

## Serum Amyloid-A Levels in Neonatal Necrotizing Enterocolitis

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We aimed to evaluate serum levels of serum amyloid-A (SAA) both in the diagnosis and monitoring the treatment response of necrotizing enterocolitis (NEC). Forty-five preterm neonates were enrolled in the study, including 15 infants with NEC, 15 with sepsis, and 15 healthy preterm infants. Pre- and posttreatment serum SAA levels were measured. Among patients with NEC, 11 had stage 1 and 4 had stage 2 disease according to the modified Bell's staging criteria. Baseline SAA levels of the infants with NEC were significantly higher than controls ( $P=0.013$ ) and were significantly lower than those with sepsis ( $P=0.004$ ). When infants with stage 1

and stage 2 NEC were analyzed separately, baseline SAA levels of the infants with stage 2 NEC were significantly higher than controls ( $P=0.027$ ) than those with stage 1 NEC ( $P=0.018$ ), but similar to those with sepsis. There was a trend that baseline SAA levels were also correlated with the Bell stage ( $r=0.501$ ,  $P=0.057$ ). Posttreatment SAA levels significantly decreased in infants with sepsis ( $P=0.002$ ). Pre- and posttreatment SAA levels were similar in patients with stage 1 and 2 NEC. In conclusion, SAA rises in early stages of NEC and may aid in diagnosis as a serum marker. *J. Clin. Lab. Anal.* 25:233–237, 2011. © 2011 Wiley-Liss, Inc.

**Key words:** necrotizing enterocolitis; preterm; serum amyloid A

### INTRODUCTION

Necrotizing enterocolitis (NEC) is one of the major causes of morbidity and mortality in the neonatal intensive care units (NICU). Incidence of NEC is 0.72/1,000 live births per year, most of them being born at <37 weeks of gestation (1). Mortality rates are also high, between 15 and 30% in various series. Furthermore, long-term complications, including poor growth, cerebral palsy, vision and hearing impairment and other neurodevelopmental disorders, can be seen in affected newborns (1). Prevention, diagnosis, and treatment of NEC are an active area of research owing to the significant mortality and morbidity.

Initial clinical findings of NEC may include delicate signs of feeding intolerance, abdominal distention/tenderness, delayed gastric emptying, vomiting, apnea, bradycardia, and lethargy. Because these symptoms are generally not specific for NEC and can be seen in other diseases of the newborn, additional tests are needed for the establishment of NEC diagnosis (2). Abdominal radiographs may demonstrate multiple dilated bowel loops, pneumatosis intestinalis. Portal venous gas and gallbladder gas are indicative of a serious disease. Pneumoperitoneum indicates a bowel perforation. Computed tomography scanning may be used to

demonstrate pneumatosis or the site of perforation (2,3). Various other laboratory findings have been investigated previously for the early diagnosis of NEC, such as leucopenia, thrombocytopenia, high ratios of immature to total leukocyte counts, metabolic acidosis, hyponatremia, high C-reactive protein (CRP) values, and hyperglycemia (4–7).

Serum amyloid-A (SAA) is an acute-phase reactant that rises in acute inflammatory conditions. SAA is synthesized in the liver upon stimulation by proinflammatory cytokines, such as tumor necrosis factor- and interleukin-6 (IL-6) (8). SAA and CRP have both increased in many disease states associated with inflammation. Moreover, SAA has been suggested to be more sensitive and specific than CRP in various inflammatory conditions (9,10). Previously, only one study assessed the diagnostic value of SAA levels in NEC. Ng et al. developed the ApoSAA score, which was computed from plasma apolipoprotein CII and des-arginine variant of

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Received 18 June 2010; Accepted 2 March 2011

DOI 10.1002/jcla.20464

Published online in Wiley Online Library (wileyonlinelibrary.com).

