Blood Level of Inter-Alpha Inhibitor Proteins Distinguishes Necrotizing Enterocolitis From Spontaneous Intestinal Perforation

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Objective To examine circulating levels of inter-alpha inhibitor protein (IaIp) in infants with necrotizing enterocolitis (NEC), spontaneous intestinal perforation (SIP), and matched controls to assess the diagnostic accuracy of IaIp to differentiate NEC from SIP and to compare receiver operating characteristics of IaIp for NEC with C-reactive protein (CRP).

Study design A prospective, nested case-control study of infants with feeding intolerance was carried out. Blood and clinical data were collected from 27 infants diagnosed with NEC or SIP and from 26 matched controls admitted to our unit. Infants with modified Bell criteria stage 2 or greater were included as NEC. Clinical, radiologic, and/or surgical findings were used to identify infants with SIP. Controls were matched for gestational age, postnatal age, sex, and birth weight.

Results Mean ± SD IaIp blood levels were 147 ± 38 mg/L, 276 ± 67 mg/L, and 330 ± 100 mg/L in infants with NEC, SIP, and matched controls, respectively ($P < .004$ and $P < .01$). Receiver operating characteristics analysis to establish the predictive value of NEC demonstrated areas under curve of 0.98 and 0.63 for IaIp and CRP, respectively.

Conclusions IaIp levels were significantly decreased in infants with NEC compared with SIP and matched controls. The diagnostic accuracy of IaIp for NEC was superior to that of CRP. *(J Pediatr 2017;180:135-40).*

Necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP) are important causes of high morbidity and mortality in infants with very low birth weight.[1-3] Neurodevelopmental impairment among survivors is substantial in both and worse for infants with surgical NEC compared with SIP.[3] NEC affects 7%-10% of infants with very low birth weight.[1,2] Early and accurate diagnosis of NEC in preterm infants remains a major challenge in neonatology because its clinical features, for example, abdominal distention and bloody stools, can be nonspecific and indistinguishable from SIP and other gastrointestinal disorders.[2] The diagnosis traditionally is established radiographically (ie, pneumatosis intestinalis); however, there is significant interobserver variability in interpreting abdominal films of neonates with clinically suspected NEC.[4] Although the exact cause of NEC is still unclear, it is widely accepted that the pathogenesis of NEC includes mucosal injury with subsequent bacterial translocation across the intestinal epithelial layer and dysregulation of innate immune defenses leading to subsequent inflammation and tissue necrosis.[2]

SIP mainly affects infants with extremely low birth weight at an early postnatal age.[5,6] SIP is characterized as an isolated perforation without surrounding necrosis or neutrophil infiltrate, often accompanied by a focal thinning or absence of the intestinal muscularis propria.[7,8] The pathophysiology of SIP is poorly understood. Studies to date are mixed on whether postnatal corticosteroids and/or nonsteroidal anti-inflammatory agents are associated with SIP.[9-11] Affected infants have relative clinical stability in the early stages, lacking systemic signs and symptoms of severe illness.[12] They do not have radiologic features of pneumatosis intestinalis and portal venous gas as seen in NEC.

Despite these differences, NEC and SIP are treated similarly, partly because of the inability to distinguish between them preoperatively, as early clinical signs and symptoms are nonspecific and overlapping.[13] Differentiating NEC from SIP would advance our understanding of these disorders and be clinically useful to assist in institutional antibiotic stewardship efforts and in identifying infants who might benefit from novel and specific therapies.

There is a paucity of data on biomarkers to distinguish NEC from SIP. C-reactive protein (CRP) has been shown to be increased inconsistently in NEC, but no studies

CRP	C-reactive protein
IaIp	Inter-alpha inhibitor protein
NEC	Necrotizing enterocolitis
ROC	Receiver operating characteristic
SIP	Spontaneous intestinal perforation
SNAPPE-II	Score for Neonatal Acute Physiology Perinatal Extension II

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have examined CRP in SIP.[14] The inter-alpha inhibitor proteins (IaIps) are serine protease inhibitors secreted by the liver.[15] Two forms of IaIp are found in blood: inter-alpha inhibitor, a heterotrimeric, 250-kDa protein complex composed of 2 heavy chains and 1 light chain held together by glycosaminoglycan bonds and pre-alpha inhibitor, a heterodimeric, 125-kDa protein complex composed of 1 heavy and 1 light chain.[16] The light chain, bikunin, has a molecular weight of 30 kDa and is the active, antiprotease component.[17] The half-life of bikunin is very short and it is excreted rapidly by the kidneys. IaIp have been shown to be decreased in neonatal sepsis and NEC.[18,19] The current nested case-control study prospectively measured the circulating IaIp levels in infants with NEC, SIP, and controls. Our a priori hypotheses were that IaIp distinguishes infants with NEC from SIP and controls and that IaIp is superior to CRP for the early detection of NEC.

