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Comparison of the efficacy of serum amyloid A, C-reactive protein, and procalcitonin in the diagnosis and follow-up of necrotizing enterocolitis in premature infants☆,☆☆

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Abstract

Purpose: The aim of this study was to compare the efficacy of serum amyloid A (SAA) with that of C-reactive protein (CRP), and procalcitonin (PCT) in diagnosis and follow-up of necrotizing enterocolitis (NEC) in preterm infants.

Methods: A total of 152 infants were enrolled into this observational study. The infants were classified into 3 groups: group 1 (58 infants with NEC and sepsis), group 2 (54 infants with only sepsis), and group 3 (40 infants with neither sepsis nor NEC, or control group). The data including whole blood count, CRP, PCT, SAA, and cultures that were obtained at diagnosis (0 hour), at 24 and 48 hours, and at 7 and 10 days were evaluated.

Results: A total of 58 infants had a diagnosis of NEC. Mean CRP (7.4 ± 5.2 mg/dL) and SAA (46.2 ± 41.3 mg/dL) values of infants in group 1 at 0 hour were significantly higher than those in groups 2 and 3. Although the area under the curve of CRP was higher at 0 hour in infants with NEC, there were no significant differences between groups with respect to the areas under the curve of SAA, CRP, and PCT at all measurement times. Levels of SAA decreased earlier than CRP and PCT in the follow-up of NEC (mean SAA levels were 45.8 ± 45.2, 21.9 ± 16.6, 10.1 ± 8.3, and 7.9 ± 5.1 mg/dL at evaluation times, respectively). Levels of CRP and SAA of infants with NEC stages II and III were significantly higher than those with only sepsis and/or NEC stage I.

Conclusions: Serum amyloid A, CRP, and PCT all are accurate and reliable markers in diagnosis of NEC, in addition to clinical and radiographic findings. Higher CRP and SAA levels might indicate advanced stage of NEC. Serial measurements of SAA, CRP, and PCT, either alone or in combination, can be used safely in the diagnosis and follow-up of NEC.

Necrotizing enterocolitis (NEC), characterized by inflammation and necrosis of the bowel, is the most common potentially fatal gastrointestinal disease in premature infants. It affects 12% of preterm infants weighing less than 1500 g [1]. The overall mortality related to NEC in very-low-birth-

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weight infants is around 20% to 30% and is inversely proportional to gestational age, approaching 40% to 50% in extremely preterm neonates [2,3]. Although the pathogenesis of NEC remains unclear, prematurity is the main and most important risk factor. Asphyxia, formula feeding, sepsis, intestinal ischemia-reperfusion injury, polycythemia, intrauterine growth retardation, and the presence of an umbilical catheter are other risk factors for NEC development [4]. Abdominal distention, visible bowel loops, tenderness, and discoloration of the abdominal wall, increased gastric residuals, occult or frank blood in the stool, lethargy, apnea, temperature instability and (in severe cases) hypotension, respiratory failure, and rapid progression to death are the most common clinical signs of NEC [5]. Because its rapid progression to perforation is among the major causes of morbidity and mortality in preterm neonates, early diagnosis of NEC is important [6]. However, initial clinical findings of NEC are often nonspecific and indistinguishable from neonatal sepsis and various other gastrointestinal diseases. Therefore, early diagnostic tools are needed for use in determining infants who would be at greater risk for NEC development and for whom early preventative measures could be targeted [7].

A limited number of studies investigated the use of various laboratory parameters, including blood glucose and electrolyte levels as well as acute-phase reactants, such as C-reactive protein (CRP) and procalcitonin (PCT) measurements, in the diagnosis of NEC [7-12]. Unfortunately, data reported thus far on the use of these inflammatory mediators in the diagnosis and follow-up of NEC are conflicting [8-12].

Because CRP levels may increase during 12 to 24 hours of inflammation, the specificity of this marker is accepted to be low; hence, altered CRP levels are recommended to be evaluated along with other acute-phase reactants, particularly in neonates [13]. Levels of PCT, an acute-phase reactant produced by monocytes and hepatocytes, start rising rapidly within 2 to 4 hours after exposure to bacterial endotoxins, peak within 6 to 8 hours, and they reach a plateau followed by a decrease to normal ranges after 24 hours. The diagnostic utility of PCT levels, which may also be increased in neonatal sepsis, is comparable with that of CRP [13,14].

