The relation between splanchnic ischaemia and intestinal damage in necrotising enterocolitis

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ABSTRACT

Objectives The underlying pathophysiology of necrotising enterocolitis (NEC) remains incompletely understood, particularly the role of intestinal perfusion. We aimed to determine the relation between cerebral and splanchnic fractional tissue oxygen extraction (FTOE), a marker for tissue underperfusion, with intestinal fatty acid-binding protein in plasma (I-FABPp), a marker for intestinal damage, in infants with NEC. Furthermore, we investigated the combined courses of cerebral and splanchnic FTOE values and I-FABPp levels in uncomplicated (conservative treatment) and complicated NEC (surgery or death).

Design This study was part of a prospective observational cohort study.

Patients We included 19 preterm infants with NEC (9 uncomplicated, 10 complicated).

Interventions Using near-infrared spectroscopy, we measured regional cerebral and splanchnic tissue oxygen saturations continuously for 48 h after NEC onset. We measured I-FABPp levels simultaneously.

Main outcome measures We used Spearman correlation tests to calculate correlation coefficients between FTOE values and I-FABPp levels in uncomplicated and complicated NEC.

Results Median (range) gestational age was 28 (25–36) weeks and median (range) birth weight was 1290 (740–2400) g. Cerebral and splanchnic FTOE values correlated strongly with I-FABPp levels (rho between .745 and 0.900; p<0.001–0.037) during the first 16 h after NEC onset. Thereafter, in uncomplicated NEC, splanchnic FTOE values increased while I-FABPp levels decreased concomitantly. In complicated NEC both splanchnic FTOE values and I-FABPp levels decreased.

Conclusions Combining cerebral and splanchnic FTOE values with I-FABPp levels, gives insight in the pathological chain of events resulting in progression or recovery of intestinal ischaemia in NEC.

Trial registration number NTR3239.

What is already known on this topic

- An impaired intestinal perfusion in necrotising enterocolitis (NEC) can be investigated by using near-infrared spectroscopy (NIRS).
- Intestinal fatty acid-binding protein in plasma (I-FABPp) is a marker for the extent of intestinal damage in NEC.
- I-FABPp measurements can accurately predict development of complicated NEC.

What this study adds

- The strong association between splanchnic fractional tissue oxygen extraction (FTOE) values and I-FABPp levels during early NEC development suggests intestinal damage to be accompanied by intestinal ischaemia.
- The combined courses of I-FABPp and splanchnic FTOE are different for complicated NEC and uncomplicated NEC, which gives insight in the pathophysiological chain of events resulting in progression or recovery of intestinal ischaemia in NEC.
- During NEC, cerebral FTOE as a measure for cerebral perfusion is related to I-FABPp levels, suggesting early systemic haemodynamic effects of intestinal damage due to NEC.

INTRODUCTION

In preterm infants necrotising enterocolitis (NEC) is the leading cause of death from gastrointestinal diseases.[1] The role of intestinal perfusion in the underlying pathophysiology of NEC remains incompletely understood.[2 3]

Intestinal perfusion in NEC can be investigated by using near-infrared spectroscopy (NIRS). NIRS is a non-invasive method used increasingly in preterm infants to assess cerebral and intestinal perfusion.[4–9] NIRS measures regional tissue oxygen saturation (rSO_2) of underlying tissue continuously.[10] When transcutaneous arterial oxygen saturation (SpO_2) is measured simultaneously, fractional tissue oxygen extraction (FTOE) can be calculated.[11] FTOE is thought to reflect the balance between tissue oxygen supply and consumption and may, therefore, be an early indicator of impaired tissue perfusion or ischaemia.[11]

A possible way of gaining more insight into the role of cerebral and intestinal perfusion during the development of NEC is to combine FTOE values with intestinal fatty acid-binding protein in plasma (I-FABPp), a marker for the extent of intestinal damage in NEC.[12–15] I-FABPp measurements can also accurately predict development of complicated disease.[15]

The overall aim of the study was to investigate the pathophysiological chain of events after NEC onset with regard to mucosal damage and reduced cerebral and splanchnic perfusion in the development of NEC. For this purpose we investigated whether cerebral and splanchnic perfusion accompanies intestinal damage due to NEC by relating cerebral and

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splanchnic FTOE values with I-FABPp levels in preterm infants with NEC. Second, we investigated whether, during the first 48 h after NEC onset, patterns of courses of cerebral and splanchnic FTOE values combined with I-FABPp levels differed between infants with uncomplicated and complicated NEC.

