The pathophysiology of necrotizing enterocolitis in preterm infants

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CHAPTER 4

TISSUE OXYGENATION AND INTESTINAL FATTY ACID-BINDING PROTEIN DURING NECROTIZING ENTEROCOLITIS

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Contents

Abstract

Objectives: The underlying pathophysiology of necrotizing 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) with intestinal fatty acid-binding protein in plasma (I-FABPp), a marker for intestinal damage, in infants with NEC. Furthermore, we investigated the 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 NIRS, we measured regional cerebral and splanchnic tissue oxygen saturations continuously for 48 hours 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) grams. Cerebral and splanchnic FTOE values correlated strongly with I-FABPp levels (rho between 0.745 and 0.900; $p<0.001$ - 0.037) during the first 16 hours 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.

Conclusion: Combining cerebral and splanchnic FTOE values with I-FABPp levels may enable us to discriminate between the progression of and recovery from intestinal damage in NEC.

Introduction

In preterm infants necrotizing 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.[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 mucosal damage.[12–15] I-FABP measurements can also accurately predict development of complicated disease.[12–15]

I-FABPp has been reported as a reliable marker for the extent of intestinal damage in NEC.[12–15] I-FABP measurements might also give insight in the progression of mucosal damage in infants with complicated NEC.[15] However, whether a decrease in I-FABP reflects healing of the mucosa or complete destruction of the mucosa remains unclear.

Our first aim was to investigate whether FTOE values reflect intestinal damage due to NEC by relating cerebral and splanchnic FTOE values with I-FABPp levels in preterm infants with NEC. Secondly, we investigated whether, during the first 48 hours after NEC onset, the courses of cerebral and splanchnic FTOE values combined with I-FABPp levels differed in infants with uncomplicated and complicated NEC.

Patients

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 ethical review board of the UMCG approved the study. 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 stabilized.

We measured I-FABPp levels repeatedly, and also continuously measured rSO_2 of cerebral and splanchnic tissue for 48 hours 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. After completing the study, a team of four consultants, blinded as to the results of the NIRS and I-FABP 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, i.e., Bell's stage ≥ 2. 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, i.e. laparotomy, were bowel perforation or lack of improvement despite optimal conservative therapy.

I-FABP 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 hours during the first 24 hours after NEC onset, and every 12 hours during the following 24 hours.
Blood samples were fractioned by centrifuging them for ten minutes at approximately 2000 x *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 hours after NEC onset. The penetration of NIRS is around 2-3 cm deep.[17]
To measure cerebral tissue oxygen saturation (r_cSO_2), 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 ($r_{liv}SO_2$), and infraumbilically on the central abdomen to measure intestinal oxygen saturation ($r_{int}SO_2$). 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 (Nellcor, Covidien). Although we intended to keep SpO2 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 that part of the arterial oxygen content that is indeed extracted by the tissue, correcting for periods of arterial hypoxia.[11] FTOE reflects the balance between oxygen supply and oxygen consumption and, thus, may serve as an indicator of impaired tissue perfusion.[11]

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 hemoglobin, 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 hemodynamically significant patent ductus arteriosus (PDA) 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 seconds and SpO_2 values every 5 minutes. We matched each SpO_2 value with the corresponding single rSO_2 value, leaving one coupled measurement every 5 minutes. 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 eight hours in the first 24 hours after NEC onset and once every 12 hours between 24 and 48 hours after NEC onset, we calculated 8-hour mean FTOE values in the first 24 hours after NEC onset and subsequently 12-hour 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 hours 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 22.0 software for Windows (IBM SPSS Statistics 22, IBM Corp., Armonk, NY, USA) for statistical analyses. We considered a p value <0.05 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 analyzing I-FABP levels (Figure 1). The median (range) time between NEC onset and the beginning of NIRS monitoring was 7 (2-31) hours.
In nine infants the course of NEC was uncomplicated. None of these infants died. Of the 10 infants with complicated NEC, two infants diagnosed with Bell's stage 3A died five and 35 days after NEC onset respectively. Eight infants with complicated NEC required surgery; seven underwent surgery during the study period, with a median of 33 hours (range 9-165) between the onset of NEC symptoms and surgery. We present the patient characteristics in Table 1.

