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Elevated Urinary iFABP Predicts Necrotizing Enterocolitis within Seven Days Prior to Clinical Onset

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Abstract

Necrotizing enterocolitis (NEC), characterized by sudden onset and rapid progression, remains the most significant gastrointestinal disorder among premature infants. In seeking a predictive biomarker, we found intestinal fatty acid binding protein, an indicator of enterocyte damage, was substantially increased within three and seven days before the diagnosis of NEC.

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in premature infants and the leading cause of aberrant growth and neurodevelopment among surviving preterm infants⁽¹⁻⁴⁾. NEC is especially problematic because of its often sudden onset and rapid progression, making it difficult to anticipate, diagnose, and treat. Along with targeting patterns of intestinal colonization and microbial signatures⁽⁵⁻⁷⁾, an early intestinal injury-specific biomarker of NEC is sought. We investigated intestinal fatty acid binding protein (iFABP) as a measure of enterocyte damage and candidate-biomarker of NEC.

iFABP is a 15-kDa cytoplasmic protein located in small intestinal enterocytes involved in the uptake and transport of polar lipids such as fatty acids from the small-bowel lumen^(8, 9). iFABP has been associated with injury to the intestinal mucosa⁽¹⁰⁾ and injury common to inflammatory bowel diseases⁽¹¹⁾, including NEC⁽¹²⁻¹⁷⁾. iFABP can be measured in serum⁽¹³⁾

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and urine^(12, 16, 17). This study assessed the early predictive relationship between urinary iFABP and NEC among very low gestational age infants.

Methods

Among a cohort of babies born prior to 29 weeks' gestation admitted to the neonatal intensive care unit at Brigham and Women's Hospital and enrolled in a study of lung biology (HL 67669), 165 of 1178 neonates born between 1997 and 2009 were diagnosed with NEC. Of these infants, 70 had urine samples available within seven days prior to NEC diagnosis. Disease severity was assessed using Bell Staging criteria⁽¹⁸⁾, and infants diagnosed with spontaneous intestinal perforation (SIP) were excluded from this study. Matching by gestational age at birth (<27 weeks or 27 to 29 weeks), we selected as a control the next baby born who did not have NEC and had a urine sample within seven days preceding the case's postnatal day of NEC diagnosis. Time of diagnosis was based on documentation of the attending neonatologist's diagnosis and initiation of treatment for NEC. Urine samples were collected from a cotton ball placed within the infant's diaper and stored at -80°C until analysis; clinical data were collected from medical records.

iFABP was measured from urine by ELISA (R&D Systems, Minneapolis, MN). For initial screening, all samples were diluted 10-fold in a bovine serum albumin-based diluent (R&D Systems) and those showing levels below or above the assay detection range were repeatedly tested undiluted or up to 100-fold dilution, respectively, to acquire accurate measurements. All measurements were performed in duplicate. A split quality control pool prepared from urine samples was tested on each plate showing inter-plate variation of 7.7%. Intra-plate variation of repeated measurements of the quality control pool was <9% [3.3% ± 3%]. Total protein concentrations were determined by BCA assay (Thermo Scientific, Rockford, IL) for iFABP normalization to mg total protein. These samples were screened at 20-fold dilution and repeated undiluted or up to 200-fold dilution, respectively.

Normality was assessed by Shapiro-Wilk test. Student t-test or Mann-Whitney U was used to analyze continuous variables, Chi-square test for categorical variables, Spearman Rank for correlation, and logistic regression for the relationship between iFABP and NEC while controlling for potential confounders. Cut-off iFABP values for predicting NEC were investigated with receiving operating characteristic analysis. SAS 9.3 (Cary, NC) was used for all statistical analyses.

Results

Demographic and clinical characteristics of NEC cases and controls were similar (Table). iFABP was not correlated with total protein levels ($r_s=0.10$) and thus is reported without adjustment. Median iFABP urine concentrations in 70 case-control pairs within seven days of NEC onset (n=140; Table and Figure, A), were higher among cases than controls ($p<0.001$). When stratified by Bell Stage, median iFABP was higher among cases and statistically significant in Bell Stages I and III ($p<0.005$). Analyses were performed using a subset of 98 infants (49 case-control pairs) who had urine samples available within three

days of NEC diagnosis. Among these, median iFABP was higher among cases ($p<0.001$), and was significant across all Bell Stages ($p<0.05$; Table and Figure, B).

Odds ratios (OR) for a 10-fold change in iFABP were 4.14, 95% CI [2.20, 7.81] ($p<0.001$) and 6.84, 95% CI [2.87, 16.31] ($p<0.001$) for seven-day and three-day analysis, respectively. ROC curves (Figure, C and D) illustrated that within seven days of NEC, iFABP>13.3 ng/mL would predict NEC with 60% sensitivity and 78% specificity (c-statistic=0.74), and within three days, iFABP>13.9 ng/mL was found to be 65% sensitive and 84% specific (c-statistic=0.80). Including mode of delivery, human milk exposure, growth restriction, antenatal steroids, and 5-minute Apgar score had minimal impact on the regression coefficient (iFABP OR 4.73 [2.44, 9.15] in the seven-day analysis) and the ROC results.