Serum amyloid A (SAA) proteins constitute a family of apolipoproteins mainly synthesized by the liver in response to cytokine release by activated monocytes/macrophages after an acute-phase stimulus such as infection and tissue injury [15]. Levels of SAA increase up to 1000 fold in the 8 to 24 hours after the onset of inflammation. Serum amyloid A has been shown to be useful in various acute diseases (bacterial, viral, traumatic, rheumatic, and ischemic heart disease) and also in neonatal sepsis [16-18]. Recently, we have shown that SAA might be used as an accurate marker in addition to clinical and laboratory findings in the diagnosis of NEC [19].

Therefore, the purpose of this study is to compare the effectiveness of SAA with that of CRP and PCT in the diagnosis and follow-up of NEC.

1. Materials and methods

A total of 162 premature infants (≤36 weeks' gestational age) born between November 2009 and September 2010 in the Neonatal Intensive Care Unit of the Pediatric Department of Uludag University, Faculty of Medicine, were enrolled into this observational study. Necrotizing enterocolitis was diagnosed according to clinical and radiographic findings, and the latter were interpreted by a pediatric radiologist and were classified according to modified Bell's criteria [20]. Neonatal sepsis was diagnosed according to the criteria defined by the International Sepsis Consensus [21]. Temperature instability, apnea, need for supplemental oxygen and ventilation, tachycardia and bradycardia, hypotension, and feeding intolerance were considered clinical signs of sepsis. Abdominal distention or tenderness, bloody stools, gastric residuals, bloody or bilious gastric aspirates, emesis, feeding intolerance, absent or decreased bowel sounds, and abdominal wall erythema were considered as clinical signs of NEC. Infants were assigned to 3 groups: group 1, infants with NEC and sepsis (NEC + sepsis group); group 2, infants with only sepsis (only-sepsis group); and group 3, infants without clinical signs or laboratory values consistent with infection (control group). Ten infants were excluded because of the following exclusion criteria: refusal of parenteral consent, lack of clinical and/or laboratory data, and major congenital abnormalities; moreover, a total of 152 infants were included in this study. Fig. 1 shows the flow diagram for the participants in the study.

The study protocol was approved by the Ethics Committee of Uludag University, Faculty of Medicine. Informed parental consent was obtained for all infants. Gestational age, birth weight, sex, mode of delivery, Apgar score at 1 and 5 minutes, prenatal demographics, premature rupture of membranes, history of chorioamnionitis, prenatal and postnatal hypoxia, and feeding properties were all recorded. Changes in the hematologic parameters were processed according to the scoring systems of Manroe et al [22] and Rodwell et al [23]. Leukopenia was defined as a leukocyte count less than 5000/mm^3; leukocytosis was defined as a leucocyte count more than 25,000/mm^3 at birth, more than 30,000/mm^3 at 12 to 24 hours, and more than 21,000/mm^3 after the second day. Thrombocytopenia was defined as a platelet count less than 150,000/mm^3. Normal absolute neutrophil count was accepted as 7800 to 14,500 /mm^3 in the first 60 hours and 1750 to 5400/mm^3 after 60 hours.

In NEC cases, abdominal radiographs were repeated every 6 hours for the first 48 hours, and then less frequently during follow-up, until the cessation of NEC antimicrobial therapy. The abdominal radiographs were interpreted by the same pediatric radiologist. According to modified Bell's criteria, radiographic findings, including intestinal dilation or mild ileus, were associated with NEC stage I; intestinal dilation, ileus, and pneumatosis intestinalis with or without ascites were associated with NEC stage II. Additional findings of pneumoperitoneum were associated with NEC