Methods

This nested case-control study was conducted at Women & Infants Hospital of Rhode Island from December 2012 to March 2015 after approval from the institutional review board. All live-born infants admitted to the neonatal intensive care unit were screened for feeding intolerance as evidenced by abdominal distention, abdominal tenderness, presence of blood in stool and/or increased gastric residuals (more than one-half the volume of feeds over the previous 3 hours) resulting in cessation of feedings, performance of abdominal radiographs, and blood sampling for complete blood count and blood cultures.[20,21] Exclusion criteria included decision made for comfort care only, major congenital anomalies, and genetic syndromes. Subsequently, infants were enrolled following written informed parental consent. Three of the eligible families did not consent to participate in the study, and no significant differences emerged between enrolled families and those that did not consent.

Infants with stage 2 or greater (modified Bell criteria) were included as NEC.[2] All of the cases with NEC had pneumatosis intestinalis. An independent pediatric radiologist who was unaware of the group assignment interpreted all abdominal radiographs. Pneumoperitoneum alone or bowel contents in the peritoneum after placement of an intraperitoneal drain in otherwise-stable infants that the attending neonatologist and attending surgeon classified as SIP and not NEC based on clinical, radiographic, and/or surgical finding were included as SIP.[13] Controls were infants who presented with nonspecific abdominal findings as described previously and were limited to Bell stage 1 (cession of feedings and/or antibiotics ≤2 days). Controls were matched for gestational age at birth ± 2 weeks, postnatal age ± 1 week, sex, and birth weight ± 500 g.

Maternal and infant clinical characteristics were recorded (**Tables I** and **II**). Bronchopulmonary dysplasia was defined as receipt of supplemental oxygen or continuous positive airway pressure at 36 weeks' postmenstrual age or death. The Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE-II) was used, because it has been shown to be a good predictor of mortality with area under the curve by using the receiver operating characteristic (ROC) analysis of 0.85 (95% CI 0.79-0.97).[22] All the physiological, laboratory, and therapy data for the first 12 hours after birth were collected prospectively to calculate SNAPPE-II, and the most aberrant values were used for scoring. The staff who collected the data from electronic medical records underwent training for consistency in recording into the database.

Circulating IaIp levels were measured quantitatively on residual blood obtained at the initial presentation. Blood was collected in tubes (Becton Dickinson Microtainer Systems, Franklin Lakes, New Jersey) containing EDTA for plasma or in plain tubes without anticoagulant for serum. The contents of collection tubes without anticoagulant were allowed to clot at room temperature for 30 minutes. Serum and plasma were separated by centrifuging at 1000-2000*g* for 10 minutes with the use of a refrigerated centrifuge and immediately transferred into a clean polypropylene tube. The samples were maintained at 2-8°C while handling and frozen at −70°C during storage. Archived blood samples were thawed at room temperature to measure IaIp. We measured IaIp on all residual samples that were available 7-10 days before and all samples after the initial diagnosis for longitudinal analysis. We used a competitive, enzyme-linked immunosorbent assay with a monoclonal antibody against human IaIp (monoclonal antibody 69.26), as described previously.[23] The assay has a sensitivity or lower limit of detection of 50 mg/L and a linear dynamic range of up to 750 mg/L. The intra-assay variability (coefficient of variation) is <3%, and the interassay coefficient of variation is <7%.[18] Previous studies have demonstrated that the IaIp levels are stable under the conditions of collection and storage used in this study.[11,12] CRP levels were measured in duplicate serum samples via the Quantikine enzyme-linked immunosorbent assay (R&D Systems Inc, Minneapolis, Minnesota). The staff who performed these assays were masked to the sample identity.

The sensitivity, specificity, positive, and negative predictive value for IaIp and CRP in diagnosis of NEC were determined by ROC analysis (MedCalc version 15.2.1, MedCalc Software, Ostend, Belgium; www.medcalc.be). The relationship between circulating IaIp and SNAPPE-II scores was assessed with Spearman correlation. Data analyses also included 1-way ANOVA and post-hoc Student-Newman-Keuls test for continuous variables. χ^2 test was performed for categorical variables as appropriate. Statistical calculations were performed using Statistica (StatSoft, Tulsa, Oklahoma) with $P < .05$ significant.

Results

During the study period, a total of 1934 neonates were admitted to the neonatal intensive care unit. Of these, 14 infants had a confirmed diagnosis of NEC (10 with Bell stage II and 4 with Bell stage III), 13 infants had SIP, and 26 infants were enrolled as matched controls.

Table I shows the maternal demographic and clinical variables. There were no significant differences between the 3 groups in maternal age, race, prenatal care, hypertension,