Discussion

Urinary iFABP may be a useful predictor of NEC within a week preceding diagnosis and is more strongly predictive within three days of NEC diagnosis. This relationship is most evident among infants with NEC stages I and III. The smaller difference in iFABP between NEC stage II cases and controls may be explained by the fact that infant pairs in this analysis were overall older at the time of measurement (median postnatal day of diagnosis was 17 compared with day 11 and 10 for stage I and III, respectively). Further analysis of iFABP based on gestational and postnatal age, reflecting both gastrointestinal maturity and clinical factors such as feeding, is needed for understanding its value as a measure of gastrointestinal function and injury in this patient population.

The findings reported here provide further evidence of the association between elevated iFABP and NEC previously observed in smaller studies^(12,13,15,17), suggesting that iFABP may be useful as a diagnostic biomarker for earlier identification of NEC. If combined with measures of intestinal inflammation (e.g. fecal calprotectin), NEC may be detected with even greater sensitivity and specificity⁽¹⁵⁾. Future work that integrates multiple measures of intestinal colonization and inflammation promises to change how we care for preterm infants so that we may prevent this devastating disease.

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Figure 1.

Urinary iFABP and NEC prior to disease onset.

Median iFABP within seven days prior to NEC onset.

Median iFABP within three days prior to NEC onset.

ROC Curve within seven days prior to NEC onset.

ROC Curve within three days prior to NEC onset.

Table 1

	NEC Stage I (n=18 pairs)			NEC Stage II (n=21 pairs)			NEC Stage III (n=31 pairs)		
	Cases	Controls	p	Cases	Controls	p	Cases	Controls	p
Gestational Age *	26.0 [25.0-27.7]	26.6 [25.0-27.3]	0.96	27.4 [26.2-28.4]	27.3 [26.3-28.0]	0.63	25.0 [24.43-26.3]	26.0 [24.57-26.71]	0.12
Birth Weight *	783 [680-975]	905 [795-1020]	0.39	1035 [830-1170]	900 [790-1090]	0.43	755 [620-885]	890 [660-940]	0.12
Male Sex **	7 (38.9)	11 (61.1)	0.18	14 (66.7)	14 (66.7)	1.0	22 (71.0)	13 (41.9)	0.02
Cesarean Birth **	10 (55.6)	11 (61.1)	0.74	14 (66.7)	18 (85.7)	0.15	20 (64.5)	23 (74.2)	0.41
Volume of First Feeding *	1.3 (1-1.7)	1.4 (1-1.9)	0.75	2 (1.1-2)	1.6 (1-2)	0.5	1.1 (1-1.4)	1 (1-1.5)	0.73
Human Milk as First Feeding *	9 [52.9]	13 [72.2]	0.24	5 [27.8]	12 [57.1]	0.07	20 [74.1]	21 [67.7]	0.6
Proportion of Human Milk Fed Prior to NEC Diagnosis **	0.63 (0-1)	1(0-1)	0.13	0.42 (0-0.96)	0.85 (0.05-0.99)	0.21	0.32 (0-1)	0.63 (0-1)	0.91
Growth Restriction **	3 (16.7)	1 (5.6)	0.60	2 (9.5)	4 (19.0)	0.66	6 (19.4)	5 (16.1)	0.74
Antenatal Steroids **	17 (94.4)	14 (77.8)	0.34	20 (95.2)	18 (85.7)	0.61	29 (93.6)	29 (93.6)	1.00
5 minute Apgar ** 0-4 5-7 8-10	3 (16.7) 9 (50.0) 6 (33.3)	1 (5.6) 9 (50.0) 8 (44.4)	0.58	2 (9.5) 10 (47.6) 9 (42.9)	1 (4.8) 12 (57.1) 8 (38.1)	0.81	5 (16.1) 18 (58.1) 8 (25.8)	3 (9.7) 17 (54.8) 11 (35.5)	0.59
iFABP (ng/mL) within 7 days of NEC *	15.4 [6.0-42.8]	3.8 [2.1-7.5]	0.005	17.8 [10.4-32.2]	10.2 [3.3-30.1]	0.125	15.9 [7.7-39.0]	2.9 [1.6-8.9]	<0.001
	NEC Stage I (n=14 pairs)			NEC Stage II (n=16 pairs)			NEC Stage III (n=19 pairs)		
iFABP (ng/mL) within 3 days of NEC *	18.4 [6.0-64.1]	4.0 [2.1-7.5]	0.006	21.8 [12.9-52.2]	8.7 [2.5-25.4]	0.036	16.7 [7.8-91.5]	2.4 [1.6-8.2]	<0.001

* Median [IQR] reported

** Number (%)