METHODS

This study was part of a prospective observational cohort study conducted at the University Medical Center Groningen (UMCG) between October 2010 and October 2012 (Dutch Trial Registry number NTR3239). The study was approved by the ethical review board of UMCG.

Patients

Following written informed parental consent, we included preterm infants who were suspected of or had recently been diagnosed with NEC. Suspected NEC was defined as the presence of non-specific NEC symptoms, such as bloody stools or distended abdomen. Definite NEC was defined if pneumatosis intestinalis, portal venous gas or both were present (NEC Bell's stage >2).[16] On first presentation of symptoms, all patients were put on a nil per os and gastric decompression regime, and received broad-spectrum antibiotics until radiographic signs of NEC resolved and clinical signs stabilised. A team of four consultants, blinded as to the results of the NIRS and I-FABPp measurements, determined the modified Bell's stage at NEC onset and end-stage Bell's stage.[16] Agreement was reached in all cases. In this paper we present data of the subgroup of preterm infants with definite NEC only, that is, Bell's stage ≥2.

We measured I-FABPp levels repeatedly, and also continuously measured rSO_2 of cerebral and splanchnic tissue for 48 h after NEC onset or until surgery, whichever came first. We defined NEC onset as the time of the first radiographic abdominal examination after clinical suspicion of NEC, including abdominal X-rays obtained in referring hospitals. We assigned the infants with NEC to one of two groups: those with uncomplicated NEC (conservative treatment) and those with complicated NEC (surgery and/or death). Indications for surgery, that is, laparotomy, were bowel perforation or lack of improvement despite optimal conservative therapy.

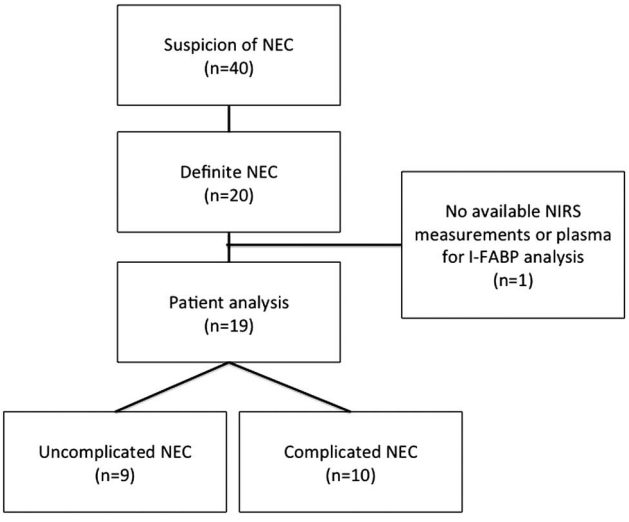

Figure 1 Flow diagram of the study. I-FABP, intestinal fatty acid-binding protein; NEC, necrotising enterocolitis; NIRS, near-infrared spectroscopy.

I-FABPp measurements

With every routine blood analysis after NEC onset, an extra sample of 100 µL was obtained in an EDTA tube. Blood samples were collected every 8 h during the first 24 h after NEC onset, and every 12 h during the following 24 h.

Blood samples were fractioned by centrifuging them for 10 min at approximately 2000× *g*. Plasma was then collected in a 5 mL Sarstedt tube and stored at −80°C. A laboratory technician, blinded to the clinical data, performed the I-FABPp measurements. We used a commercially available ELISA for determining I-FABPp levels (Human FABP2 kit from R&D systems, Minneapolis, USA).

