurodevelopmental outcomes. However, the link between cTOI and brain injury or neurodevelopmental impairment has already been demonstrated[28,36,47,48].

Conclusion

Our results demonstrate that infants who develop NEC had significantly lower cTOI throughout their neonatal intensive care stay. This may suggest an underlying mechanistic relation between NEC and their worse neurodevelopmental outcome. We believe our study offers novel information as it highlights that it is not just the acute transition period for intra to extra uterine life that is important, but the cTOI throughout their neonatal intensive care course.

As well as offering a potential mechanism for their worse neurodevelopmental outcome, our findings also potentially pave a way to target neurodevelopmental follow up in infants with NEC. Continuous cerebral NIRS monitoring may benefit the clinical outcomes of preterm infants because NIRS has the potential to alert clinicians to when cerebral hypoxia occurs, allowing time for prompt interventions to ameliorate their cerebral hypoxia, and, potentially reduce their long-term neurodevelopmental impairment. In addition, it could allow infants with NEC with lower cTOI to be targeted for specific neurodevelopmental interventions. However further trials are needed to examine if NIRS monitoring coupled with intervention can improve outcomes.

Figures/tables in separate files

Table 1: Infant characteristics and their relevant antenatal details

Table 2: Characteristics of infants with NEC and those without NEC.

Table 3: Mean Cerebral NIRS measurements in those infants with NEC compared to those infants without NEC (excluding infants with HPI) across the whole study.

Figure 1: Mean cTOI across the study period divided into infants with NEC and without NEC

Figure 2: Mean cFTOE across the study period divided into infants with NEC and without NEC

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Figure 1: Mean cTOI across the study period divided into infants with NEC and without NEC

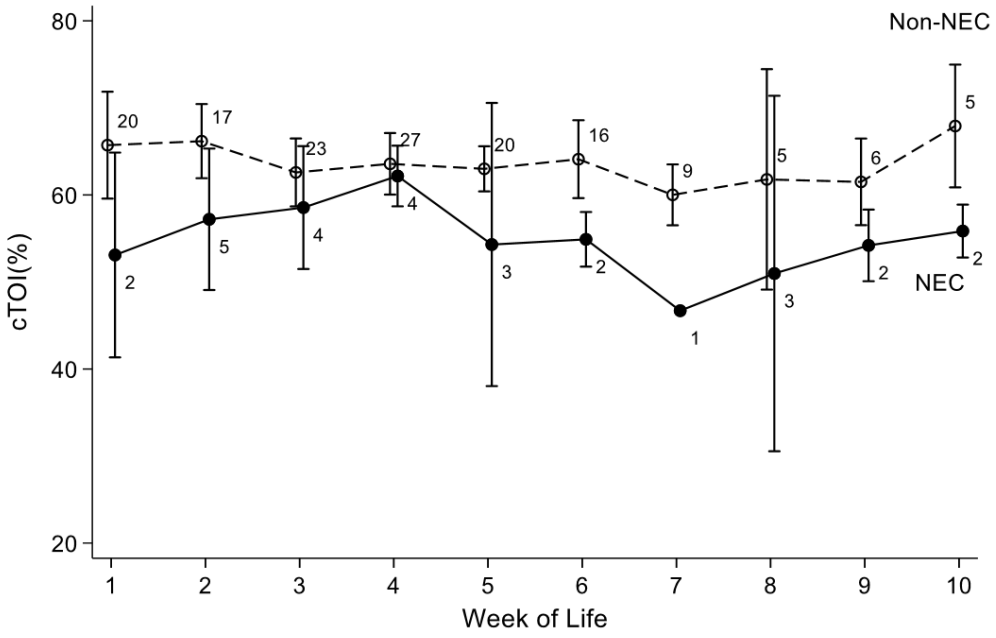

Figure 2: Mean cFTOE across the study period divided into infants with NEC and without NEC

