The Role of Inter-Alpha Inhibitor Proteins in the Diagnosis of Neonatal Sepsis

Hala Chaaban, MD, Kultar Singh, MD, Juliza Huang, Ed Siryaporn, Yow-Pin Lim, MD, PhD, and James F. Padbury, MD

We evaluated Inter-alpha inhibitor proteins (IaIp) as a diagnostic marker in neonatal sepsis. Samples were collected from 573 neonates who were examined for suspected sepsis. IaIp level was significantly lower in the septic group (121 ± 71 mg/L) than in the non-septic group (322 ± 91 mg/L). The optimal cutoff value with the receiver operating characteristic curve was ≤177 mg/L (sensitivity, 89.5%; specificity, 99%; positive predictive value, 95%; negative predictive value, 98%) with area under the curve of 0.94. IaIp is a more reliable diagnostic marker for neonatal sepsis than other available tests. *(J Pediatr 2009;154:620-2)*

Neonatal sepsis is an important cause of neonatal morbidity and mortality, despite the major advances in neonatal management.[1] Early diagnosis is a challenge because the clinical signs are non-specific. Currently, there are no reliable markers for confirmation of sepsis.[2] Blood culture tests are still considered the gold standard for bacterial sepsis, but results are not available until at least 48 hours. Hematologic indices, acute phase reactants, protein markers, and cytokines have been extensively examined as adjunctive tests for diagnosis of sepsis. None have shown sensitivity, specificity, positive predictive values (PPV), or negative predictive values (NPV) that are sufficiently robust to guide clinical management.[3,4]

Inter-alpha inhibitor proteins (IaIp) are a family of structurally related serine protease inhibitors found in relatively high concentration in human plasma. The major forms in human plasma are products of 4 unique genes: inter-α inhibitor (IaI), consisting of 2 heavy chains (H1 and H2) and a single light chain, and pre-α inhibitor (PaI), consisting of 1 light chain and 1 heavy chain (H3). The shared light chain, also known as bikunin, is known to inhibit several serine proteases, such as trypsin, human leukocyte elastase, plasmin, and cathepsin G.[5] IaIp are involved in numerous biologic activities, including tumor invasion, extracellular matrix stabilization, inflammation, and wound healing, and play an important anti-inflammatory and regulatory role in infection.[6] We previously reported that circulating IaIp levels are significantly decreased in adult and neonatal sepsis and that the total IaIp levels correlated inversely with the mortality rate in adult patients with severe sepsis.[7,8] These findings suggest the potential clinical usefulness of IaIp as a marker in sepsis. The aim of this study was to evaluate the diagnostic usefulness of IaIp in neonatal sepsis.

METHODS

This study was conducted in the neonatal intensive care unit of Women & Infants' Hospital of Rhode Island between August 2003 and January 2004. Residual blood samples of neonates who were examined for suspected sepsis were collected. An evaluation for neonatal sepsis was done at the discretion of the neonatal providers for a variety of reasons, including maternal risk factors and signs of neonatal sepsis. Routine sepsis evaluations included a full blood count, platelet count, and blood culture. Previously validated hematologic criteria were used as indicators of sepsis[4]: absolute neutrophils count <7500 or $>14\,500$ cells/mm^3, absolute band count >1500 cells/mm^3, immature/total neutrophil ratio >0.16, and platelet count $<150\,000$ cells/mm^3. Cerebrospinal fluid and urine cultures were performed in cases of late-onset sepsis and when clinically indicated. Positive blood culture results were considered the gold standard for diagnosis of sepsis. Plasma IaIp levels were measured quantitatively by using a competitive enzyme-

AUC	Area under the curve	NPV	Negative predictive value
IaI	Inter-α inhibitor	PPV	Positive predictive value
IaIp	Inter-alpha inhibitor proteins	ROC	Receiver operating curve
PaI	Pre-α inhibitor		

From Women & Infants Hospital of Rhode Island, Department of Pediatrics, Brown University, Providence, RI (H.C., K.S., J.P.); and Prothera Biologics, East Providence, RI (J.H., E.S., Y-P. L.).

Lim holds a significant interest in Prothera Biologics, which supported these studies by performing the IaIp assays. None of the other authors declare conflicts of interest

Submitted for publication Jul 19, 2008; last revision received Sep 18, 2008; accepted Oct 3, 2008.

Reprint requests: James F. Padbury, MD, Women & Infants Hospital of Rhode Island, 101 Dudley St, Providence, RI 02906.