SAA concentrations and was effective in identifying sepsis/NEC cases (11). As exaggerated inflammatory response to gastrointestinal injury remains the leading hypothesis in the pathophysiology of NEC (12,13), we sought to investigate the utility of major inflammatory markers, i.e., SAA, CRP, and IL-6 in the diagnosis of NEC. We aimed to evaluate serum levels of SAA both in the diagnosis and monitoring the treatment response of NEC.

## PATIENTS AND METHODS

This study with a case-control design was performed between March 2007 and September 2007 in the NICU in Zekai Tahir Burak Maternity Teaching Hospital in Ankara, Turkey. Forty-five preterm neonates were included in the study. Initially, 15 patients with NEC were included, according to the following criteria: gestational week <35 weeks, Bell's stage I-III NEC diagnosis, no congenital malformations. The diagnosis of NEC was made based on clinical and radiological findings and graded according to the modified Bell's staging criteria. All infants were re-evaluated at 48 hr after disease onset to determine the diagnosis of NEC, because clinical findings of NEC are nonspecific. As SAA is a well-established acute phase reactant in sepsis, 15 infants with sepsis were also included in the study as positive controls. Sepsis diagnosis was made in the presence of clinical and biochemical signs as well as positive blood cultures. Fifteen healthy preterm infants, who did not have a severe illness, sepsis, or NEC, and who were hospitalized for feeding purposes, were also included as a negative control group. In all patients, peripheral venous blood samples were collected. Serum was separated from blood cells by centrifugation at 4,000 cycles per minute. All samples were stored at  $-70^{\circ}\text{C}$  until analyzed. Serum SAA levels of the subjects were measured with enzyme-linked immunosorbent assay method (Biosource Hu SAA, CA). Upper detection limit for SAA was 600 mg/l. CRP and IL-6 levels were only measured in the patient groups, but not

controls, using particle-enhanced immunoturbidimetric assay for CRP (CRP latex HS, Cobas, IN), and chemiluminescent sequential immunometric assay for IL-6 (Immulite 1000 systems, Los Angeles, CA). SAA levels of the infants with NEC and sepsis were also measured in the posttreatment period after complete clinical resolution.

Statistical analysis was performed with SPSS software version 13.0 and statistical significance was set at  $P$  less than 0.05. Kruskal-Wallis and Mann-Whitney  $U$  test was used to compare independent variables, Wilcoxon test for dependent variables, Spearman test for correlations, and Chi-square test for comparison of ratios. The study was approved by the institutional review board of the hospital and informed consent was obtained from the parents of the participants.

## RESULTS

Forty-five premature infants were involved in the study. Patient characteristics are shown in Table 1. Gestational ages, postnatal ages, birth weights, and gender distribution were similar between infants with NEC and those in other groups. Among patients with NEC, 11 had stage 1 (suspected NEC) and 4 had stage 2 disease according to the modified Bell's staging criteria. Table 2 shows baseline SAA, CRP, and IL-6 levels of the infants in three groups.

Baseline SAA levels of the infants with NEC were significantly higher than controls ( $P = 0.013$ ) and were significantly lower than those with sepsis ( $P = 0.004$ ). When infants with stage 1 and stage 2 NEC were analyzed separately, baseline SAA levels of the infants with stage 2 NEC were significantly higher than controls ( $P = 0.027$ ) and than those with stage 1 NEC ( $P = 0.018$ ), but similar to those with sepsis ( $P = 0.357$ ) (Table 2). The distribution of baseline SAA levels of the groups is demonstrated in Figure 1.

Among all patients, baseline SAA levels were correlated with CRP and IL-6 levels ( $r = 0.506$ ,  $P = 0.004$  for CRP;  $r = 0.437$ ,  $P = 0.026$  for IL-6). There was a

**TABLE 1. Baseline Characteristics of the Infants<sup>a</sup>**

	NEC ( $n = 15$ )	Sepsis ( $n = 15$ )	Control ( $n = 15$ )	$P$
Gestational age (weeks)	30 (29–33)	32 (30–33)	32 (30–32)	$P > 0.05$
Birth weight (grams)	1,510 (1,290–1,710)	1,400 (1,230–1,680)	1,490 (1,310–1,740)	$P > 0.05$
Gender (male/female)	10/4	9/6	7/8	$P > 0.05$
Postnatal age (days)	10 (7–26)	13 (7–24)	7 (14–9)	$P = 0.024$
NEC Stage		–	–	
1A	9			
1B	2			
2A	4			

<sup>a</sup>Values are median (interquartile range) or number.

