Fecal Phagocyte-Specific S100A12 for Diagnosing Necrotizing Enterocolitis

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Objective To determine whether longitudinal measurements of fecal S100A12, a fecal marker of intestinal inflammation, can identify very low birth weight infants at risk for necrotizing enterocolitis (NEC).

Study design This prospective study included 145 preterm infants with birth weight <1500 g. Meconium and stool samples (n = 843) were collected prospectively on alternate days for 4 weeks, and fecal S100A12 and calprotectin were measured by enzyme-linked immunosorbent assay.

Results Eighteen patients (12.4%) developed NEC. Gestational age and birth weight were significantly lower in the patients with NEC compared with unaffected reference infants. Fecal S100A12 levels were significantly higher in patients with severe NEC at onset of disease and also, in contrast to fecal calprotectin, at 4-10 days before onset of NEC compared with unaffected reference infants (ideal cutoff value, 65 μg/kg; sensitivity, 0.76; specificity, 0.56).

Conclusions Fecal S100A12 level may be a helpful marker for predicting disease severity and early risk assessment for subsequent development of NEC. However, the use of fecal S100A12 as a predictive biomarker for NEC in very low birth weight infants may be limited due to a high interindividual and intraindividual variability in S100A12 fecal excretion. *(J Pediatr 2012;161:1059-64)*.

Necrotizing enterocolitis (NEC) is a serious gastrointestinal disorder of preterm infants,[1] with an incidence of 10%-15% and a mortality rate of 10%-30% in very low birth weight (VLBW) infants (birth weight <1500 g), as well as a high rate of long-term complications.[2-4] The pathogenesis of NEC remains largely unknown but is suspected to be multifactorial, involving such factors as gastrointestinal ischemia, enteral alimentation, and microorganisms in combination with immature gastrointestinal functions and host defense mechanisms. Early detection of NEC and adequate intervention could possibly improve the prognosis; however, initial clinical manifestations of NEC are nonspecific and are difficult to distinguish from other gastrointestinal disorders and neonatal sepsis. Thus, diagnosis is often delayed and limited by insufficient serum-based laboratory tests and current imaging modalities. Conventional fecal inflammatory markers (eg, calprotectin, lactoferrin) or urinary markers for enterocyte damage (eg, intestinal fatty acid binding protein) have not met the initially high expectations for diagnosing NEC.[1,5]

More recently, fecal S100A12 (calgranulin C) has been identified as a fecal marker of intestinal inflammation. S100A12 belongs to a novel group of proinflammatory molecules that play important roles in the mechanisms of innate immunity. In contrast to pathogen-associated molecular patterns proteins as exogenous factors initiating inflammation, S100A12 is one of the damage-associated molecular pattern proteins, endogenous molecules released by activated or damaged cells under conditions of cell stress. Phagocyte-specific damage-associated molecular pattern proteins of the S100 family are released from neutrophils or monocytes, followed by proinflammatory activation of pattern recognition receptors.[6-9] Thus, in intestinal inflammation, S100A12 is readily detectable in feces and plasma.[10,11] Furthermore, S100A12 is remarkably resistant to degradation by fecal bacteria, making fecal S100A12 a suitable marker for gut wall inflammation, as has been reported for inflammatory bowel disease.[12] The small amount of feces (50-100 mg) required for the measurement of fecal S100A12 by enzyme-linked immunosorbent assay (ELISA) makes this test potentially useful in preterm infants. We analyzed fecal S100A12 levels in VLBW infants to obtain baseline expression characteristics and to evaluate this protein's role as biomarker of intestinal inflammation in the screening for NEC and prediction of disease severity.

CRP	C-reactive protein
GA	Gestational age
ELISA	Enzyme-linked immunosorbent assay
IL-6	Interleukin 6
NEC	Necrotizing enterocolitis
ROC	Receiver operating characteristic
VLBW	Very low birth weight

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Funded by the HELIOS Research Center (Germany), which had no involvement in the study design; the collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the manuscript for publication. The authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2012.06.003

Methods

Patients were recruited from 5 German tertiary neonatal intensive care units (Wuppertal, Schwerin, Erfurt, Münster, and Krefeld) between April 2008 and December 2009. Ethical approval was obtained from the Ethics Committee of Witten/Herdecke University for all participating hospitals, and fully written informed consent was obtained from all legal guardians. All preterm infants with a birth weight <1500 g were included. Meconium and stool samples were collected prospectively on alternate days for at least 28 days. On admission, baseline characteristics and maternal information for enrolled infants were recorded. In addition, postnatal age, daily feeding regimen, respiratory support, laboratory and radiograph results, and clinical findings were recorded at the collection of each stool sample throughout follow-up.