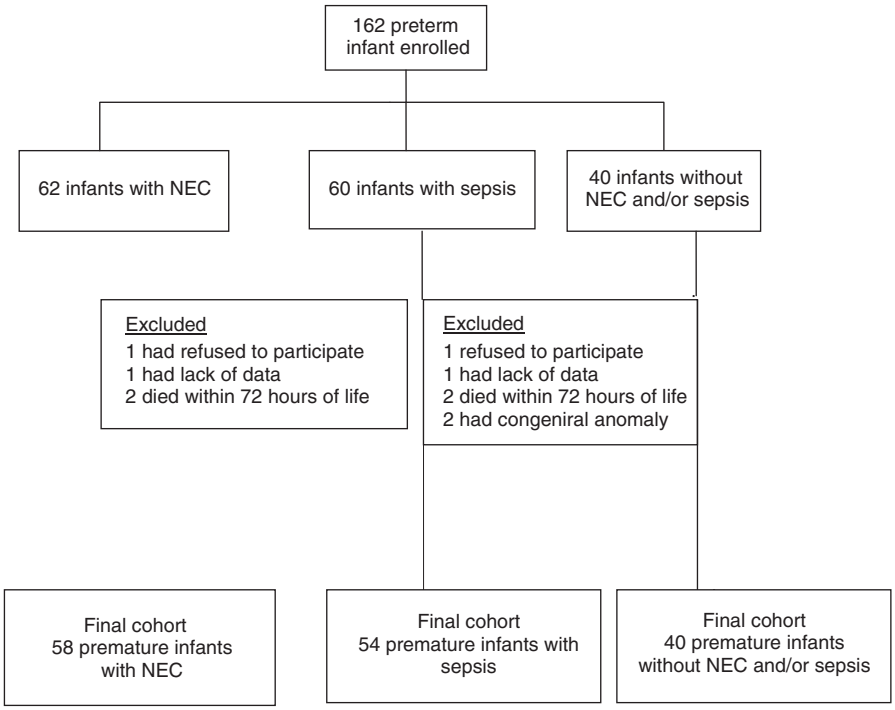

Fig. 1 Flow diagram of participants in the study.

stage III [20]. Infants were also given appropriate therapies, such as mechanical ventilation, gastric decompression, or volume expansion with fluid or blood products.

When sepsis or NEC was suspected, blood samples for whole blood count, CRP, PCT, SAA, and culture were obtained from all 3 groups of infants before initiating antimicrobial therapy. Levels of CRP, PCT, and SAA were measured at 0 (at diagnosis), 24, and 48 hours and at 7 and 10 days in groups 1 and 2 to determine the efficacy of these laboratory parameters in predicting the sepsis or NEC. Cerebrospinal fluid, urine, and tracheal and gastric materials were also sent out for culture, if obtained. Blood smears of all infants were also evaluated for findings of sepsis.

Whole blood count, PCT level, CRP level, SAA level, and cultures were studied immediately. Whole blood count was performed using an automatic counter, the Cell Dyn 3700 (Abbott Diagnostics Division, Santa Clara, CA, USA). Levels of CRP and SAA were determined by an immunonephelometric method using a BN II device (Dade Behring Marburg GMBH, Marburg, Germany). Total SAA was measured with a Siemens kit (Siemens, Deerfield, IL). Procalcitonin was measured by monoclonal immunoluminometric assay (Lumitest PCT; Brahm Diagnostica GMBH, Berlin, Germany), which is specific for the PCT molecule. In this assay, 2 different antibodies, one directed to calcitonin and another directed to katacalcin, were used. Levels greater than 0.5 ng/mL was accepted as pathological. Levels of SAA greater than 6.8 mg/dL were referred to as positive, whereas levels of SAA below 6.8 mg/dL were considered negative, according to the SAA kit's manufacturer instructions. Therefore, infants who had SAA levels greater than 6.8 mg/dL were considered to have abnormal SAA levels. Levels less than 0.5 ng/mL for PCT and CRP and 6.8 mg/dL for SAA were accepted as zero for statistical analysis. Blood and cerebrospinal fluid cultures were analyzed using the fully automatic BACTEC method with the BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany).

Infants were given antibiotic therapies. Neonates who had positive cultures were treated with appropriate antibiotics based on the result of a culture antibiogram. The antimicrobial therapy was stopped after clinical and laboratory improvement. Infants who had negative cultures were started on broad-spectrum combination antibiotic therapy, including penicillin, gentamicin, and metronidazole, to cover gram-positive, gram-negative, and anaerobic organisms. These therapies continued until the infant was stable and abdominal radiographic findings were normal. All groups were compared with regard to demographic features and clinical and laboratory findings.

