Published in final edited form as:

J Pediatr. 2017 January ; 180: 135–140.e1. doi:10.1016/j.jpeds.2016.09.016.

Blood Level of Inter-alpha Inhibitor Proteins Distinguishes Necrotizing Enterocolitis from Spontaneous Intestinal Perforation

Birju A Shah, MD, MPH^{1,2}, Alison Migliori, BS², Itsuka Kurihara, BS², Surendra Sharma, PhD², Yow-Pin Lim, MD, PhD^{3,4}, and James Padbury, MD²

¹Department of Pediatrics, Division of Neonatal-Perinatal Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK

²Department of Pediatrics, Women and Infants Hospital, Alpert Medical School of Brown University, Providence RI

³ProThera Biologics Inc., Providence, RI

⁴Department of Pathology and Laboratory Medicine, Alpert Medical School of Brown University, Providence RI

Abstract

Objective—To examine circulating levels of inter-alpha inhibitor protein (IaIp) in infants with necrotizing enterocolitis (NEC), spontaneous intestinal perforation (SIP) and matched controls in order to assess the diagnostic accuracy of IaIp to differentiate NEC from SIP; and to compare receiver operating characteristics (ROC) 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's criteria stage 2 or higher were included as NEC. Clinical, radiological 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<0.004 and P<0.01). ROC 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.

Correspondence: Birju A Shah, Birju-Shah@ouhsc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The other authors declare no conflicts of interest.

Portions of the study were presented as an abstract at the annual meeting of the Pediatric Academic Societies, San Diego, CA, April 25–28, 2015.

Keywords

preterm; neonate; intensive care unit; diagnosis; biomarker; NEC; neurodevelopment; CRP

Necrotizing enterocolitis (NEC) and spontaneous intestinal perforation (SIP) are important causes of high morbidity and mortality in very-low-birth-weight (VLBW) infants.(1–3) Neurodevelopmental impairment (NDI) among survivors is substantial in both, and worse for infants with surgical NEC as compared with SIP.(3) NEC affects 7% to 10% of VLBW infants.(1, 2) Early and accurate diagnosis of NEC in preterm infants remains a major challenge in neonatology as its clinical features, for example, abdominal distention and bloody stools, can be nonspecific and indistinguishable from SIP and other GI disorders.(2) The diagnosis is traditionally established radiographically (ie, pneumatosis intestinalis). However, there is significant inter-observer 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 NEC pathogenesis 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 extremely low-birth-weight infants (ELBW) 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 non-steroidal 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 due to inability to distinguish between them preoperatively, as early clinical signs and symptoms are non-specific 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 inconsistently increased in NEC but no studies have examined CRP in SIP.(14) The inter-alpha inhibitor family of proteins (IaIp) are serine protease inhibitors secreted by the liver.(15) Two forms of IaIp are found in blood: Inter-alpha inhibitor, a heterotrimeric, 250 kilo Dalton (kD) protein complex composed of two heavy chains and one light chain held together by glycosaminoglycan bonds and Pre-alpha inhibitor, a heterodimeric, 125 kD protein complex composed of one heavy and one light chain.(16) The light chain, Bikunin, has a molecular weight of 30 kD and is the active, anti-protease component.(17) The half-life of Bikunin is very short and it is rapidly excreted 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 NEC from SIP and controls, and that IaIp is superior to CRP for 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 Institutional Review Board approval. All live-born infants admitted to the neonatal intensive care unit (NICU) were screened for feeding intolerance as evidenced by abdominal distention, abdominal tenderness, presence of blood in stool and/or increased gastric residuals (more than half the volume of feeds over prior three 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 higher (modified Bell's criteria) were included as NEC.(2) All of the NEC cases 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 which 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 above and were limited to Bell stage 1 (cessation of feedings and/or antibiotics 2 days). Controls were matched for gestational age at birth $\pm$ 2 weeks, postnatal age $\pm$ 1 week, sex, and birth weight $\pm$ 500 grams.