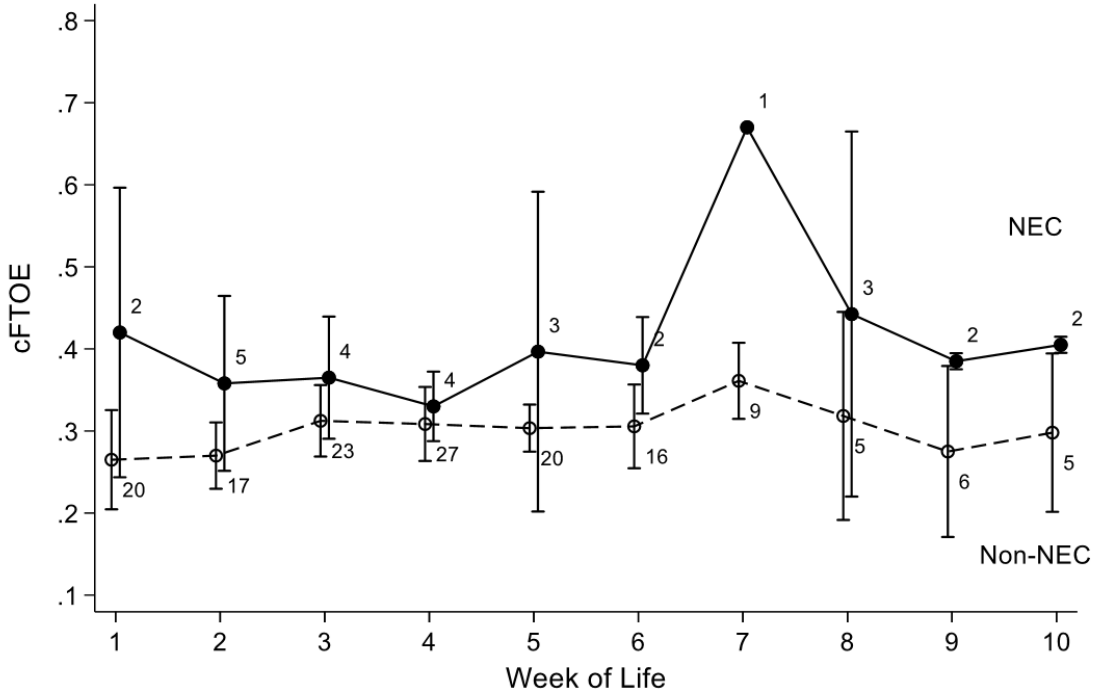

Table 1: Infant characteristics and their relevant antenatal details

Characteristic	Infants studied (n = 48)
Birthweight (g) [median (range)]	883.5 (460-1600)
Gestational age (wks) [median (range)]	26^{+3} (23^{+0}-29^{+6})
Gender [number (%)]	
Male	23 (47.9)
Female	25 (52.0)
Ethnicity [number (%)]	
White (white British /white other)	30 (62.5)
Black (Afro Caribbean/ Black other)	10 (20.8)
Asian (Indian/Pakistani /Asian other)	8 (16.7)
Singletons [number (%)]	38 (79)
Twins [number (%)]	10 (21)
Antenatal steroids [number (%)]	
Complete course	30 (62.5)
Incomplete course	14 (29.2)
No steroids	4 (8.3)
Antenatal Magnesium Sulphate	36 (75)
Placental histology	
Chorioamnionitis	26 (54.2)
Normal	17 (35.4)
Unknown	5 (10.4)

Table 2: Characteristics of infants with NEC and those without NEC.

Characteristic	NEC (n = 7)	Non NEC (n = 41)	P value
Gestational age at birth (wk) [median (range)]	25^{+2} (23^{+4} – 27^{+3})	26^{+4} (23^{+0} – 29^{+6})	0.13
Birth weight (g) [median (range)]	700 (600-1015)	900 (460-1600)	0.14
Maternal chorioamnionitis (%)	85.7	53.6	0.21
Antenatal steroids (%)	85.7	92.6	0.48
Days of ventilation [median (range)]	26 (6 – 48)	6 (1-65)	**0.01**
Use of inotropes in the first week (%)	29	14.6	0.33
Days to achieve full feeds [median (range)]	23 (9 – 26)	15 (6-41)	0.29
Ethnicity (White, black, Asian/other) (%)	71.4, 14.3,14.3	61.0, 17.0, 22.0	0.86

Table 3: Mean Cerebral NIRS measurements in those infants with NEC compared to those infants without NEC (excluding infants with HPI) across the whole study.

NIRS Measurement	NEC infants Mean (CI) n = 6	Non - NEC infants Mean (CI) n = 35	Mean difference (CI)	P value
cTOI (%)	56.9 (53.2 – 60.6)	63.9 (62.2 – 65.5)	-6.6 (-11.7 to -1.5)	**0.011**
cFTOE	0.38 (0.33 – 0.43)	0.30 (0.28 – 0.32)	0.08 (0.02 to 0.14)	**0.009**