The SPSS 16.0/Windows program was used for data analyses (SPSS, Chicago, IL). Descriptive statistics were given as mean, standard deviation of the mean, and percentage. The differences between groups were evaluated with χ^2 tests for qualitative data and with Mann-Whitney U and t tests for quantitative data. Receiver operating characteristic analyses were performed with the MedCalc version 9.3.9.0 statistical program (Mariakerke, Belgium). Values of $P < .05$ were considered significant. The Kruskal-Wallis test was used in comparison of 2 groups that did not have normal distributions. For the Mann-Whitney U test that was used in the comparison of CRP, PCT, and SAA for NEC stages, P values less than .017 were considered statistically

Table 1 Birth and clinical characteristics of study group

	Sepsis + NEC (group 1), n = 58	Only sepsis (group 2), n = 54	No sepsis (group 3), n = 40
Gestational age (wk) [a]	28.6 ± 1.6	31.2 ± 1.5	32.6 ± 1.4
Birth weight (g) [a]	1190 ± 325	1580 ± 430	1705 ± 440
Male/female	34:24	28:26	20:20
Apgar minute 1 [b]	4 (1-9)	5.5 (2-9)	7.5 (4-9)
Apgar minute 5 [b]	6 (5-9)	7 (5-9)	7.5 (6-10)
Cesarean delivery [c]	37 (64%)	39 (72%)	20 (50%)
PROM [c]	10 (17%)	9 (17%)	0 (0) *
Choriamnionitis [c]	4 (6%)	4 (7%)	0 (0) *
Asphyxia [c]	8 (14%)	7 (13%)	0 (0) *
Apnea [c]	39 (67%)	29 (54%)	1 (2.5%)
Feeding intolerance [c]	49 (84%)	35 (65%)	2 (5%)
Mortality [c]	8 (14%)	6 (11%)	0 (0)
Gastric residue [c]	31 (53%)	12 (22%)	1 (2.5%)
Abdominal distention [c]	30 (52 %) *	3 (5%)	0 (0)

PROM indicates premature rupture of membranes.
[a] Mean ± SD.
[b] Median (range).
[c] n (%).
* $P < .05$ between sepsis and non–sepsis group.

significant according to the Bonferroni correction. Correlations between variables were calculated using Pearson correlation and Spearman correlation tests. In general, $\alpha = .05$ ($P < .05$) was considered significant.

2. Results

Although infants with NEC had a lower gestational age and birth weight as expected, no statistically significant difference was found between the groups with respect to gestational age, birth weight, sex, Apgar scores at 1 and 5 minutes, and the mode of delivery. Table 1 shows the demographic features of the 3 groups of infants.

A total of 58 infants were diagnosed with NEC, and 32 (55%) were male. The mean gestational age and birth weight values for these infants were 28.6 ± 1.6 weeks and 1190 ± 325 g, respectively. In group 1, 20 infants (34%) had stage I NEC, 23 (40%) had stage II NEC, and 15 (26%) had stage III NEC. In group 1, 34 infants (59%) had respiratory distress syndrome and were given surfactant. Surgery was performed in 5 infants with stage III NEC, 3 of these infants underwent laparotomy with bowel resection, and primary peritoneal drainage was performed in 2 of them. These infants underwent surgery within 2.3 ± 1.1 days after NEC onset. A total of 8 infants in group 1 (5 with NEC III, 2 with NEC II, and 1 with NEC I) and 6 infants in group 2 died during follow-up.

White blood cell and platelet counts were also evaluated. At 0, 24, and 48 hours, the mean leucocyte counts in group 1 were lower than those in groups 2 and 3, but this difference was not statistically significant (in all comparisons, $P > .05$). Mean platelet counts at time 0, 24, and 48 hours and on days 7 and 10 were also significantly lower in infants with NEC than those without NEC (in all comparisons, $P < .05$; Figs. 2 and 3).