Maternal and infant clinical characteristics were recorded (Tables I and II). Bronchopulmonary dysplasia was defined as receipt of supplemental oxygen or CPAP at 36 weeks' postmenstrual age or death. The Score for Neonatal Acute Physiology Perinatal Extension II (SNAPPE-II) was used as 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 prospectively collected 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, N.J.) 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 min. Serum and plasma were separated by centrifuging at 1,000–2,000 $\times$ g for 10 minutes using 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 [CV]) is <3%, and the inter-assay CV 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 using the Quantikine enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, MN, USA). 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 using Spearman correlation. Data analyses also included one-way analysis of variance and post-hoc Student-Newman-Keul test for continuous variables. χ^2 test was performed for categorical variables as appropriate. Statistical calculations were performed using Statistica® (StatSoft, Tulsa, Oklahoma) with $P<0.05$ significant.

RESULTS

During the study period, a total of 1934 neonates were admitted to the NICU. Of these, fourteen infants had a confirmed diagnosis of NEC (ten with Bell stage II and four with Bell stage III), thirteen infants had SIP and twenty-six 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, insulin-dependent diabetes mellitus, prolonged rupture of membranes, histologic chorioamnionitis, antibiotics, antenatal steroids, and breast feeding.

Table II shows the clinical characteristics of the infant study population. The mean $\pm$ SD corrected gestational age for initial presentation of NEC in our study was 29.6 $\pm$ 2 weeks. The SIP infants had lower mean gestational age and birth weight than others. They also had a mean day of initiation of feeds that was later than that for the other groups and higher days on parenteral nutrition, but these differences were not statistically significant. The majority of infants with SIP had not been fed or had only recently initiated feedings. There were significantly more infants who received initial antibiotic treatment for more than 48 hours in SIP group compared with NEC and controls. Additionally, there were significant differences among the three groups in culture-proven sepsis, age at initiation of enteral feeds, duration of parenteral nutrition and the incidence of retinopathy of prematurity.

When compared with SIP and matched controls, circulating IaIp levels were significantly lower in infants with NEC ($P<0.004$ and $P<0.01$) at the time of initial presentation (Figure 1). Blood levels of IaIp remained significant on subgroup analysis of the infants with

diagnosis established by tissue pathology ($P<0.05$). CRP levels were not statistically different among the three groups ($p>0.05$). There were no statistically significant differences in blood levels of IaIp between NEC Bell stage II and stage III ($p>0.05$).

ROC analysis for NEC yielded an area under the curve (AUC) of 0.98 ($P<0.0001$, 95% confidence interval, CI; 0.84–0.99) with a sensitivity of 100%, specificity of 88.2%, positive predictive value (PPV) of 41%, and a negative predictive value (NPV) of 100% for IaIp (<207 mg/L); the AUC for CRP (<4 mg/L) was 0.65 ($P=0.01$, 95% CI; 0.54–0.90) with a sensitivity of 100%, specificity of 64.7%, PPV of 18.7% and NPV of 98% (Figure 2).

The mean $\pm$ SD IaIp levels in blood after initial presentation were significantly lower in infants with NEC when compared with infants with SIP ($P<0.05$) (Figure 3; available at www.jpeds.co). We also found a significant negative correlation between circulating IaIp levels at disease presentation and SNAPPE-II scores (first 12 hours of life) in all studied infants ($r=-.32$, $P<0.05$) (Figure 4; available at www.jpeds.com).

DISCUSSION

This study reports the circulating IaIp levels in infants with NEC, SIP and matched controls evaluated prospectively and longitudinally. Circulating IaIp levels were significantly reduced in infants with NEC compared with infants with SIP and controls with feeding intolerance at presentation. This was followed by a gradual increase during subsequent therapy to normal levels when measured serially. These data demonstrate a striking difference in the degree of innate immune system dysregulation (lower IaIp levels) associated with NEC.

