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Intestinal fatty acid-binding protein levels in Necrotizing Enterocolitis correlate with extent of necrotic bowel: results from a multicenter study☆,☆☆,★,★★

F.H. Heida [a,*], J.B.F. Hulscher [a], M. Schurink [a], A. Timmer [b], E.M.W. Kooi [c], A.F. Bos [c], J.L.M. Bruggink [a], D.C. Kasper [e], M. Pones [d], T. Benkoe [d]

[a] Department of Pediatric Surgery, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, The Netherlands
[b] Department of Pediatric Pathology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, The Netherlands
[c] Department of Pediatric Neonatology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, The Netherlands
[d] Department of Pediatric Surgery, Medical University of Vienna, Austria
[e] Research Core Unit for Pediatric Biochemistry and Analytics, Medical University of Vienna, Austria

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ABSTRACT

Background: Intestinal fatty acid-binding protein (I-FABP) is considered as a specific marker for enterocyte damage in necrotizing enterocolitis (NEC).
Objective: The purpose of this study was to evaluate the association of plasma and urinary I-FABP levels with the extent of macroscopic intestinal necrosis in surgical NEC.
Methods: We combined data from prospective trials from two large academic pediatric surgical centers. Nine and 10 infants with surgical NEC were included, respectively. Plasma and urinary of I-FABP at disease onset were correlated with the length of intestinal resection during laparotomy.
Results: Median length of bowel resection was 10 cm (range 2.5–50) and 17 cm (range 0–51), respectively. Median I-FABP levels were 53 ng/mL (range 6.3–370) and 4.2 ng/mL (range 1.1–15.4) in plasma in cohort 1 respectively cohort 2 and 611 ng/mL (range 3–23,336) in urine. The length of bowel resection significantly correlated with I-FABP levels in plasma (Rho 0.68; $p = 0.04$ and Rho 0.66;$p = 0.04$) and in urine (Rho 0.92; $p = 0.001$).
Conclusion: This 'proof of concept' study demonstrates that plasma and urine I-FABP levels at disease onset was strongly associated with the length of intestinal resection in surgical NEC. This offers further evidence that I-FABP levels are a promising biomarker for assessing intestinal necrosis in infants with advanced NEC.

Necrotizing enterocolitis (NEC) is a severe intestinal inflammatory disorder of newborns associated with high mortality rates [1]. Initial treatment consists of discontinuation of enteral feeding, nasogastric suction, intravenous administration of broad-spectrum antibiotics, and cardiopulmonary support [2]. In the case of perforation or clinical deterioration despite maximal conservative treatment, resection of the affected bowel is often the treatment of choice [2,3]. The fact that symptoms and laboratory results are often unspecific during the early stages of disease makes a timely diagnosis of NEC challenging [4]. The assessment of intestinal necrosis and the timing of surgery, especially in the absence of perforation, remain as key problems in NEC. Furthermore, the decision of early surgical intervention might lead to an unnecessary laparotomy (including general anesthesia with its associated risks), while postponing surgery might lead to further disease progression with severe sepsis and eventual death [3,4]. Consequently, novel diagnostic and prognostic tools are in great demand.

In recent years, several promising diagnostic and prognostic markers for NEC have been identified. One of these markers is intestinal-fatty acid binding protein (I-FABP). I-FABP is a cytoplasmic protein with high organ sensitivity found in the enterocytes located at the tip of the villi. I-FABP plays a central role in the fat-metabolism processes of these cells [5,6]. In the context of progressive gut wall barrier failure in NEC, enterocytes are damaged and I-FABP is released in the circulation with subsequent secretion by the kidneys. In several studies I-FABP levels have been identified as an early marker and also as a predictor for the severity (including the need of surgical intervention) of NEC [5–7]. These studies assume a correlation between I-FABP levels and the degree of intestinal involvement. However, no study confirming the

Abbreviations: NEC, Necrotizing enterocolitis; ELISA, Enzyme-linked-Immune sorbent assay; I-FABP, Intestinal fatty acid-binding protein; NICU, Neonatal intensive care unit; UMCG, University Medical Centre Groningen; MUV, Medical University of Vienna.

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★★ Clinical Trial Registration: NoNEC trial, registered under trial number NTR3239 in the Dutch Trial Registry.

* Corresponding author at: Department of Pediatric Surgery, University of Groningen, University Medical Center of Groningen, Hanzeplein 1, 9713 GZ Groningen. Tel.: +31 613935058.

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correlation between I-FABP levels and the length of resected bowel is available. Therefore, we aimed to investigate the relation between I-FABP levels, as measured in plasma and urine, and the length of resected bowel (as a surrogate for the extent of tissue necrosis) in neonates with surgical NEC.

1. Methods

1.1. Patients

We conducted this multicenter study at the University Medical Center of Groningen (UMCG), termed as cohort 1, and at the Medical University of Vienna (MUV), termed as cohort 2. (See Table 1.)

In the UMCG all parents of neonates with suspected NEC admitted to the NICU were asked to participate in a prospective study (registered under trial number NTR3239) focusing on the diagnostic value of several biomarkers in suspected NEC cases between October 2010 and October 2012. The Institutional Review Board approved this study.