Mean levels of CRP at 0 hours of infants in group 1 (7.4 ± 5.2 mg/dL) were significantly higher than those in group 2 (2.6 ± 1.8 mg/dL) and group 3 (0.5 ± 0.3 mg/dL; $P = .04$ and $P = .01$, respectively). Similarly, the mean SAA levels of infants in group 1 at 0 hours (46.2 ± 41.3 mg/dL) were also significantly higher than those in group 2 (16.1 ± 9.8) and group 3 (4.1 ± 2.0 mg/dL; $P = .03$ and $P = .01$, respectively). The mean CRP levels of infants in group 1 at 24 hours (5.7 ± 3.8 mg/dL), 48 hours (6.4 ± 4.1 mg/dL), and 7 days (3.8 ± 1.6 mg/dL) were significantly higher than those of infants in group 2 (2.9 ± 1.5, 3.2 ± 1.2, and 1.9 ± 0.8 mg/dL, respectively; $P = .01$, $P = .01$, $P = .02$, respectively). Although the SAA levels of infants in group 1 at 24 hours (45.8 ± 38.2 mg/dL), at 48 hours (21.9 ± 13.6 mg/dL), and at

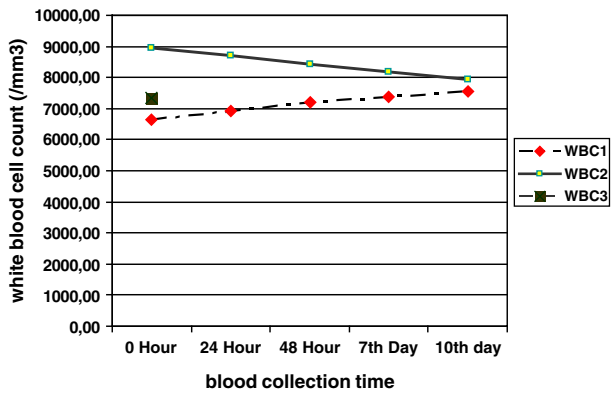

Fig. 2 Trends of white blood count in 3 groups at different sample collection times. WBC1 indicates white blood cell count of infants in group 1; WBC2, white blood cell count of infants in group 1; WBC3, white blood cell count of infants at 0 hour.

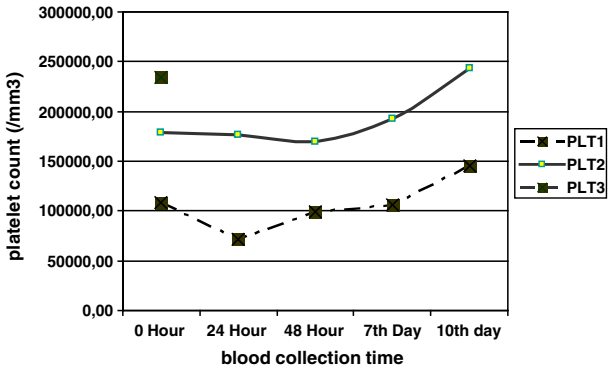

Fig. 3 Trends of platelet count in 3 groups at different sample collection times. PLT1 indicates platelet count of infants in group 1; PLT2, platelet count of infants in group 1; PLT3, platelet count of infants at 0 hour.

7 days (10.1 ± 6.3) were higher than group 2 (28.5 ± 21.2, 17.0 ± 9.6, and 8.4 ± 2.4 mg/dL), the differences were not significant (all P values > .05). Similarly, there were no significant differences in terms of PCT levels between groups 1 and 2 at 0 hours (6.1 ± 4.3 and 7.0 ± 4.4 ng/mL), 24 hours (4.3 ± 3.6 and 4.4 ± 3.2. ng/mL), 48 hours (3.8 ± 2.2 and 3.5 ± 2.4 ng/mL), or day 7 (1.7 ± 1.9 and 1.0 ± 0.5 ng/mL; all P values > .05). Figs. 4 and 5 show the mean CRP, PCT, and SAA levels of infants in 3 groups at different times.

Table 2 shows the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) value, and area the under curve (AUC) for CRP, PCT, and SAA at 0 hours.