Inter-alpha Inhibitor proteins (IaIp) are endogenous serine protease inhibitors found in human plasma at relatively high concentrations.(23) Their circulating levels are independent of gestational age, postnatal age and are similar to adult levels.(19) They are significantly reduced in patients with sepsis in both adults and neonates.(19, 23) IaIp are considered negative acute-phase proteins (24) that, as part of the innate immune response, protect against the damaging effects of proteases released during acute systemic inflammation following severe infections.(17) As a consequence, these proteins are rapidly “consumed” and excreted in the urine, leading to a rapid decrease in circulatory levels. In addition, in several adult and newborn animal models of sepsis, IaIp replacement therapy has been demonstrated to reverse the decrease in systemic IaIp levels and reduce sepsis related mortality. (25–29)

At least three distinct mechanisms in the inflammatory pathway appear to contribute to protective role of IaIp: 1) inhibition of destructive serine proteases such as elastase, cathepsin G, and several others (17); 2) down-regulation of pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-6 (25, 26); and 3) blocking excess complement activation and generation of circulating C5a (30). Because NEC is associated with systemic inflammation, IaIp are involved in modulating the host response to systemic immune system activation that accompanies inflammation. More recently, IaIp were shown to bind and inhibit the damaging effects of extracellular histones both in vitro and in vivo. (31) Histones elicit major cytotoxic, thrombotic, and inflammatory effects and serve as

danger-associated molecular patterns (DAMPs/alarmins).(32) This is further supported by animal studies in IL-10^{-/-} mice in which newborns housed under non-aseptic conditions develop NEC-like symptoms. (33)

Hematological indices, cytokines and other mediators have been examined for diagnosis of NEC. Individual markers like serum amyloid A, anaphylatoxin (C5a), urinary intestinal fatty acid-binding protein, claudin-3, stool PAF, and calprotectin may support the diagnosis of NEC.(34–37) Downregulation of micro-RNA (miRNA) such as miR-31 and miR-203 has been proposed to potentiate pro-inflammatory signaling mediated by bacterial toll-like receptor (TLR)-4. In contrast, upregulation of miR-451 and miR-223 in SIP suggests modulation of G-protein-mediated muscle contraction.(38) However, none of these markers have shown to be reliable and clinically useful.

CRP is an acute phase reactant synthesized by the liver and has a half-life of 24–48 hours. Because it takes 10–12 hours to change significantly after the onset of infection, the sensitivity of CRP is low during the early phase of sepsis.(15) It can be elevated in non-infectious conditions like meconium aspiration syndrome, traumatic or ischemic tissue injuries, hemolysis, or histologic chorioamnionitis.(15) Nonetheless, the association between persistently high levels of CRP and NEC related complications has been shown.(39) Here we used the unbiased estimate (ROC modeling) of IaIp to determine a cutoff value of <207 mg/L. With this value, assessment of IaIp level would have a robust diagnostic accuracy with a high sensitivity, specificity, and negative predictive value. We also found the diagnostic accuracy of IaIp to be superior to CRP for NEC.

A limitation of this study is the modest sample size. There may be confounders of which we remain unaware. It is also possible that the disease processes start before clinical signs and symptoms develop when the blood samples were collected. But because there are no reliable biomarkers currently available this is difficult to evaluate. Moreover, serial samples were not available for all infants with NEC and SIP at similar time points. Our study was not powered to distinguish “classic” NEC from the disease that presents within 48 of a packed RBC transfusion. Finally, the diagnosis of NEC vs. SIP was not confirmed histologically in all cases, as five infants randomized to bedside peritoneal drain as per the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development necrotizing enterocolitis surgery trial. A strength of this study is the prospective, population-based, unbiased collection of samples from all patients in a single, regional center over a defined time period. Moreover, these data may allow for early discrimination of NEC from SIP, which will be an important consideration for proper assignment and follow-up as new clinical therapies are tested.

In summary, we demonstrated IaIp may assist in early detection of NEC and distinguish NEC from SIP and other, nonspecific abdominal disorders of preterm infants. We confirmed that circulating IaIp levels were significantly decreased in patients with NEC. As a biomarker, this distinction at presentation may lead to earlier effective treatments and improved outcomes. Because IaIp correlated with disease progression when measured serially, it may serve as a surrogate to monitor response to therapy in NEC. Further

multicenter studies with large sample sizes are needed to validate these findings in other populations.