FIGURE 1:
Flow diagram of the study

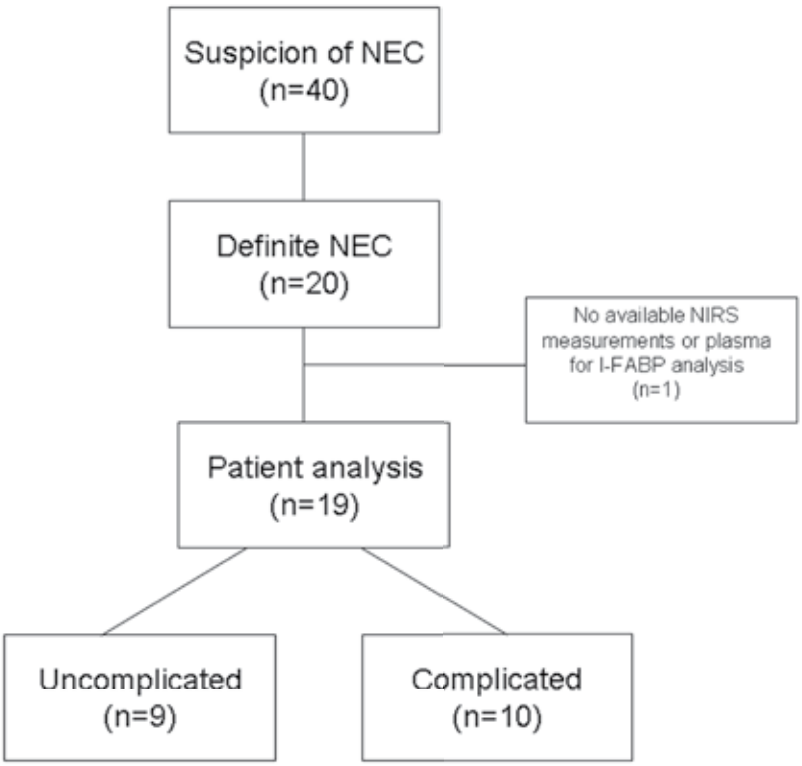


TABLE 1:
Patient characteristics

	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)
Hemoglobin	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)
Hemodynamically significant	-	2 (20)
RBC transfusion during NEC (%)	3 (33)	4 (40)
Fluid resuscitation	4 (44)	8 (80)
Inotropes (%)	-	6 (60)*
Surgery (%)	1 (11)	8 (80)*
Mortality (%)	-	7 (70)*

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

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

During the first 16 hours, 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 and 36 hours after NEC onset.

TABLE 2:

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

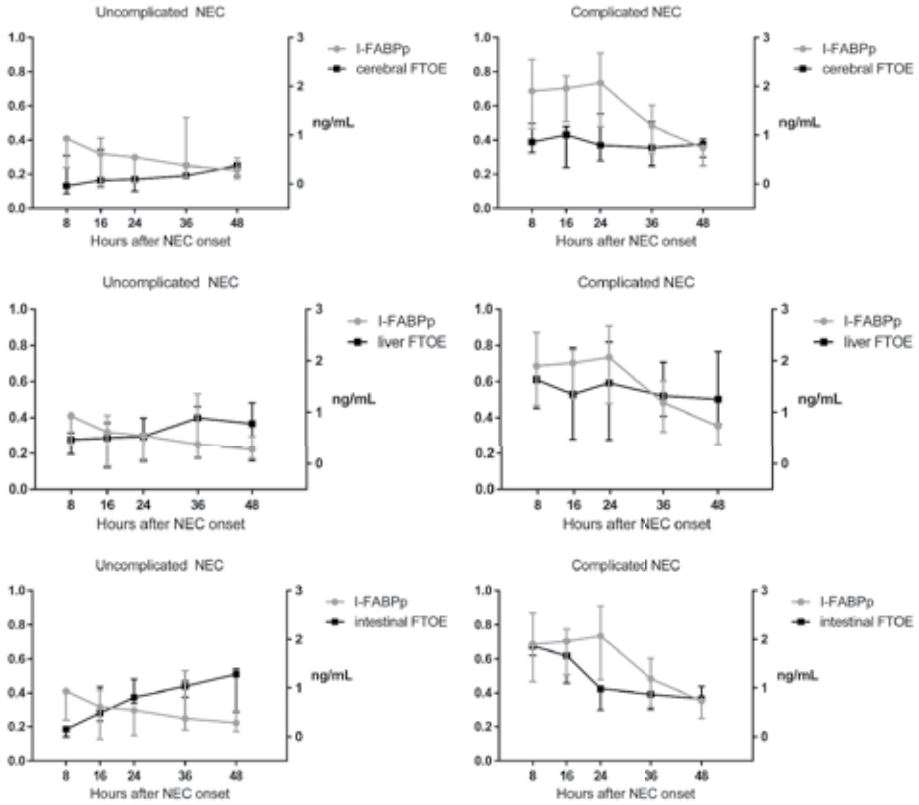
	cFTOE	livFTOE	intFTOE
0-8 hours			
I-FABPp	0.786* n=7	0.600 n=5	0.900* n=5
8-16 hours			
I-FABPp	0.891** n=11	0.881* n=8	0.745* n=10
16-24 hours			
I-FABPp	0.750 n=7	0.771 n=6	0.800 n=4
24-36 hours			
I-FABPp	0.731* n=13	0.573 n=11	-.0285 n=10
36-48 hours			
I-FABPp	0.510 n=12	0.100 n=11	0.188 n=10