The AUC for CRP at 0 hours was higher (0.729) than that for SAA (0.625) and PCT (0.616); however, the difference was not statistically significant (P = .18). In addition, no statistically significant difference was found between CRP, SAA, and PCT at other measurement times. The AUC for SAA at 24and 48 hours and at 7 and 10 days were found to be lower compared with CRP and PCT, but the difference was not statistically significant. There were no significant differences between the sensitivity, specificity, PPV, NPV, and AUC of CRP, PCT, or SAA levels at 0, 24, or 48 hours; nor were there significant differences at 7 and 10 days in group 1 (data not shown).

The mean CRP, PCT, and SAA levels of infants in group 1 for NEC stages are also shown in Table 3. As shown in Table 3, both CRP and SAA levels of infants with NEC stages II and III were significantly higher than those with sepsis or NEC stage I. In the correlation analyses, levels of SAA, PCT, and CRP at 0 hours were all found to be associated with NEC stages because all of them increased with NEC stage (r = 0.41, P = .003, for CRP; r = 0.28, P = .004, for PCT; and r = 0.36, P = .011). Also, both CRP and SAA levels of infants with stage II and III NEC persisted significantly higher at 48-hour and 7-day evaluation compared with infants who had stage I NEC and/or had only sepsis.

3. Discussion

To the best of our knowledge, this is the first study to compare the efficacy of SAA with CRP and PCT in the diagnosis and follow-up of NEC in premature infants. The results of this study showed that SAA was an accurate and reliable marker as CRP in the diagnosis of NEC because the mean SAA and CRP values at 0 hours were significantly higher in infants with NEC. This study also showed that CRP and SAA levels were significantly higher in infants with stage II and stage III NEC.

The diagnosis of NEC is based on both clinical findings and characteristic radiographic abnormalities. Hematologic and biochemical abnormalities with a high concurrent bacteremia incidence have been reported in infants with both NEC and sepsis [24]. Necrotizing enterocolitis is

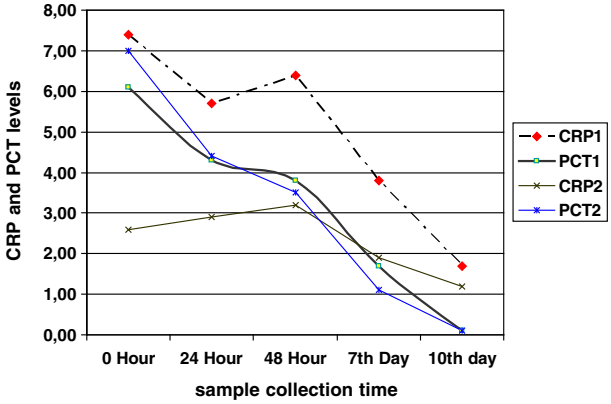

Fig. 4 Trends of CRP and PCT levels of infants in groups 1 and 2 at different sample collection time points. CRP1 indicates CRP levels of infants in group 1; PCT1, PCT levels of infants in group 1; CRP2, CRP levels of infants in group 2; PCT2, PCT levels of infants in group 2.

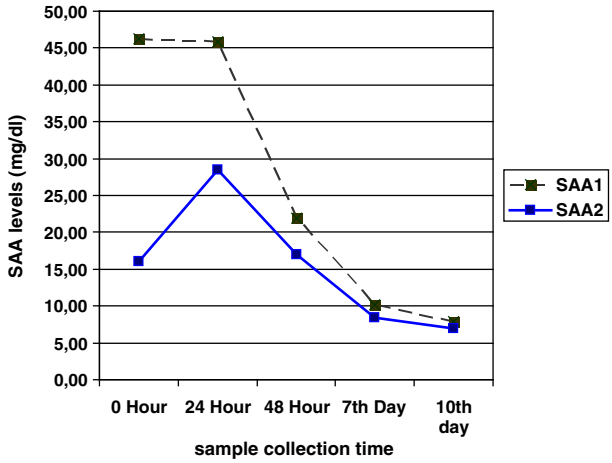

Fig. 5 Trends of SAA levels in infants in groups 1 and 2 at different sample collection time points. SAA1 indicates SAA levels of infants in group 1; SAA2, SAA levels of infants in group 2.