0022-3476/$ - see front matter

10.1016/j.jpeds.2008.10.008

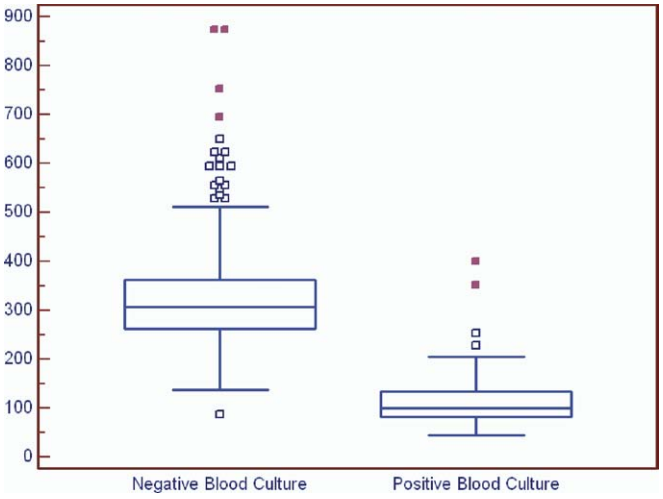

Figure 1. Ialp level in culture proven sepsis and culture negative newborns. Ialp levels was significantly lower in the blood culture proven sepsis (121 ± 71 mg/L; 95% CI = 100–143, n = 45) compared to the blood culture negative group (322 ± 91 mg/L, 95% CI; 314–330, n = 528) $P < .0001$.

linked immunosrbent assay with a monoclonal antibody against human IaIp (MAb 69.26), as described previously.[7,8]

Statistical Methods

The septic and non-septic groups were compared by using the student *t* test or χ^2 test when appropriate. All values are given as mean plus or minus SD or SEM. Sensitivity, specificity, PPV, and NPV were determined to evaluate plasma IaIp level as a predictor of neonatal sepsis. The receiver operating curve method (ROC) was used to determine the optimal cutoff value by plotting the sensitivity on the y axis and 1-specificity on the x axis. The area under the curve (AUC) was used as an estimator of the overall diagnostic accuracy. All statistical tests were performed with MedCalc software (version 9.3.2; MariaKerke, Belgium). A *P* value <.05 was considered to be significant.

RESULTS

A total of 573 neonates were examined for sepsis, 45 of whom had blood culture-proven sepsis (7.5%). The identified bacteria included *Staphylococcus aureus* (n = 2), group B Streptococcus (n = 2), *Escherichia coli* (n = 5), Citrobacter species (n = 2), *Enterococcus fecalis* (n = 4), Klebsiella species (n = 2), coagulase-negative Staphylococcus (n = 13), other gram-positive cocci (n = 9), and Candida species (n = 7). Patients in the septic group had a lower gestational age compared with patients in the non-septic group and had a higher age at sepsis evaluation. They were characterized by significantly higher white blood cell count, absolute band count, and immature/total neutrophils ratio (I:T ratio), compared with the non-septic group (Table I; available at www.jpeds.com).

IaIp levels were significantly lower ($P < .0001$) in the sepsis group (121 ± 71 mg/L; 95% CI, 100-143; n = 45) compared with the non-septic group (322 ± 91 mg/L; 95% CI, 314-330; n = 528; Figure 1). The cutoff level with the optimal diagnostic efficiency derived from the ROC model was ≤177 mg/L. Table II compares the sensitivity, specificity, PPV, NPV, and AUC of the hematologic indices and IaIp. IaIp <177 mg/L was superior to the hematologic indices, with a sensitivity rate of 89.5%, specificity rate of 99%, PPV of 95%, and NPV of 98% (Table II). The AUC was 0.94 (95% CI, 0.92-0.96; Figure 2).

Table II. Comparison of sensitivity, specificity, positive and negative predictive values, and area under the receiver operating characteristic curve between nematologic indicies and inter-alpha inhibitor proteins *P* level

Test	Cutoff value	Sensitivity	Specificity	PPV	NPV	AUC
Absolute band count	≥1500	38	82	15	94	0.63
Absolute neutrophilic count	≤7500	59	46	8.5	93	0.53
Absolute neutrophilic count	≥14 500	23	87	13	93	0.55
I:T ratio	≥0.16	54	81	19	95	0.675
Platelets	<150 000 cells/mm^3	30	89	19	94	0.6
Ialp	≤177 mg/L	89.5	99	95	98	0.94

DISCUSSION

The IaIp family is a group of plasma-associated serine protease inhibitors synthesized mainly in the liver.[9] In adult patients with sepsis, plasma levels are significantly decreased (by 20%-90%) and inversely correlated with unfavorable outcome.[7] In neonates, we previously studied a cohort of newborn infants with a gestational age between 24 and 42 weeks and found the circulating concentration of IaIp to be endogenously produced independent of gestational age, independent of postnatal age, and similar to the levels seen in adults. IaIp levels were also significantly decreased in neonatal sepsis, followed by a gradual increase during antibiotic therapy to reach normal levels within 4 to 12 days.[8] Similarly, this study showed a significant decrease in IaIp levels in proven neonatal sepsis, with a mean ± SD of 121 ± 71 mg/L compared with non-septic patients with mean ± SD of 322 ± 91 mg/L ($P <$ .0001). We also found the diagnostic reliability of IaIp to be superior to the frequently used hematologic markers. We used an unbiased estimate (ROC modeling) to determine a cutoff value of <177 mg/L. With this value and blood culture as the gold standard, assessment of plasma IaIp level would have a diagnostic reliability with a sensitivity rate of 89.5%, specificity rate of 99%, PPV of 95%, and NPV of 98%. Hematologic indices, acute phase reactants, protein markers, and cytokines have been extensively examined as adjunctive tests for diagnosis of sepsis. So far none has shown sensitivity, specificity, PPV, or NPV that can guide clinical management. The greatest predictability usually results from a combination of