NIRS measurements

We used the INVOS 5100C near-infrared spectrometer (Covidien, Mansfield, USA) in combination with the neonatal SomaSensors (Covidien) to measure cerebral and splanchnic oxygen saturation continuously for 48 h after NEC onset.

To measure cerebral tissue oxygen saturation, we placed the neonatal SomaSensor on the right or left frontoparietal side. We measured splanchnic oxygen saturation at two locations: just below the right costal arch to measure liver oxygen saturation, and infraumbilically on the central abdomen to measure intestinal oxygen saturation. The SomaSensors were held in place by elastic bandaging or Mepitel (Mölnlycke, Sweden). During routine nursing care, clinical assessments and radiographic examinations the sensor was temporarily removed and replaced in the same location. The data, collected prospectively, were stored off-line for future analysis. Additionally, we collected SpO_2-values (Nellcor, Covidien-Medtronic, Minneapolis, USA). Although we intended to keep SpO_2 within the former guideline range of 85–92%, periods of hypoxia may have occurred. By using the formula: $FTOE=(SpO_2-rSO_2)/SpO_2$, we calculated the part of the arterial oxygen content that is indeed extracted by the tissue, correcting for periods of arterial hypoxia. FTOE reflects the balance between oxygen supply and oxygen consumption and, thus, may serve as an indicator of impaired tissue perfusion.[11] The path length of near-infrared light through cerebral tissue in infants is at least 5 cm[17] suggesting a penetration depth of approximately 2 cm. It remains unclear how this relates to abdominal tissue.

Demographic data

We collected the following patient characteristics: gestational age, birth weight, postnatal age at NEC onset, gender, whether surgery was required and mortality. Furthermore, we documented the first concentrations during the study period of haemoglobin, thrombocytes, pH, C reactive protein and lactate. We documented the need for mechanical ventilation and treatment of circulatory failure (volume expansion, vasoactive drugs). We also documented the presence of a haemodynamically significant patent ductus arteriosus during the study period, defined as either diastolic forward flow in the branches of the pulmonary artery, diastolic backflow in the descending aorta or left ventricular end diastolic diameter >p 95.

Data and statistical analysis

Cerebral and splanchnic regional tissue oxygen saturations were collected once every 6 s and SpO_2 values every 5 min. We matched each SpO_2 value with the corresponding single rSO_2 value, leaving one coupled measurement every 5 min. Next, we calculated FTOE values using these combined SpO_2 and rSO_2 values for the cerebral, liver and intestinal regions separately.

Since I-FABPp levels were collected once every 8 h in the first 24 h after NEC onset and once every 12 h between 24 h and 48 h after NEC onset, we calculated 8-h mean FTOE values in the first 24 h after NEC onset and subsequently 12-h mean FTOE values for the remaining study period.

For the first aim of this study, we calculated correlation coefficients between cerebral and splanchnic FTOE values and I-FABPp levels during the first 48 h after NEC onset using the Spearman rank correlation test. For our second aim, we constructed courses of cerebral and splanchnic FTOE values together with I-FABPp levels for infants with uncomplicated NEC and complicated NEC after logarithmic transformation of I-FABPp levels.

We used SPSS V.22.0 software for Windows (IBM SPSS Statistics 22, IBM, Armonk, New York, USA) for statistical analyses. We considered a p value <0.05 as statistically significant.

RESULTS

We enrolled 19 preterm infants with NEC Bell's stage ≥2 in whom we were able to measure cerebral and splanchnic tissue oxygen saturation simultaneously and collected plasma for analysing I-FABPp levels (figure 1). The median (range) time between NEC onset and the beginning of NIRS monitoring was 7 (2–31) h.