TABLE 2. SAA, CRP, and IL-6 Levels of the Infants<sup>a</sup>

	NEC Stage I (n = 11)	NEC Stage II (n = 4)	Sepsis (n = 15)	Control (n = 15)	P
SAA (mg/l)	40.6 (13.2–600)	152.2 (20.2–600)	600 (192.30–600.00)	6.65 (1.00–120.13)	$P < 0.001^b$
CRP (mg/ml)	0.8 (0.16–5.00)	11.8 (1.64–30.1)	16.2 (3.90–32.40)	–	$P < 0.001^c$
IL-6 (mg/ml)	14.2 (5.50–25.0)	32.0 (28.0–97.3)	268 (26.05–1,000)	–	$P = 0.004^c$

<sup>a</sup>Values are median (interquartile range).

<sup>b</sup>Kruskal–Wallis test.

<sup>c</sup>Mann–Whitney U test.

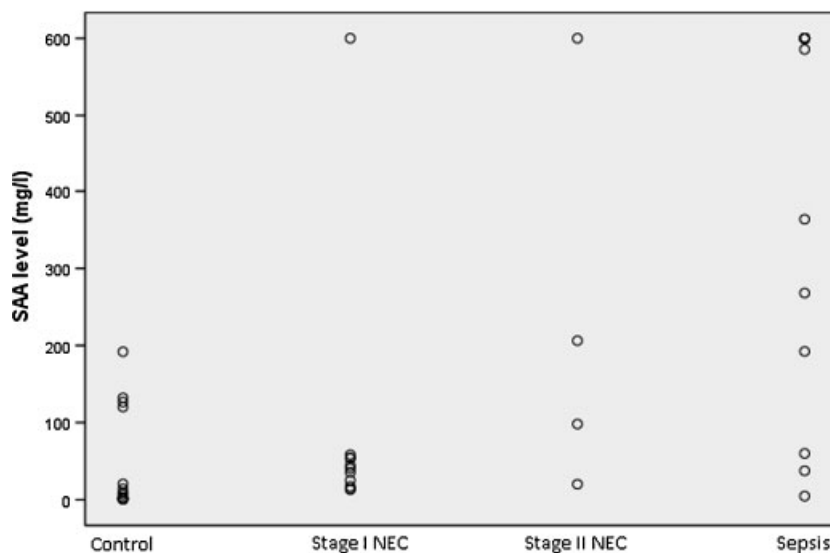


Fig. 1. Baseline SAA levels of the patients with NEC, sepsis, and controls.

TABLE 3. Pre- and Post Treatment SAA Levels of the Patients With NEC or Sepsis

	Pretreatment SAA (mg/l)	Posttreatment SAA (mg/l)	$P^a$
NEC stage 1	40.6 (13.2–600)	16.3 (3.6–562.4)	0.594
NEC stage 2	152.2 (20.2–600)	104.7 (25.9–562.4)	0.273
Sepsis	600 (4.7–600)	11.3 (1.0–393.8)	0.002

Data show median (interquartile range) values.

<sup>a</sup>Wilcoxon test.

trend that baseline SAA levels were also correlated with the Bell stage ( $r = 0.501$ ,  $P = 0.057$ ). Posttreatment SAA levels significantly decreased in infants with sepsis ( $P = 0.002$ ). Pre- and posttreatment SAA levels were similar in patients with stage 1 and 2 NEC (Table 3).

## DISCUSSION

In this study, we found that baseline SAA levels of the infants with NEC were significantly higher than healthy preterms. Moreover, baseline SAA levels were correlated with NEC stage. Together, these data suggest

that SAA may have a potential role in diagnosis of the patients with NEC.