Diagnosis of NEC was established based on typical clinical features and radiologic and laboratory findings. Disease stage (I, II, or III) was determined using the modified Bell classification scheme.[3,13] In brief, this classification scheme differentiates infants with NEC stage I (suspect), stage IIa (definite, mildly ill), stage IIb (definite, moderately ill), stage IIIa (advanced, severely ill with intact bowel), and stage IIIb (advanced, severely ill with bowel perforation).

The reference group consisted of all infants without signs and symptoms of intestinal distress. To obtain clear case definitions, patients without signs of NEC but with signs of other gastrointestinal disorders (eg, spontaneous intestinal perforation) were excluded from this study. Patients with an insufficient amount of stool to allow measurements of S100 proteins were excluded as well. Meconium was defined as the first stool passed after birth but no later than 72 hours after birth.

All stool samples were stored at −80°C before being analyzed within 24 hours of collection. S100A12 concentrations were determined by double-sandwich ELISA, as described previously.[14] Fecal calprotectin concentrations were determined by ELISA (Immundiagnostik, Bensheim, Germany). Analyses were performed by investigators in Münster, Germany who were blinded to the diagnosis and disease stage. All analyses were performed in triplicate.

Statistical Analyses

Statistical comparisons of the data between groups (unaffected control vs NEC) were performed using the Mann-Whitney *U* test. Data are presented as median and range except when stated otherwise. To determine the accuracy of S100A12 measurements, receiver operating characteristic (ROC) curves were drawn by plotting sensitivity against 1 - specificity. Overall accuracy of the markers in detecting NEC is represented by the area under the ROC curve with 95% CI. The best cutoff point is defined as the maximum sum of sensitivity and specificity. These cutoff points were used to calculate sensitivity, specificity, and positive and negative predictive values. All tests of significance were 2-tailed. A *P* value of <.05 was considered significant. All calculations were performed using SPSS version 14 (SPSS Inc, Chicago, Illinois).

Results

NEC in Patients

We enrolled 145 infants, including 18 (12.4%) who subsequently developed NEC and 127 without NEC or any other gastrointestinal distress (reference group) (Table). We excluded 5 patients with spontaneous intestinal perforation and 8 patients with insufficient stool samples. A total of 843 meconium and postmeconium stool samples were collected and analyzed. Gestational age (GA) and birth weight were significantly lower in the patients with NEC. According to the modified Bell scheme, 5 patients were classified as NEC stage IIa, 3 as stage IIb, 5 as stage IIIa, and 5 as stage IIIb. Eleven patients with NEC (61%) required surgery (all laparotomy), which confirmed the diagnosis of NEC. The mortality rate was 25% (n = 2) for patients in NEC stage II, 30% (n = 5) for those in NEC stage III, and 0.7% (n = 1) for those without NEC or gastrointestinal disorders. Causes of death were sepsis (*Klebsiella oxytoca*) with multiorgan failure, septic shock (*Clostridium difficile*) during surgery, and total intestinal necrosis (duodenum to rectum) with respiratory insufficiency in the patients with NEC stage III; septic shock without detectable pathogens and severe sepsis (*Candida glabrata*) in those with NEC stage II; and severe cerebral and pulmonary hemorrhage in the reference group.

S100A12 in Meconium

S100A12 concentrations were higher in meconium samples from VLBW infants before the onset NEC stage III (median, 398 μg/kg; range, 70-94 000 μg/kg; n = 10; $P < .05$) and lower

Table. Patient characteristics

	Control	NEC	*P* value
Patients, n (%)	127 (87.6)	18 (12.4)	NA
GA, weeks, median (range)	28.4 (23-35)	25.5 (23-33)	<.0005
Birth weight, g, median (range)	1082 (436-1490)	773 (354-1300)	<.0005
Sex ratio, M/F	0.91	1.71	NS
Maternal age, years, median (range)	30 (17-45)	31 (22-41)	NS
Apgar score at 1 minute, median (range)	6 (1-9)	5 (1-8)	NS
Apgar score at 5 minutes, median (range)	7 (2-10)	6 (4-9)	NS
Apgar score at 10 minutes, median (range)	9 (5-10)	8 (6-10)	NS
Umbilical artery pH, median (range)	7.34 (7.04-7.50)	7.34 (7.20-7.47)	NS
Vaginal birth, %	7.4	5.6	NS
Stool samples, n	671	172	NA
NEC stage IIa/IIb/IIIa/IIIb, n	NA	5/3/5/5	NA
Weight at diagnosis, g, median (range)	NA	834 (506-1520)	NA
Age at diagnosis, days, median (range)	NA	13 (2-28)	NA

NA, not applicable; *NS*, nonsignificant.