In nine infants the course of NEC was uncomplicated. None of these infants died. Of the 10 infants with complicated NEC, 6 infants died. Eight infants with complicated NEC required surgery; seven underwent surgery during the study period, with a median of 33 (range 9–165) h between the onset of NEC symptoms and surgery. The other infant required surgery 35 days after onset of NEC symptoms due to ongoing feeding difficulties and signs of ongoing infection, and died shortly afterwards due to total necrosis of the small and large intestines. We present the patient characteristics in table 1.

Table 1 Patient characteristics

	Uncomplicated NEC (n=9)	Complicated NEC (n=10)
Gestational age, weeks	31.6 (25.7–35.9)	26.7 (25.0–34.0)
Birth weight, grams	1520 (740–2400)	980 (790–2280)
Male:female	6:3	8:2
PNA at NEC diagnosis, days	8 (3–29)	9 (7–22)
Haemoglobin	8.7 (7.0–12.4)	8.2 (6.0–10.3)
Thrombocytes	235 (131–491)	202 (42–405)
pH	7.34 (7.19–7.39)	7.24 (7.09–7.42)
C reactive protein, mg/L	33 (0–166)	30 (0–95)
Lactate, mmol/L	2.7 (1.2–4.5) (n=4)	2.0 (1.0–11.9) (n=8)
Mechanical ventilation (%)	3 (33)	7 (70)
PDA (%)	1 (11)	3 (30)
Haemodynamically significant	–	2 (20)
RBC transfusion (%)	3 (33)	4 (40)
Fluid resuscitation	4 (44)	8 (80)
Inotropes (%)	–	6 (60)*
Surgery (%)	1 (11)	8 (80)*
Mortality (%)	–	6 (60)*

Data are expressed as median (range) or as numbers unless specified otherwise.
Statistical differences between the two groups are marked by *(<0.05).
NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; PNA, postnatal age; RBC, red blood cell.

Correlation between cerebral and splanchnic FTOE values and I-FABPp levels

During the first 16 h, cerebral and splanchnic FTOE values correlated strongly with I-FABPp levels (table 2). Thereafter, cerebral FTOE values correlated significantly with I-FABPp levels between 24 h and 36 h after NEC onset.

Courses of cerebral and splanchnic FTOE values and I-FABPp levels in infants with complicated and uncomplicated NEC

Figure 2 shows courses of cerebral, liver and intestinal FTOE values and I-FABPp levels in preterm infants with uncomplicated NEC and infants with complicated NEC. In complicated NEC, the initially higher intestinal FTOE values decreased after onset of NEC (figure 2). Uncomplicated NEC cases, on the other hand, exhibited increasing intestinal FTOE values after disease onset. We observed these changes in FTOE values particularly in the intestinal region, rather than in the liver region. Cerebral FTOE values remained relatively stable during the first 48 h after onset in both uncomplicated and complicated NEC. I-FABPp levels started out higher in the complicated groups and decreased after 24 h after NEC onset, whereas in the uncomplicated NEC group, the lower initial I-FABPp levels decreased over time from NEC onset onwards.

DISCUSSION

The current study can be regarded as a 'proof of concept' that combining intestinal FTOE and I-FABPp levels may reveal the underlying pathophysiological chain of events during early NEC.

Our results demonstrated a strong association between splanchnic FTOE values and I-FABPp levels during the first 16 h after NEC onset, suggesting intestinal damage to occur simultaneously with decreased splanchnic perfusion. We offer several explanations for the strong associations between splanchnic FTOE values and I-FABPp levels during the first 16 h after NEC onset. First, the presence of ischaemia may cause intestinal epithelial cell damage. Second, intestinal ischaemia and hypoxia may develop due to circulatory insufficiency in the presence of intestinal epithelial cell damage. Finally, intestinal circulation

Table 2 Correlation coefficients between cerebral and splanchnic FTOE values and I-FABP levels in plasma

	cFTOE	livFTOE	intFTOE
0–8 h			
I-FABPp	0.786* n=7	0.600 n=5	0.900* n=5
8–16 h			
I-FABPp	0.891** n=11	0.881* n=8	0.745* n=10
16–24 h			
I-FABPp	0.750 n=7	0.771 n=6	0.800 n=4
24–36 h			
I-FABPp	0.731* n=13	0.573 n=11	−0.285 n=10
36–48 h			
I-FABPp	0.510 n=12	0.100 n=11	0.188 n=10