Table 2 Sensitivity, specificity, PPV, NPV, and AUC of CRP, PCT, and SAA at 0 hour in group 1

Marker	Time (h)	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	P [a]
CRP (mg/dL)	0	0.45	100	79.6	78.4	100	0.89	<.0001
PCT	0	0.24	92.5	98.1	97.4	94.6	0.98	<.0001
SAA	0	6.7	100	72.2	72.2	100	0.84	<.0001

[a] P value of receiver operating characteristic analysis that shows the significant sensitivity and specificity of these markers at reported cutoff values.

usually diagnosed by clinical and abdominal radiographic findings during the progression of the disease [25]. Therefore, the absence of a consistent, reliable, and early marker of intestinal inflammation in the peripheral blood has been one of the great challenges for the diagnosis and management of NEC [7,26]. Although some serum markers such as CRP, interleukin-6, and platelet-activating factor have been studied, all of them showed a lack of specificity [9,27,28]. There are also conflicting data about the use of these inflammatory mediators in the diagnosis and follow-up of NEC [8-12].

Serum amyloid A increases rapidly and significantly at the onset of inflammation and promptly returns to baseline levels with the resolution of the inflammation. It has been reported that SAA has a very short half-life of between 75 and 90 minutes [29]. We previously showed that SAA was an accurate and reliable marker for the diagnosis and follow-up of neonatal sepsis [18]. In addition, adult studies reported that the clinical value of SAA was superior or equal to CRP in inflammatory abdominal disorders such as acute pancreatitis [30,31]. Therefore, recently, we evaluated SAA levels in preterm infants with NEC and found that SAA could be used in NEC diagnosis in preterm infants [19]. In addition, in a recent study, Apolipoprotein SAA (ApoSAA) scores computed from plasma proapolipoprotein C2 and SAA concentrations were found to be effective in identifying both sepsis and NEC [32]. Although the pathophysiology of NEC is multifactorial, an exaggerated inflammatory response to intestinal injury formed by immature intestinal epithelial cells has been one of the leading hypotheses for NEC [33]. Bacterial overgrowth and translocation, hydrogen gas accumulation caused by bacterial fermentation, and endotoxin absorption secondary to intestinal mucosal damage have all been suggested as mechanisms for infection and inflammation [34]. In agreement with these hypotheses, this study also showed that SAA might be used in the diagnosis and follow-up of NEC with a similar accuracy as CRP. However, no significant differences were found between SAA, CRP, and PCT in terms of AUC, PPV, and NPV values at all evaluation time points. These data might also indicate that these markers had no superiority to the others. Therefore, we suggest that all of these markers might be used together combined with other laboratory, clinical, and radiographic findings for an accurate and reliable diagnosis of NEC in premature infants. Because SAA levels decreased earlier than CRP and PCT, this advantage might facilitate evaluation of the response to therapy in infants with NEC.

C-reactive protein is an acute-phase reactant that increases in the serum in the presence of inflammation caused by infection or tissue injury. It is produced by the liver in response to inflammation caused by infection or tissue injury. In an early report, CRP was found to increase in 83% of infants with definite NEC at the time of onset compared with those without NEC [11]. In another study, although abnormal CRP values were reported within 24 hours of the onset of gastrointestinal signs in infants with stages II and III

Table 3 Mean CRP, PCT, and SAA levels at different time points according to NEC stages

	Only sepsis, n = 54	NEC stage I, n = 20	NEC stage II, n = 23	NEC stage III, n = 15
CRP at 0 h	2.6 ± 1.8	3.6 ± 1.2	5.5 ± 1.1 *, †	10.4 ± 2.0 ‡, §
CRP at 24 h	2,9 ± 1.5	3,0 ± 0.9	5.8 ± 1.1 *, †	8.3 ± 1.7 ‡, §
CRP at 48 h	3.2 ± 1.2	2.0 ± 0.5	7.9 ± 1.5 *, †	8.2 ± 2.6 ‡, §
CRP at day 7	1.9 ± 0.8	1.3 ± 0.3	4.5 ± 1.3 *, †	5.3 ± 0.8 ‡, §
SAA at 0 h	16.1 ± 9.8	39.6 ± 11.3 ‖	39.4 ± 9.5 *	52.5 ± 15.8 §
SAA at 24 h	28.5 ± 21.2	27.5 ± 6.9	56.2 ± 22.5 *, †	50.2 ± 15.1 ‡, §
SAA at 48 h	17.0 ± 9.6	20.2 ± 5.8	29.1 ± 6.9	33.3 ± 11.8 ‡, §
SAA at day 7	8.4 ± 2.4	11.6 ± 2.5	14.0 ± 3.2	19.8 ± 5.2 ‡, §
PCT at 0 h	7.0 ± 4.4	5.4 ± 0.5	7.2 ± 1.8 †	8.0 ± 2.2 §
PCT at 24 h	4.4 ± 3.2	2.0 ± 0.4	5.3 ± 1.1	5.3 ± 1.3
PCT at 48 h	3.5 ± 2.4	1.8 ± 0.3	3.5 ± 1.0	5.3 ± 1.1 ‡, §
PCT 7 at day 7	1.1 ± 0.5	0.7 ± 0.1	1.5 ± 0.4	2.5 ± 0.5 ‡, §