Acknowledgments

Supported in part by the National Institutes of Health (R43 HD069243). Y.-P.L. is the chief executive officer at ProThera Biologics, where the IaIp assays used in these experiments were carried out, blinded to the design of the study, and he has an equity interest in the company.

We acknowledge the parents of the infants who participated in the study and entire NICU staff for their help with patient enrollment. We also express our appreciation to Bethany McGonnigal and Rakesh Singh (Women & Infants Hospital), Joseph Qiu and Andre Santoso (ProThera Biologics), and Edgardo Szyld (University of Oklahoma) for their technical assistance.

Abbreviations

CRP	C-reactive protein
IaIp	Inter-alpha Inhibitor proteins
LBW	Low-birth-weight
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
SIP	Spontaneous Intestinal Perforation
ROC	Receiver operating characteristic

REFERENCES

1. Lin PW, Stoll BJ. Necrotising enterocolitis. *Lancet*. 2006; 368(9543):1271–1283. [PubMed: 17027734]
2. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011; 364(3):255–264. [PubMed: 21247316]
3. Wadhawan R, Oh W, Hintz SR, Blakely ML, Das A, Bell EF, et al. Neurodevelopmental outcomes of extremely low birth weight infants with spontaneous intestinal perforation or surgical necrotizing enterocolitis. *J Perinatol*. 2014; 34(1):64–70. [PubMed: 24135709]
4. Mata AG, Rosengart RM. Interobserver variability in the radiographic diagnosis of necrotizing enterocolitis. *Pediatrics*. 1980; 66(1):68–71. [PubMed: 7402793]
5. Meyer CL, Payne NR, Roback SA. Spontaneous, isolated intestinal perforations in neonates with birth weight less than 1,000 g not associated with necrotizing enterocolitis. *J Pediatr Surg*. 1991; 26(6):714–717. [PubMed: 1941464]
6. Okuyama H, Kubota A, Oue T, Kuroda S, Ikegami R, Kamiyama M. A comparison of the clinical presentation and outcome of focal intestinal perforation and necrotizing enterocolitis in very-low-birth-weight neonates. *Pediatr Surg Int*. 2002; 18(8):704–706. [PubMed: 12598969]
7. Lai S, Yu W, Wallace L, Sigalet D. Intestinal muscularis propria increases in thickness with corrected gestational age and is focally attenuated in patients with isolated intestinal perforations. *J Pediatr Surg*. 2014; 49(1):114–119. [PubMed: 24439593]
8. Izraeli S, Freud E, Mor C, Litwin A, Zer M, Merlob P. Neonatal intestinal perforation due to congenital defects in the intestinal muscularis. *Eur J Pediatr*. 1992; 151(4):300–303. [PubMed: 1499584]