Statistical differences are marked by *(<0.05) or **(<0.001).
cFTOE, cerebral fractional tissue oxygen extraction; I-FABPp, intestinal fatty acid-binding protein in plasma; intFTOE, intestinal fractional tissue oxygen extraction; livFTOE, liver fractional tissue oxygen extraction.

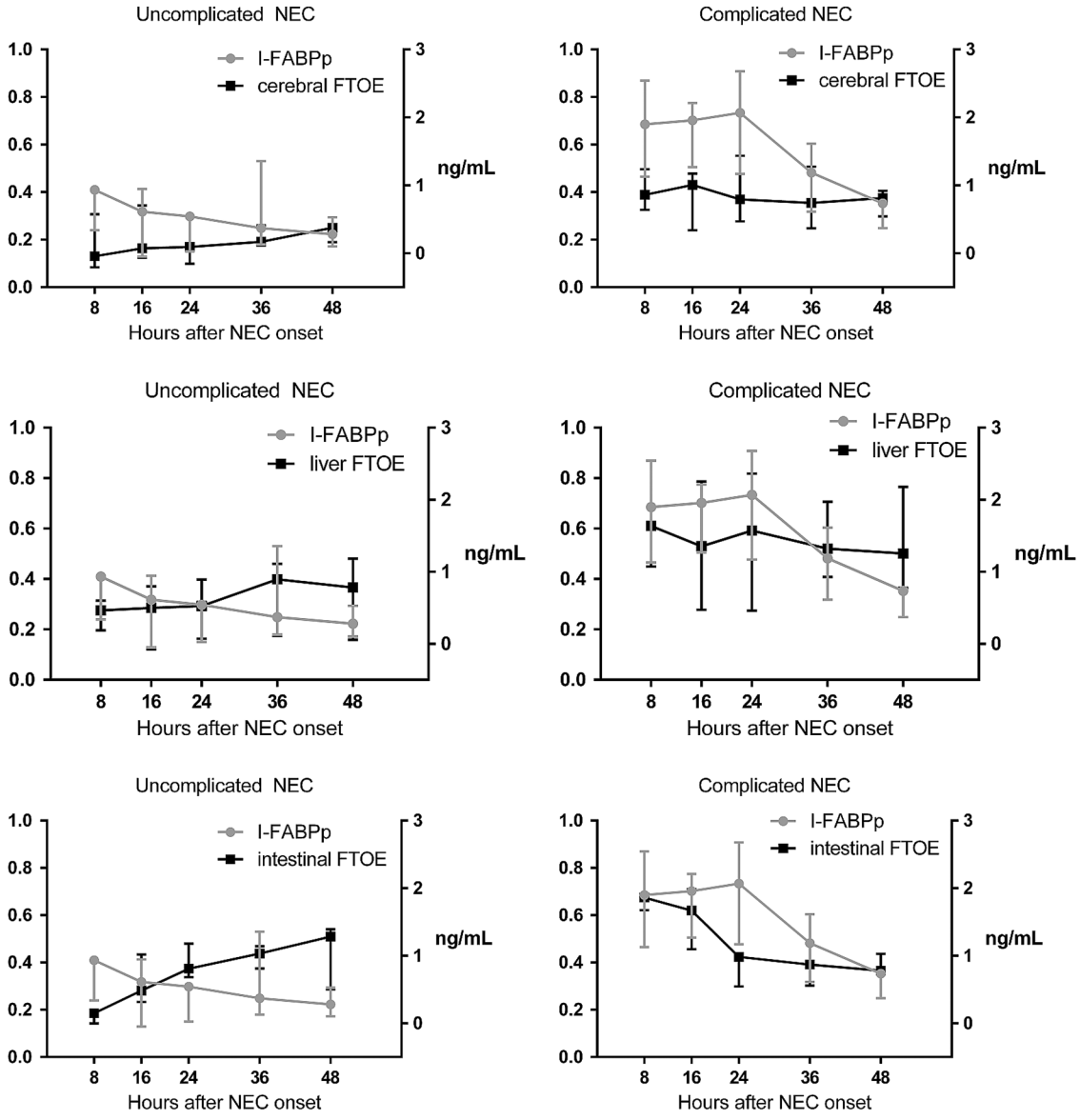

Figure 2 Graphs showing the median (dots and squares) and IQR (lines) of splanchnic and cerebral fractional tissue oxygen extraction (FTOE) values and intestinal fatty acid-binding protein in plasma (I-FABPp) levels of infants with uncomplicated and complicated necrotising enterocolitis (NEC).

may be affected locally as a result of intestinal injury. NEC is characterised by coagulation necrosis of the intestinal wall, suggesting that intestinal ischaemia is involved in its pathogenesis.[18] Whatever the cause, our data suggested that splanchnic FTOE values can be used to gain information about the degree of intestinal damage during NEC.

Interestingly, we also observed strong associations in the total group, between cerebral FTOE values and I-FABPp levels during the early phase of NEC and between 24 h and 36 h after NEC onset. This finding may be a reflection of the fact that by the time NEC becomes clinically evident, it already has systemic effects on haemodynamics. Combined with an impaired cerebrovascular autoregulation (CAR) in these preterm infants, cerebral underperfusion may occur.[19–21]

In all infants with NEC, we found decreasing I-FABPp levels up to 24 h after NEC onset. This may result from one of two mechanisms: either expansion of damage or recovery of intestinal tissue. In complicated NEC, it could be caused by intestinal necrosis leaving no villi to secrete I-FABP or the absence of blood flow through a demarcated necrotic bowel segment.[13 15] Conversely, when the infant's condition is ameliorating and the intestinal tissue is not injured any further, secretion of I-FABP into the circulation will diminish.[15] With simultaneous knowledge of FTOE values we could differentiate between the aforementioned supposed mechanisms.