In the MUV blood samples were collected from infants exhibiting clinical signs and radiographic findings of NEC between January 2010 and July 2013. This prospective observational study aimed to evaluate the diagnostic value of different biomarkers in infants with proven NEC. The Institutional Review Board approved this study (study number EK875/2009).

Only infants with proven NEC (Bell's stage ≥ II) who needed surgery were included in the present study. Infants with isolated intestinal perforations were excluded in both centers. Indications for surgery were bowel perforation (NEC IIIb) or lack of improvement despite optimal conservative therapy [8]. The decision to go to the operating theater was always a multidisciplinary decision by the neonatology and pediatric surgery team caring for the neonate. Those specialists were not aware of I-FABP levels during the clinical course of the disease.

1.2. Surgery and resection specimens

All operations were performed by or under close supervision of the consultant pediatric surgeons in both centers. Only macroscopic necrotic tissue was resected, thereby saving as much as possible viable bowel. Resection material was measured two times in those neonates who underwent a laparotomy. During laparotomy the surgeon measured (using a tape-measure) or estimated the length of the affected bowel at its anti-mesenterial border before resecting it. The surgeon tried to avoid stretching of the tissue. After resection the specimen was fixed in 10% buffered formalin solution. To improve measurement objectivity, the length of the bowel was measured again by the pathologist with a tape-measure and any abnormalities were recorded. Representative sections of normal and diseased bowel were taken out and embedded in paraffin. Tissue sections of 4 μm were stained using haematoxylin and eosin using standard staining protocols. The same pathologist, who was blinded for the I-FABP data, examined the slides.

Table 1
Patient characteristics.

	UMCG	MUV
Patients (n)	9	10
Gender (m/f)	7/2	6/4
Gestational age (weeks + days)	26 + 5 (25–34)	27 (24–40)
Birth weight (grams)	1000 (670–2280)	1130 (590–3200)
Time of surgery after first symptoms (hours)	79 (3–816)	36 (8–172)
Final Bell's stage (n)	NEC 3a: 2 NEC 3b: 7	NEC 3a: 7 NEC 3b: 3
Resection (n)	Small intestine: 3 Colon: 5 Both: 1	Small intestine: 2 Colon: 1 Both: 4 Covered perforation: 3
Length of resection (cm)	10.7 (2.5–50)	17 (0–51)
I-FABP at onset of disease (ng/mL)	Plasma: 53 (6.3–370) Urine: 1,132 (3.3–23,335)	Plasma: 4.2 (1.1–15.4)

**Values are expressed as median (range) if applicable.

1.3. Sampling for I-FABP

Initial I-FABP levels were defined as the first recorded I-FABP levels in plasma and urine available after the onset of NEC. In cohort 1, an extra sample of 100 μL was obtained for study purposes with every routine blood analysis after NEC diagnosis. In cohort 2, I-FABP levels in plasma were collected only at onset of disease, and no I-FABP levels in urine were collected.

Blood samples were fractionated by centrifugation for 10 minutes at 2000 x *g*. Plasma was then collected in a 0.5 mL Sarstedt tube and stored at −80 °C. Approximately 1.5 mL of urine was transferred to a 2 mL Sarstedt tube and also stored at −80 °C. Urine samples were collected at regular intervals by placing a cotton wool swab in the diaper of the patients. Once saturated with urine the cotton wool was gently squeezed into a sterile syringe. In patients with an indwelling catheter, urine was collected directly from the catheter. Plasma and urinary I-FABP measurements were performed by a laboratory technician without knowledge of the clinical data. We used commercially available ELISAs for both urine and plasma I-FABP measurements (in cohort 1: Human FABP2 kit from R&D Systems, Minneapolis, America and in cohort 2: Hycult Biotech, Uden, the Netherlands). Sample workup was done according to the manufacturer's recommendations. Plasma samples were diluted at 1:20. Absorption was determined on a microplate reader (Statfax 3200, Awareness Technology Inc., Palm City, FL, US) at 450 nm.

1.4. Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics 21, IBM Corp., Armonk, New York, USA). All data are presented as median with range, unless otherwise specified. We compared baseline variables between the two centers using the Mann Whitney U test or the Chi squared test, where appropriate. For testing correlations between I-FABP levels and length of bowel resection the Spearman's correlation test was used. Testing correlations between I-FABP levels in plasma at onset of disease and the length of bowel resection in both cohorts were separated because the ELISAs used were not the same and therefore I-FABP levels might not be interchangeable. To test this correlation for the whole population a regression analysis was performed with the correction for differences between the cohorts. Two sided p-values less than 0.05 were considered statistically significant.

2. Results

2.1. Patients

We included 9 (m/f: 7/2) respectively 10 (m/f: 6/4) neonates with surgical NEC in both centers. All infants underwent a transverse laparotomy of which none were treated by percutaneous drainage. In cohort 1, median gestational age was 26 + 5 weeks (range 25–34) and median birth weight 1000 grams (range 670–2280). In cohort 2 this was 27 + 0 weeks (range 24–40) and 1130 grams (range 590–3200) respectively.

2.2. Surgery and length of specimens

Resections included: five neonates with a small intestine resection, six neonates with a colon resection, and five neonates with both a small intestine and colon resection. Three infants presented with characteristic morphologic changes of NEC (e.g. mosaic like pattern of affected intestine and pneumatosis intestinalis) and intestinal perforations in