SAA proteins are involved in acute phase responses; these are the immediate early-host responses to inflammation. The human SAA gene family maps to chromosome 11. Liver is the major site of synthesis, although extrahepatic synthesis macrophages, endothelial cells adipocytes, and smooth muscle cells have been reported (8,14). The exact function of SAA is not known. It has immunomodulatory actions, aids in tissue regeneration by activating collagenases, acts as a chemoattractant for monocytes, T lymphocytes, mast cells, and neutrophils (8). SAA is a sensitive acute phase reactant both in the diagnosis and monitoring of inflammatory and infectious diseases. Elevated SAA levels are reported in various infectious and inflammatory disorders, including viral and bacterial infections, sepsis, rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel diseases, lymphoma, and solid cancers (15–18). Studies in patients with chronic obstructive pulmonary disease, acute coronary syndromes, and acute appendicitis revealed SAA to aid better in diagnosis and a better predictor of outcomes (10,19,20).

SAA is most widely studied in sepsis in newborns. In one study, SAA has been investigated in early-onset sepsis. When compared with CRP, SAA was more sensitive at onset of sepsis, rose earlier, and returned faster to normal values in those infants who recovered. They concluded that it can be used as a reliable tool for detection of neonatal early-onset sepsis in full-term infants (17). It was also reported as a useful inflammatory marker during late-onset sepsis in preterm infants (21). For this reason, we included patients with sepsis as positive controls. Consistent with the literature, we also observed high levels of SAA in infants with sepsis that has decreased significantly after the treatment. SAA levels of the infants with NEC were not as high as those with sepsis. This may be because all our patients with diagnosis of NEC had early-stage disease, i.e., either stage I or II. When stage I and II patients were analyzed separately, patients with stage II NEC and those with sepsis had similar SAA levels, whereas those with stage I NEC had lower SAA levels. Nevertheless, SAA levels of the infants with NEC were higher than controls even in early stages. Thus, it can be possible to use SAA as a serum marker to aid in the early diagnosis of NEC when the clinical signs are subtle. A recent study used the proteomic approach to identify potential biomarkers in the diagnosis of sepsis/NEC in preterm infants. Among 180 proteomic peaks obtained in patients with NEC/sepsis by quantitative proteomics profiling, Ng et al. identified 25 peaks that were significantly higher and 40 peaks that were significantly lower in the sepsis/NEC group compared with nonsepsis infants. Multivariate logistic regression analysis identified plasma apolipoprotein CII ( $P=0.011$ ) and SAA ( $P=0.007$ ) as the only two nonredundant biomarkers. Thus, immunoassay concentrations of plasma apoC2 and SAA were used to construct an equation for calculating the "ApoSAA score," which was the diagnostic score used for identification of sepsis and/or NEC cases. The diagnostic value of the ApoSAA score was subsequently validated by the independent case-control cohort as well as in a prospective cohort study. In this study, the ApoSAA score has also provided a definitive strategy for safe and effective use of antimicrobial therapy for suspected preterm infants. The authors proposed that this triage strategy would enable neonatologists to prescribe antibiotics rationally and to judiciously allocate resources by targeting those who truly require urgent treatment and intensive care (11).

Ng et al. found SAA levels returned to normal after recovery of NEC (11). In our study, baseline and posttreatment levels of SAA were similar in patients with both stage 1 and stage 2 NEC. However, 11 of 15 patients with NEC had median 76% reduction in SAA levels after recovery. Therefore, we feel that we

could not demonstrate the difference between pre- and posttreatment SAA levels because of the limited number of patients. Studies with a higher number of patients may show the value of SAA on follow-up of patients with NEC.

In conclusion, SAA rises in early stages of NEC and may provide early diagnosis. The role of SAA in follow-up of these patients and whether SAA was more sensitive than other inflammatory markers should be investigated in larger patient series. Preliminary results in this study deserve further studies with more patients to search the utility of SAA in diagnosis and follow-up of the patients with NEC.

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