Looking at the courses of I-FABPp and FTOE, we identified two distinct patterns during the first 48 h in which the disease was developing, differentiating uncomplicated NEC from complicated NEC. In uncomplicated NEC, we observed low splanchnic FTOE values at NEC onset that increased over time. We speculate that hyperaemia is present during the 1st hours after NEC onset due to an inflammatory response but this hyperaemia gradually disappears. This course of FTOE values in combination with decreasing I-FABPp levels could represent recovery of intestinal tissue.

The opposite occurred in complicated NEC, that is, relatively high initial splanchnic FTOE values with high I-FABPp levels, both decreasing during disease progression. We speculate that

the initial high splanchnic FTOE values were the result of compromised intestinal perfusion. A decreasing or absent intestinal metabolism and a reduced oxygen extraction due to the presence of necrotic bowel might result in the decreasing intestinal FTOE values. Decreasing I-FABPp levels after 24 h in this case could have been the result of absent venous return from the necrotic bowel,[12 13] rather than recovery of intestinal injury.

Various locations, including the liver and infraumbilical regions, have been selected for placing NIRS sensors to investigate the splanchnic oxygen saturation.[22] In a previous study, Schat *et al* observed that liver derived and intestinal derived FTOE values are not interchangeable.[22] In the present study, in the intestinal region the increase and decrease of FTOE values was more distinct than in the liver region. This could be explained by the liver's dual blood supply: in addition to receiving partially deoxygenated blood through the portal vein it receives oxygenated blood from the hepatic artery.[23]

This was the first study to relate FTOE values with a marker for intestinal damage. The strength of this study was the regularly obtained plasma samples and simultaneously measured cerebral and splanchnic rSO_2 values. Furthermore, we measured cerebral and splanchnic oxygenation for 48 consecutive hours.