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

FIGURE 2:

I-FABP en FTOE courses over time

Graphs showing the median (dots and squares) and interquartile range (lines) of splanchnic and cerebral FTOE values and I-FABPp levels of infants with uncomplicated and complicated NEC.



Courses of FTOE values and I-FABPp levels in infants with (un)complicated 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. During the first 16 hours after NEC onset we found little differences between infants with uncomplicated and complicated NEC. From 16 hours after NEC onset, however, the courses differed: in preterm infants with complicated NEC both splanchnic FTOE values and I-FABPp levels decreased and in infants with uncomplicated NEC splanchnic FTOE values increased whilst I-FABPp levels decreased. We observed this increase and decrease of splanchnic FTOE values particularly in the intestinal region, and not in the liver region.

Discussion

Our results demonstrated a strong association between cerebral and splanchnic FTOE values and I-FABPp levels during the first 16 hours after NEC onset. Furthermore, from 16 hours after NEC onset, we observed decreasing splanchnic FTOE values and I-FABPp levels in infants with complicated NEC, whilst infants with uncomplicated NEC showed increasing splanchnic FTOE values concomitant with decreasing I-FABPp levels.

NEC is characterized by coagulation necrosis of the intestinal wall, suggesting that intestinal ischemia is involved in its pathogenesis.[18] Our data suggested that high intestinal FTOE values in infants with complicated NEC reflect low intestinal perfusion during the first 8 to 16 hours of the development of NEC, because they concurred with high levels of I-FABPp, a marker for enterocyte damage.[12,13]

We offer several explanations for the strong associations between splanchnic FTOE values and I-FABPp levels during the first 16 hours after NEC onset. First, the presence of ischemia may cause intestinal epithelial cell damage. Second, intestinal ischemia and hypoxia may develop due to circulatory insufficiency in the presence of intestinal epithelial cell damage. Finally, intestinal circulation may be affected locally as a result of intestinal injury. Whatever the cause, our data suggested that splanchnic FTOE values could be used to gain information about the degree of intestinal damage during NEC.
Interestingly, we also observed strong associations between cerebral FTOE values and I-FABPp levels during the early phase of NEC and between 24 and 36 hours 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 hemodynamics. Thereby affecting cerebral oxygenation. Another explanation is that cerebrovascular autoregulation (CAR) in these preterm infants is impaired.[19,20]

In all infants with NEC, we found decreasing I-FABPp levels 16 hours 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. We identified two distinct patterns during the first 48 hours in which the disease was developing, differentiating uncomplicated NEC from complicated NEC.

In uncomplicated NEC, we observed low splanchnic FTOE values during the first 16 hours after NEC onset that increased over time. We hypothesize that hyperemia is present during the first hours after NEC onset due to an inflammatory response but this hyperemia 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, i.e., relatively high initial splanchnic FTOE values, and gradually decreasing intestinal FTOE during disease progression. We speculate that the initial high splanchnic FTOE values were the result of compromised intestinal perfusion. From 16 hours after NEC onset, a decreasing or absent intestinal metabolism due to the presence of necrotic bowel might result in decreasing splanchnic FTOE values. Decreasing I-FABPp levels in this case could have been the result of absent venous return from the necrotic bowel, and not 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.[21] In a previous study, Schat et al.[21] observed that liver-derived and intestinal derived FTOE values are not interchangeable. In the present study, 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.[22]

This was the first study to correlate 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 NEC in our cohort prospectively. Irrespective of some missing data, we found some very strong associations. Future research should investigate differences in intestinal perfusion measures between infants with NEC and healthy preterm infants. 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 cerebral and splanchnic tissue and I-FABPp levels during the first 16 hours after NEC onset, suggesting that FTOE values can be used to gain information about the degree of intestinal damage. Additionally, during the first 48 hours after NEC onset, we identified distinct splanchnic FTOE and I-FABPp courses in preterm infants with uncomplicated and complicated NEC. This finding suggests that impaired intestinal perfusion plays an important role early on in the development of complicated NEC.

Acknowledgments

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