9. Kelleher J, Salas AA, Bhat R, Ambalavanan N, Saha S, Stoll BJ, et al. Prophylactic indomethacin and intestinal perforation in extremely low birth weight infants. *Pediatrics*. 2014; 134(5):e1369–e1377. [PubMed: 25349317]
10. Attridge JT, Clark R, Gordon PV. New insights into spontaneous intestinal perforation using a national data set (3): antenatal steroids have no adverse association with spontaneous intestinal perforation. *J Perinatol*. 2006; 26(11):667–670. [PubMed: 17024144]
11. Attridge JT, Clark R, Walker MW, Gordon PV. New insights into spontaneous intestinal perforation using a national data set: (2) two populations of patients with perforations. *J Perinatol*. 2006; 26(3):185–188. [PubMed: 16493433]
12. Pumberger W, Mayr M, Kohlhauser C, Weninger M. Spontaneous localized intestinal perforation in very-low-birth-weight infants: a distinct clinical entity different from necrotizing enterocolitis. *J Am Coll Surg*. 2002; 195(6):796–803. [PubMed: 12495312]
13. Blakely ML, Tyson JE, Lally KP, McDonald S, Stoll BJ, Stevenson DK, et al. Laparotomy versus peritoneal drainage for necrotizing enterocolitis or isolated intestinal perforation in extremely low birth weight infants: outcomes through 18 months adjusted age. *Pediatrics*. 2006; 117(4):e680–e687. [PubMed: 16549503]
14. Evennett N, Alexander N, Petrov M, Pierro A, Eaton S. A systematic review of serologic tests in the diagnosis of necrotizing enterocolitis. *J Pediatr Surg*. 2009; 44(11):2192–2201. [PubMed: 19944232]
15. Shah BA, Padbury JF. Neonatal sepsis: an old problem with new insights. *Virulence*. 2014; 5(1): 170–178. [PubMed: 24185532]
16. Wachter E, Hochstrasser K. Kunitz-type proteinase inhibitors derived by limited proteolysis of the inter-alpha-trypsin inhibitor, IV. The amino acid sequence of the human urinary trypsin inhibitor isolated by affinity chromatography. *Hoppe Seylers Z Physiol Chem*. 1981; 362(10):1351–1355. [PubMed: 6171496]
17. Fries E, Blom AM. Bikunin--not just a plasma proteinase inhibitor. *Int J Biochem Cell Biol*. 2000; 32(2):125–137. [PubMed: 10687949]
18. Chaaban H, Shin M, Sirya E, Lim YP, Caplan M, Padbury JF. Inter-alpha inhibitor protein level in neonates predicts necrotizing enterocolitis. *J Pediatr*. 2010; 157(5):757–761. [PubMed: 20955849]
19. Baek YW, Brokat S, Padbury JF, Pinar H, Hixson DC, Lim YP. Inter-alpha inhibitor proteins in infants and decreased levels in neonatal sepsis. *J Pediatr*. 2003; 143(1):11–15. [PubMed: 12915817]
20. Jadcherla SR, Kliegman RM. Studies of feeding intolerance in very low birth weight infants: definition and significance. *Pediatrics*. 2002; 109(3):516–517. [PubMed: 11875150]
21. Schanler RJ, Shulman RJ, Lau C, Smith EO, Heitkemper MM. Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. *Pediatrics*. 1999; 103(2):434–439. [PubMed: 9925837]
22. Harsha SS, Archana BR. SNAPPE-II (Score for Neonatal Acute Physiology with Perinatal Extension-II) in Predicting Mortality and Morbidity in NICU. *J Clin Diagn Res*. 2015; 9(10):SC10–SC12. [PubMed: 26557585]
23. Lim YP, Bendelja K, Opal SM, Siryaporn E, Hixson DC, Palardy JE. Correlation between mortality and the levels of inter-alpha inhibitors in the plasma of patients with severe sepsis. *J Infect Dis*. 2003; 188(6):919–926. [PubMed: 12964125]
24. Salier JP, Rouet P, Raguenez G, Daveau M. The inter-alpha-inhibitor family: from structure to regulation. *Biochem J*. 1996; 315(Pt 1):1–9. [PubMed: 8670091]
25. Yang S, Lim YP, Zhou M, Salvemini P, Schwinn H, Josic D, et al. Administration of human inter-alpha-inhibitors maintains hemodynamic stability and improves survival during sepsis. *Crit Care Med*. 2002; 30(3):617–622. [PubMed: 11990925]
26. Singh K, Zhang LX, Bendelja K, Heath R, Murphy S, Sharma S, et al. Inter-alpha inhibitor protein administration improves survival from neonatal sepsis in mice. *Pediatr Res*. 2010; 68(3):242–247. [PubMed: 20520583]
27. Opal SM, Artenstein AW, Cristofaro PA, Jhung JW, Palardy JE, Parejo NA, et al. Inter-alpha-inhibitor proteins are endogenous furin inhibitors and provide protection against experimental anthrax intoxication. *Infect Immun*. 2005; 73(8):5101–5105. [PubMed: 16041026]