* $P < .05$, between NEC stage II and only-sepsis group.
† $P < .05$, between NEC stage II and NEC stage I.
‡ $P < .05$, between NEC stage III and only-sepsis group.
§ $P < .05$, between NEC stage III and NEC stage I.
‖ $P < .05$, between NEC stage I and only-sepsis group.

of NEC, CRP levels persisted in normal levels in infants with stage II NEC [33]. Recently, Pourcyrous et al [9] performed serial CRP measurements in a large population to identify premature infants with NEC. They reported abnormal CRP levels in both stages II and III of NEC. They concluded that persistence of high CRP levels might suggest ongoing disease and/or complications in infants with NEC and proposed serial CRP measurements for the follow-up of NEC [9]. In accordance with previous studies, in the present study, mean CRP levels at the time of NEC diagnosis were significantly higher and remained high at 24- and 48-hour as well as at 7-day evaluations in infants with NEC compared with other infants. Therefore, we agree that CRP is an accurate marker for the diagnosis of NEC in premature infants. However, because the number of infants in our study was relatively small, we did not evaluate CRP levels according to NEC stages.

Procalcitonin, another acute-phase reactant, increases within 2 to 3 hours in response to bacterial infection. In a previous study from our department, we concluded that serum PCT levels seemed to be superior to serum CRP levels in terms of the early diagnosis of neonatal sepsis, in detecting the severity of the illness, and in evaluation of the response to antibiotic treatment [35]. To our knowledge, there is only one study in the literature that evaluated serial PCT levels during NEC episodes [12]. The authors found low PCT levels in all infants with NEC compared with the control group and suggested that bacterial proliferation in NEC did not extend beyond the local mucosa and was not extensive enough to trigger a systemic response of PCT; therefore, they concluded that PCT was not a good marker for NEC diagnosis. In the present study, although mean PCT levels in infants with NEC and sepsis were significantly higher than those in control group, there was no significant difference between infants with NEC and sepsis at all measurements. Therefore, in agreement with the previous study, we might suggest that PCT is an accurate marker comparable with CRP and SAA in the diagnosis of NEC.

In this study, we also evaluated white blood cell and platelet counts at similar measurement points. The value of whole blood count evaluation in NEC has been reported previously. Neutropenia or high leukocyte counts were reported to be pathognomic in infants with NEC to predict mortality and intestinal perforation [8]. Similarly, thrombocytopenia was reported to be an important marker in infants with sepsis and/or NEC. It was also suggested that thrombocytopenia might predict the need for surgical intervention in NEC [8,36]. In agreement with these data, our results showed that infants with NEC had lower leukocyte counts and significantly lower platelet counts. These values were also concordant with the increase of SAA, CRP, and PCT levels. Therefore, we suggest that whole blood count evaluation combined with SAA, CRP, and SAA measurements might help physicians to diagnose NEC initially and to predict possible NEC complications in the follow-up period.

This is the first study to demonstrate that the use of SAA in the diagnosis and follow-up of NEC shows similar effectiveness to CRP and PCT. Higher levels of CRP and SAA might also indicate an advanced stage of NEC. Although the diagnosis of NEC depends on clinical, radiologic, and laboratory parameters, we suggest that serial measurements of SAA, CRP, and PCT, either alone or in combination, can be used safely and accurately by clinicians and surgeons in the diagnosis and follow-up of NEC.

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