Stool Biomarkers to Diagnose Necrotizing Enterocolitis in Preterm Infants: A Pilot Case-Control Study

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Necrotizing Enterocolitis (NEC):

- Acute inflammatory disease of the intestine with high morbidity and mortality
- Most common in preterm infants with higher risk (2-10%) in VLBW (< 1500 g) infants
- Histopathologic hallmark is ischemic/coagulation necrosis
- Pathophysiology of NEC is characterized by defects in the intestinal epithelial barrier and gut wall inflammation
Problem Statement:

- Staging relies on non-specific clinical findings, radiological signs and laboratory data (Bell’s staging).
- It is sometimes difficult to diagnose NEC (prior to fulminant disease) because of its similarity in presentation to other clinical conditions.
- Signs and symptoms can overlap with other diseases (spontaneous intestinal perforation, feeding intolerance, allergic enteropathy, sepsis, intussusception).
Clinical Concern for NEC

Bell’s Stage I (Suspected Disease)
Medical Rx

Bell’s Stage II (Definitive Disease)
Medical Rx 7-14 days

Bell’s Stage III (Advanced Disease)
Consider Surgical Rx

DIAGNOSTIC DECISION

BIOMARKERS ??

THERAPEUTIC DECISION
Potential Utility of Biomarker?

- Make a definitive diagnosis
- Identify cases prior to onset of fulminant disease
- Identify mild (medical) from severe (surgical)
- Determine when inflammation has resolved
  - Timing of antibiotic discontinuation
  - Resumption of enteral feeding
Markers identified in stool have the advantage of being specific to intestinal pathology

- Loss of membrane integrity $\rightarrow$ release of I-FABP
- Released by phagocytes followed proinflammatory activation $\rightarrow$ S100A12
- Intestinal inflammation is characterized by sequestration of neutrophils $\rightarrow$ release of Calprotectin
Previous Studies:

• Urinary I-FABP levels of neonates with intestinal necrosis leading to surgery were significantly higher than conservatively treated neonates (Thuijls G et al. Ann Surg. 2010)

• Stool S100A12 were higher with severe NEC at onset of disease and higher 4-10 days before onset of NEC (Dabritz J, et al. J Pediatr 2012)

• Stool calprotectin was elevated at onset of NEC and calprotectin levels correlated with increasing severity of NEC (Dabritz J, et al. J Pediatr 2012; Ehab AM et al. J Clin Neonatol. 2014)

• Combination of urine I-FABP and stool calprotectin is promising for diagnosing NEC with improved diagnostic accuracy (Kostan WR et al. J. Peds Surg. 2012)
Hypothesis:

As compared to matched non-GI disease control infants, infants with NEC will have elevated stool levels of:

- I-FABP
- S100A12
- Calprotectin
Methods:

- Prospective case-control (1 case:2 controls)
- NEC cases: Bell’s staging ≥ 2
- Matched controls in concurrent patient registry:
  - Birth date (within 1 month)
  - Gestational age (± 1 week)
  - Birth weight (± 300 grams)
  - Post-menstrual age (± 2 weeks)
- Samples recruited from two level IIIB NICUs
- Institutional and Nursing Research Review Board approval
- Written informed consent was obtained from mother (and father, if available)
Sample processing and biomarker measurements

ELISA
I-FABP
S100A12
Calprotectin
Statistical analysis:

• Comparisons between cases and controls were made using Students t-test and Fisher’s exact test

• The diagnostic accuracy of each marker was evaluated with a receiver-operating characteristic (ROC) curve analysis

• $P < 0.05$ was considered statistically significant

• Results are presented as mean ± SEM
### Clinical Characteristics of Study Subjects:

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=8)</th>
<th>Controls (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (weeks)</td>
<td>28.2 ± 11.0#</td>
<td>28.6 ± 0.6</td>
</tr>
<tr>
<td>BW (grams)</td>
<td>1205 ± 168#</td>
<td>1216 ± 96</td>
</tr>
<tr>
<td>PMA (weeks)</td>
<td>29.7 ± 0.8#</td>
<td>30.6 ± 0.5</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>2/6#</td>
<td>8/8</td>
</tr>
</tbody>
</table>

*#P = NS vs. controls*
### Results:
**Mean Stool Biomarkers**

<table>
<thead>
<tr>
<th></th>
<th>I-FABP (ng/mg)</th>
<th>S100A12 (ng/mg)</th>
<th>Calprotectin (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases</strong></td>
<td>2.74 ± 1.4*</td>
<td>65.7 ± 29.3*</td>
<td>131.6 ± 34.7#</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>0.28 ± 0.02</td>
<td>10.3 ± 4.7</td>
<td>119.0 ± 12.9</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. controls; #P = NS vs. controls
I-FABP

7/8 cases showed greater stool I-FABP
5/8 cases showed greater S100A12 values
Calprotectin
I-FABP generated a statistically significant ROC curve with S100A12 approaching statistical significance.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Likelihood Ratio (LR+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-FABP (0.35 ng/mg)</td>
<td>75%</td>
<td>94%</td>
<td>12</td>
</tr>
<tr>
<td>S100A12 (54.9 ng/mg)</td>
<td>38%</td>
<td>94%</td>
<td>6</td>
</tr>
<tr>
<td>Calprotectin (60.5 ng/mg)</td>
<td>25%</td>
<td>94%</td>
<td>4</td>
</tr>
</tbody>
</table>
Summary

• Stool I-FABP and S100A12 show promise in diagnosing NEC in preterm infants with good specificity and moderate to large relative predictive values.

• However, the overlap of values with controls yield low sensitivity. This overlap may be due to the exact timing of sample collection relative to the intestinal inflammation and onset of NEC.

• Our study suggests that stool I-FABP is a good marker for diagnosing NEC with high diagnostic accuracy and LR >10.
Future…

• A larger longitudinal study is required to identify the appropriate timing of sample collection

• Combine described markers (stool I-FABP and S100A12) to evaluate usefulness in improving the diagnostic accuracy of early noninvasive detection of NEC
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