28. Opal SM, Lim YP, Cristofaro P, Artenstein AW, Kessimian N, Delsesto D, et al. Inter-alpha inhibitor proteins: a novel therapeutic strategy for experimental anthrax infection. *Shock*. 2011; 35(1):42–44. [PubMed: 20523269]
29. Wu R, Cui X, Lim YP, Bendelja K, Zhou M, Simms HH, et al. Delayed administration of human inter-alpha inhibitor proteins reduces mortality in sepsis. *Crit Care Med*. 2004; 32(8):1747–1752. [PubMed: 15286553]
30. Garantziotis S, Hollingsworth JW, Ghanayem RB, Timberlake S, Zhuo L, Kimata K, et al. Inter-alpha-trypsin inhibitor attenuates complement activation and complement-induced lung injury. *J Immunol*. 2007; 179(6):4187–4192. [PubMed: 17785858]
31. Chaaban H, Keshari RS, Silasi-Mansat R, Popescu NI, Mehta-D'Souza P, Lim YP, et al. Inter-alpha inhibitor protein and its associated glycosaminoglycans protect against histone-induced injury. *Blood*. 2015; 125(14):2286–2296. [PubMed: 25631771]
32. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. *Blood*. 2014; 123(18):2768–2776. [PubMed: 24366358]
33. Emami CN, Chokshi N, Wang J, Hunter C, Guner Y, Goth K, et al. Role of interleukin-10 in the pathogenesis of necrotizing enterocolitis. *Am J Surg*. 2012; 203(4):428–435. [PubMed: 22450025]
34. Tayman C, Tonbul A, Kahveci H, Uysal S, Koseoglu B, Tatli MM, et al. C5a, a complement activation product, is a useful marker in predicting the severity of necrotizing enterocolitis. *Tohoku J Exp Med*. 2011; 224(2):143–150. [PubMed: 21628869]
35. Thuijls G, Derikx JP, van Wijck K, Zimmermann LJ, Degraeuwe PL, Mulder TL, et al. Non-invasive markers for early diagnosis and determination of the severity of necrotizing enterocolitis. *Ann Surg*. 2010; 251(6):1174–1180. [PubMed: 20485148]
36. Rabinowitz SS, Dzakpasu P, Piecuch S, Leblanc P, Valencia G, Kornecki E. Platelet-activating factor in infants at risk for necrotizing enterocolitis. *J Pediatr*. 2001; 138(1):81–86. [PubMed: 11148517]
37. Yang Q, Smith PB, Goldberg RN, Cotten CM. Dynamic change of fecal calprotectin in very low birth weight infants during the first month of life. *Neonatology*. 2008; 94(4):267–271. [PubMed: 18784422]
38. Ng PC, Chan KY, Leung KT, Tam YH, Ma TP, Lam HS, et al. Comparative MiRNA Expressional Profiles and Molecular Networks in Human Small Bowel Tissues of Necrotizing Enterocolitis and Spontaneous Intestinal Perforation. *PLoS One*. 2015; 10(8):e0135737. [PubMed: 26274503]
39. Pourcyrus M, Korones SB, Yang W, Boulden TF, Bada HS. C-reactive protein in the diagnosis, management, and prognosis of neonatal necrotizing enterocolitis. *Pediatrics*. 2005; 116(5):1064–1069. [PubMed: 16263990]

Figure 1.
Circulating levels of IaIp (A) and CRP (B) in infants with NEC (n=14), SIP (n=13) & matched controls (n=26) at initial presentation

Figure 2.

Receiver operating characteristic (ROC) curves of IaIp and CRP for NEC

Figure 3.
Serial circulating IaIp levels in infants with NEC & SIP (n=128)

Figure 4. Circulating IaIp at disease presentation negatively correlated with SNAPPE-II (first 12 hours of life) in infants with NEC (n=14), SIP (n=13) and matched controls (n=26)