A limitation was the relatively small sample size. We also did not include a control group. We included, however, all infants with definite NEC in our cohort prospectively. Irrespective of some missing data, we found some very strong associations. Unfortunately, although this was a prospective design, we were not always able to draw blood or place the NIRS sensor in these very ill and unstable infants, when clinical care dictated otherwise. Furthermore, the first 8 h after onset of first symptoms sometimes occurred in a referring hospital. At the end of the 48 h study period, some infants had already gone for surgery or died. This fact may have induced a selection bias.

While this study gives a proof of concept using both NIRS and I-FABPp to gather information on the pathophysiological chain of events resulting in progression or recovery of the intestinal tissue, it does not give insight on whether the intestinal perfusion plays a causal role in the development of complicated NEC or whether complicated NEC is the cause of poor intestinal perfusion. Future research in larger populations should investigate the exact cause of disease progression and differences in intestinal perfusion measures between infants with NEC and healthy preterm infants, since there is limited data of splanchnic FTOE values in healthy infants matched for (gestational) age and weight.

Another limitation is that intestinal NIRS measurements are possibly influenced by peristalsis, gut movements, feedings, air and stools, which all could complicate the interpretation of intestinal NIRS values. Because the feedings were withheld in all of the studied infants, it is unlikely that the different courses of FTOE between infants with complicated and uncomplicated NEC were related to the absence of enteral feedings. Furthermore, we cannot be sure which part of the intestine is specifically measured when using NIRS. However, because of lack of a gold standard, multisite NIRS has been shown to correlate strongly with other derivates of intestinal perfusion, such as venous oxygen saturation,[24 25] lactate levels[26] and SMA flow[7] measured with Doppler.

Our findings may have clinical implications. Combining splanchnic FTOE values and I-FABPp levels during early NEC might indicate infants who are at high risk of developing intestinal perforations. Early detection of impaired intestinal perfusion and hypoxia would be most helpful, because the assessment of intestinal necrosis and the timing of surgery for NEC, especially in the absence of perforation, remain difficult. It may also lead to new interventions, other than surgical ones, aimed at counteracting the progression of NEC into complicated disease. Further research is warranted to confirm this hypothesis.

CONCLUSION

We found strong associations between FTOE values of splanchnic tissue and I-FABPp levels early after NEC onset, suggesting that changes in intestinal perfusion accompanies intestinal damage. Also, cerebral FTOE as a measure for cerebral perfusion is related to I-FABPp levels, suggesting early systemic haemodynamic effects of NEC in preterm infants often lacking CAR.

Additionally, assessing both splanchnic FTOE as a measure for intestinal perfusion on the one hand and intestinal oxygen demand on the other, and I-FABPp levels as a measure for intestinal mucosal damage, we observed distinct patters between complicated and uncomplicated NEC. These patterns suggest ischaemia and necrosis of the intestines in complicated NEC versus intestinal hyperaemia and recovery in uncomplicated NEC.

This study can be regarded as a 'proof of concept' that assessing the combination of splanchnic FTOE and I-FABPp values gives insight in the pathological chain of events resulting in progression or recovery of intestinal ischaemia in NEC.

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Contributors TES conceptualised and designed the study, performed the study, drafted the initial manuscript, and approved the final manuscript as submitted. FHH: conceptualised and designed the study, performed the study, drafted the initial manuscript, and approved the final manuscript as submitted. MS conceptualised and designed the study, and approved the final manuscript as submitted. MEvdL contributed to the performance of the study, reviewed and revised the manuscript the manuscript, and approved the final manuscript as submitted. CVH reviewed and revised the manuscript and approved the final manuscript as submitted. AFB: reviewed and revised the manuscript and approved the final manuscript as submitted. EMWK: reviewed and revised the manuscript and approved the final manuscript as submitted. JBFH: supervised the study, reviewed and revised the manuscript and approved the final manuscript as submitted.

Competing interests None declared.

Patient consent Obtained.

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