Table 1

Maternal demographic and clinical variables

Variable	NEC (n=14)	SIP (n=13)	Controls (n=26)
Maternal age, mean±SD	27.1 ± 5.9	25.8 ± 6.7	29.5 ± 6.8
Race, n (%)			
White	7 (50)	9 (69)	14 (54)
African American	1 (7)	1 (8)	6 (23)
Hispanic	5 (36)	3 (23)	4 (15)
Asian	1 (7)	0 (0)	1 (4)
Other race	0 (0)	0 (0)	1 (4)
Married, n (%)	8 (57)	1 (8)	14 (54)*
At least one prenatal visit, n (%)	14 (100)	12 (92)	26 (100)
Hypertension, n (%)	4 (29)	3 (23)	6 (23)
Diabetes (insulin dependent), n (%)	0 (0)	1 (8)	0 (0)
Twin gestation, n (%)	2 (14)	5 (39)	9 (35)
Fetal growth restriction, n (%)	4 (29)	1 (8)	6 (23)
Prolonged rupture of membranes [†] , n (%)	3 (21)	2 (15)	3 (12)
Chorioamnionitis, histologic, n (%)	4 (29)	3 (23)	6 (23)
Antibiotics during pregnancy, n (%)	10 (71)	11 (85)	16 (62)
Group B streptococcal colonization	4 (29)	3 (23)	5 (19)
Urinary tract infection	2 (14)	2 (15)	3 (12)
Antenatal steroids, n (%)	13 (93)	13 (100)	22 (85)
Inborn, n (%)	14 (100)	13 (100)	26 (100)
Cesarean delivery, n (%)	8 (57)	10 (77)	20 (77)

* P<.05;

[†]PROM: > 24 hours

Abbreviation: PROM – prolonged rupture of membranes

Table 2

Clinical characteristics of the study population

Characteristic	NEC (n=14)	SIP (n=13)	Controls (n=26)
Gestational age, weeks, mean±SD	27.6 ± 3.3	26.3 ± 1.6	28 ± 3.1
Body weight, grams, mean±SD	988 ± 536	792 ± 185	1053 ± 516
Male sex, n (%)	5 (36)	9 (69)	14 (54)
Apgar, n (%)			
1-minute 3	5 (36)	4 (31)	12 (46)
5-minute 3	2 (14)	1 (8)	3 (12)
Postnatal age at presentation, days, mean±SD	13 ± 10	10 ± 7	11 ± 11
Antibiotics during first 48 hours after birth, n (%)	13 (93)	12 (92)	22 (85)
Prolonged (>48h) antibiotics after birth, n (%)	2 (14)	8 (62)	5 (19) *
Sepsis [†] , n (%)	9 (64)	11 (85)	10 (39) *
Respiratory distress syndrome, n (%)	11 (79)	13 (100)	25 (96)
Mechanical ventilation, n (%)	13 (93)	13 (100)	25 (96)
Duration, days, mean±SD	76 ± 60	100 ± 37	81 ± 50
Postnatal steroids, n (%)	7 (50)	6 (46)	9 (35)
Age, days, mean±SD	39 ± 19	31 ± 16	60 ± 39
Parenteral nutrition, n (%)	14 (100)	13 (100)	26 (100)
Duration, days, mean±SD	46 ± 34	76 ± 41	27 ± 30 *
Age at initiation of enteral feeds, days, mean±SD	6 ± 6	10 ± 12	3 ± 3 *
Feeding, n (%)			
Breast milk	1 (7)	2 (15)	5 (19)
Formula	0 (0)	0 (0)	1 (4)
Both	13 (93)	11 (85)	20 (77)
Patent ductus arteriosus, n (%)	2 (14)	5 (39)	3 (12)
Medical treatment, n (%)	1 (7)	1 (8)	2 (8)
Surgery ligation, n (%)	1 (7)	0 (0)	0 (0)
pRBC transfusion, n (%)	8 (57)	12 (92)	16 (62)
Within prior 48 hours of presentation, n (%)	2 (14)	3 (23)	1 (4)
Length of NICU stay, days, mean±SD	98 ± 52	110 ± 32	94 ± 45
Death, n (%)	1 (7)	1 (8)	2 (8)
Severe IVH [‡] or PVL, n (%)	3 (21)	1 (8)	1 (4)
Retinopathy of prematurity, n (%)	8 (57)	11 (85)	11 (42) *
Bronchopulmonary dysplasia, n (%)	8 (57)	9 (69)	10 (39)

* P<.05;

[†] Culture-positive sepsis;[‡] Severe IVH: Papile grade 3 or 4

Abbreviations: IVH – periventricular-intraventricular haemorrhage; pRBC – packed red blood cells; PVL – periventricular leukomalacia

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript