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ORIGINAL ARTICLE

Comparison of serum amyloid A concentrations with those of C-reactive protein and procalcitonin in diagnosis and follow-up of neonatal sepsis in premature infants

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Objective: The purpose of this study was to determine the role of serum amyloid A (SAA) in diagnosis of neonatal sepsis and evaluation of clinical response to antibiotic therapy. We also aimed to compare the efficiency of SAA with that of C-reactive protein (CRP) and procalcitonin (PCT) in diagnosis and follow-up of neonatal sepsis in preterm infants.

Study Design: A total of 163 infants were enrolled in this prospective study. The infants were classified into four groups: group 1 (high probable sepsis), group 2 (probable sepsis), group 3 (possible sepsis) and group 4 (no sepsis, control group). Blood samples for whole blood count, CRP, PCT, SAA and culture were obtained before initiating antibiotic treatment. This procedure was repeated three times at 48 h, 7 and 10 days.

Result: Initial CRP, PCT and SAA levels were found to be positive in 73.2, 75.6 and 77.2% of all infants, respectively. Sensitivities of CRP, PCT and SAA at 0 h were 72.3, 74.8 and 76.4%, respectively. Although it was not statistically significant, SAA was found to be more sensitive than CRP and PCT in diagnosis of neonatal sepsis. The area under the curve (AUC) for CRP, PCT and SAA at 0 h were 0.870, 0.870 and 0.875, respectively. Although the AUC for SAA at 0 h was higher than PCT and CRP, the difference was not statistically significant.

Conclusion: SAA is an accurate and reliable marker for diagnosis and follow-up of neonatal sepsis. It is especially useful at the onset of inflammation for rapid diagnosis of neonatal sepsis and can be safely and accurately used in combination with other sepsis markers such as CRP and PCT in diagnosis and follow-up of neonatal sepsis in preterm infants. *Journal of Perinatology* (2009) **29,** 225–231; doi:10.1038/jp.2008.207; published online 11 December 2008

Keywords: serum amyloid A; C-reactive protein; procalcitonin; neonatal sepsis; newborn

Introduction

Neonatal sepsis remains as an important cause of neonatal morbidity and mortality, despite the major advances in the management of newborn infants.[1] Early warning signs and symptoms of neonatal sepsis are often nonspecific and subtle especially at the onset of infection, and can easily be confused with other common noninfectious causes.[2] The incidence of sepsis ranges from 1 to 10 cases in every 1000 live births, with high mortality rates despite antibiotic treatment.[3] One of the most important aspects is the ability to obtain an early diagnosis and thus early therapy.[3] Therefore, antibiotics are started immediately in newborn infants who have nonspecific findings of infection and are continued until the final result of the blood culture is obtained.[4,5] Blood culture is the most valuable and gold-standard diagnostic method, but it may yield false-positive results because of contamination. Also, blood culture can remain negative despite generalized bacterial infection. Body fluid cultures, determination of bacterial antigens, white blood cell count, acute-phase proteins (C-reactive protein (CRP), haptoglobin, fibrinogen, α1-antitrypsin), interleukin (IL) and inflammatory cytokines and procalcitonin (PCT) are other laboratory tests, which are used to support the neonatal sepsis diagnosis.[6–8]

Serum amyloid A (SAA) term groups a family of 12 to 14 kDa polymorphic apolipoproteins that are mainly produced by the liver. They are regarded as acute-phase proteins because they increase considerably during infection. They can show as much as a 1000-fold increase in 8 to 24 h after the onset of sepsis. SAA has been shown to be useful in various acute diseases (bacterial, viral, traumatic, rheumatic and ischemic heart disease) and also in the diagnosis of sepsis in neonates.[3,9–11] Although the data about its use in diagnosis of neonatal sepsis is limited, recently it has been suggested as a superior marker compared with CRP.[12]

CRP is a protein produced by the liver in response to inflammatory and/or infectious stimuli, and for this reason it is regarded as an acute-phase protein. It may increase 12 to 24 h after the exposure to endotoxins. CRP may also increase in a number of prenatal conditions such as fetal distress, stressful delivery and

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maternal fever in the absence of systemic infection. Therefore, its specificity is accepted low and it is preferably used in combination with another serum marker.[12]

PCT is the precursor protein of calcitonin and is produced by monocytes and hepatocytes. It begins to rise 2 to 4 h after exposure to bacterial endotoxins, increases rapidly peaking in 6 to 8 h, and reaches a plateau and then decreases to normal levels after 24 h. Serum PCT levels appear to correlate with the severity of microbial invasion and they decrease rapidly after appropriate antibiotic therapy. Normal serum and plasma levels of PCT are less than 0.5 ng ml^{-1}. Levels above this value have been accepted to be pathological.[13–16] It has also been suggested that PCT levels may be increased in neonatal sepsis and its diagnostic utility is comparable that of with CRP.[12]

As stated previously, clinical signs of neonatal sepsis are nonspecific and there is no sensitive and specific laboratory test for detecting an early-stage infection. The purpose of this study was to determine the role of SAA in diagnosis of neonatal sepsis and in evaluation of clinical response to antibiotic therapy. In addition, we also aimed to compare the efficiency of SAA with that of CRP and PCT in diagnosis and follow-up of neonatal sepsis in preterm infants.

Methods

In this prospective study, 185 premature infants born between January 2006 and January 2008 in the neonatal intensive care unit (NICU) of the Pediatric Department of Uludag University, Faculty of Medicine were initially planned to be enrolled. However, 22 infants were excluded from the study population because parents of 14 infants (4 in study group and 10 in control group) rejected inclusion of their children and 8 infants (all from the study group) were already receiving antibiotics on admission. Therefore, a total of 163 premature infants (123 infants with neonatal sepsis and 40 controls) were included in this study. This study was originally planned to investigate 120 infants in each group. However, the number of patients in control group did not reach the initially planned level because most infants hospitalized during the study period had major respiratory, cardiac and neurological problems partly because of the fact that our NICU is a tertiary care neonatal referral unit in South Marmara region of Turkey. The infants were classified into four groups according to the criteria defined by Gitto *et al*.[17] group 1 (high probable sepsis), group 2 (probable sepsis), group 3 (possible sepsis) and group 4 (no sepsis, control group). Infants with neonatal convulsion, neonatal hypoglycemia, neonatal hyperbilirubinemia who had no signs of clinical and laboratory infection were referred to as the control group. Table 1 lists the criteria for classifying study groups.

The study protocol was approved by the Ethics Committee of Uludag University, Faculty of Medicine. Informed parental consent was obtained for all infants. Exclusion criteria included

Table 1 Criteria employed for defining the sepsis score

Groups	*Criteria*
Group 1	At least 3 sepsis-related clinical signs[a]
High probable sepsis	CRP >1 mg per 100 ml
	At least 2 other altered serum parameters in addition to CRP[b]
	Blood culture; positive or negative
Group 2	Less than 3 sepsis-related clinical signs[a]
Probable sepsis	CRP > 1 mg per 100 ml
	At least 2 other altered serum parameters in addition to CRP
	Blood culture; negative
Group 3	Less than 3 sepsis-related clinical signs[a]
Possible sepsis	CRP <1 mg per 100 ml
	Less than 2 other altered serum parameters
	Blood culture; negative
Group 4	No sepsis-related clinical signs[a]
No sepsis	CRP <1 mg per 100 ml
	No altered serum parameters
	Blood culture; negative

Abbreviation: CRP, C-reactive protein.
[a]Sepsis-related clinical signs: temperature instability, apnea, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distension, necrotizing enterocolitis.
[b]Serum parameters other than CRP: white blood cell count, absolute neutrophil count, platelet count.

administration of antibiotic therapy at admission and refusal of parental consent. Gestational age, birth weight, gender, mode of delivery, Apgar score at 1 and 5 min, prenatal demographics, premature rupture of membranes (PROM) and history of chorioamnionitis were all recorded. Temperature instability, apnea, need for supplemented oxygen, need for ventilation, tachycardia/bradycardia, hypotension, feeding intolerance, abdominal distension, necrotizing enterocolitis were considered among clinical signs of sepsis. The changes in the hematologic parameters were processed according to the Manroe and Rodwell scoring systems.[18,19] Leukopenia was defined as leukocyte count <5000 mm^{-3}; leukocytosis was defined as leukocyte count >25 000 mm^{-3} at birth, >30 000 mm^{-3} at 12 to 24 h and >21 000 mm^{-3} after the second day. Thrombocytopenia was defined as platelet count <150 000 mm^{-3}. Normal absolute neutrophil count was accepted as 7800 to 14 500 mm^{-3} in the first 60 h and 1750 to 5400 mm^{-3} after 60 h. Before initiating the antimicrobial therapy, blood samples for whole blood count, CRP, PCT, SAA and culture were obtained both from neonates with sepsis and from control patients.

This procedure was repeated three times at 48 h, 7 and 10 days. Cerebrospinal fluid (CSF), urine, and tracheal and gastric materials were also sent out for culture, if obtained. Blood smears of all infants were also evaluated for the findings of sepsis when

Table 2 Birth and clinical characteristics of study group

	Group 1 (n = *108*)	*Group 2+3* (n = *15*)	*Group 4* (n = *40*)
Gestational age (week)[a]	31.2 ± 3.19 (25–37)	31.6 ± 2.9 (26–36)	32.7 ± 1.56 (32–35)
Birth weight (g)[b]	1745 (690–2700)	1685 (750–2550)	1725 (1200–2250)
Males/females	58/50	10/5	22/18
Apgar min 1[b]	5.7 (1–9)	5.6 (2–9)	7.4 (4–9)
Apgar min 5[b]	6.7 (5–9)	7.0 (5–9)	7.5 (6–10)
Cesarean delivery[c]	72 (66.7)	11 (73.3)	20 (50)
PROM[c]	12 (11.1)	2 (13.3)	0 (0)*
Choriamnionitis[c]	4 (3.7)	1 (6.7)	0 (0)*
Hypoxia[c]	6 (5.6)	6 (40)	0 (0)*
Apnea[c]	6 (5.6)	4 (26.7)	0 (0)*
Feeding intolerance[c]	36 (33.3)	4 (26.3)	0 (0)*
Jaundice[c]	22 (20.4)	9 (60)	1 (2.5)*
Mortality[c]	25 (23.1)	1 (6.7)	0 (0)*

Abbreviation: PROM, premature rupture of membrane.
*$P<0.05$ between sepsis and nonsepsis group.
[a]Median ± standard deviation (range).
[b]Median (range).
[c]*n* (%).

blood samples obtained. Meningitis was diagnosed according to the cell count, glucose and protein levels of CSF along with CSF culture.

Whole blood count, PCT, CRP, SAA levels and cultures were studied immediately. Whole blood count was performed using an automated counter, Cell Dyn 3700 (Abbott Diagnostics Division, Santa Clara, CA, USA). CRP and SAA were determined by an immunonephelometric method using BN II device (Dade Behring Marburg GMBH, Marburg, Germany). Detection limits were 0.5 and 6.8 mg per 100 ml for CRP and SAA, respectively. PCT was measured by monoclonal immunoluminometric assay (Lumitest PCT; Brahm Diagnostica GMBH, Berlin, Germany), which is specific for PCT molecule. In this assay, two different antibodies, one directed to calcitonin and other directed to katacalcin were used. Levels greater than 0.5 ng ml^{-1} were accepted as pathological. Levels lower than 0.5 ng ml^{-1} for PCT and CRP and 6.8 mg per 100 ml for SAA were accepted as 0 for statistical analysis. Blood and CSF cultures were analyzed using fully automated BACTEC method by BACTEC 9240 device (Becton Dickinson, Heidelberg, Germany).

Infants were treated with appropriate antibiotic therapies. Neonates who had positive cultures were treated with antibiotics according to the culture antibiogram. The antimicrobial therapy was stopped after clinical and laboratory improvement. All of the groups were compared according to demographic features, clinical and laboratory findings.

SPSS 12.0/Windows program was used for data analyses. Descriptive statistics were given as mean, median, standard deviation, minimum, maximum and percentage. The significance between groups were evaluated with χ^2-test and McNemar's test for qualitative data and with Friedman and Wilcoxon tests for quantitative data. Correlations between quantitative data were analyzed by Spearman's correlation test. Values of $P<0.05$ were considered to be significant. In subgroup comparisons, Bonferroni correction was used for *P* significance levels.

Results

A total of 163 preterm infants were enrolled in this study. The demographic and clinical characteristics of the study population are shown in Table 2. There were 108, 5, 10 and 40 neonates in groups 1, 2, 3 and 4, respectively. But, as the number of infants in groups 2 and 3 were too small for statistical analysis, they were grouped together as group 2 + 3. There were no differences between the groups with respect to gestational age, birth weight, gender, Apgar scores at 1 and 5 min, the mode of delivery and presence of PROM. Hypoxia, apnea, jaundice, feeding intolerance and chorioamnionitis were also found to be significantly higher in group 2 + 3 than those in group 1. However, all these differences were associated with the small number of infants in group 2 + 3 compared with those in group 1. There were no differences between infants with sepsis with respect to other characteristics.

In group 1, 10 infants (9.3%) had early-onset sepsis (EOS) and 98 infants (90.7%) had late-onset sepsis (LOS). There were eight infants (53.3%) with EOS and seven infants (47.7%) with LOS in group 2 + 3. Pneumonia was diagnosed in 52.8 and 33% of the infants in group 1 and group 2 + 3, respectively. Neonatal meningitis was determined in 23.1 and 38.5% of infants in group 1 and group 2 + 3, respectively. There were no statistically significant differences between two groups with respect to

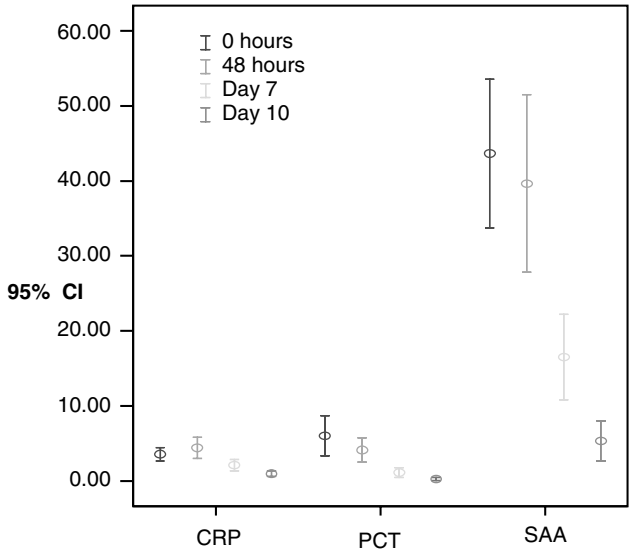

Figure 1 Figure showing C-reactive protein (CRP), procalcitonin (PCT) and serum amyloid A (SAA) levels measured baseline, and trends of these levels during 48 h, day 7 and day 10.

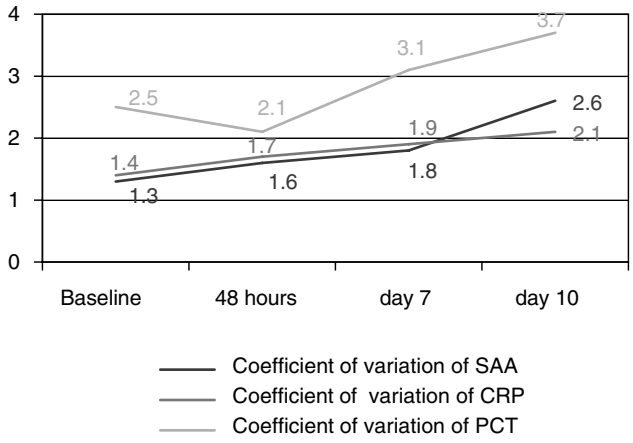

Figure 2 Figure showing the coefficient of variations of C-reactive protein (CRP), procalcitonin (PCT) and serum amyloid A (SAA).

meningitis and pneumonia. Total 72 infants had positive blood culture for gram-positive sepsis (66 *Staphylococcus epidermidis*, 4 group B *Streptococcus*, 1 *Enterococcus faecalis*, 1 *Corynebacterium matruchotii*), 13 for gram-negative sepsis (6 *Escherichia coli*, 4 *Pseudomonas aeruginosa*, 3 *Klebsiella pneumonia*) and 14 for fungal sepsis (10 *Candida parapsilosis*, 4 *Candida albicans*). Out of 123, 26 (21.1%) infants in sepsis group died during the study period. Nine of them died because of noninfectious causes. Twenty-five of them were in group 1, only one was in group 2 + 3. The difference between mortality rates was found to be statistically significant.

Mean initial leukocyte counts were determined as $14\,377 \pm 11\,065$, $14\,913 \pm 7788$ and $12\,575 \pm 2763\ mm^{-3}$ in groups 1, 2 + 3 and 4, respectively. No significant differences were found between sepsis groups in terms of mean leukocyte counts. Although the mean leukocyte counts in sepsis group was higher than those in control group, the difference was not statistically significant. Mean leukocyte counts in sepsis group significantly decreased at 48 h, 7 and 10 days. There were no differences between sepsis groups with respect to platelet counts and thrombocytopenia. There were also no differences between infants with positive or negative blood cultures with respect to CRP, PCT and SAA levels ($P > 0.05$).

Initial CRP levels were positive in 73.2% of infants in sepsis group. CRP levels reached to peak levels at 48 h and then decreased significantly. PCT levels were found to be positive in 75.6% of infants in sepsis group and these levels declined throughout the study period. SAA levels were positive in 77.2% of infants with sepsis and it also returned to normal levels after 48 h. Figure 1 shows the baseline levels of CRP, PCT and SAA with their trends throughout the study period.

SAA values of 92.2% of patients with CRP values beyond upper limits were higher than normal limits, however, the results were not statistically significant ($P = 0.359$). SAA values of 7 (7.8%) patients with CRP values beyond upper limits were within normal limits, and CRP values of 12 (12.6%) patients with SAA values beyond upper limits were within normal limits. CRP results showed 87.8% correlation with PCT being beyond upper limits ($P = 0.690$). SAA levels of 93.5% of patients with PCT values beyond upper limits also were higher than normal, and there was no statistical difference between groups ($P = 0.791$). Statistical analyses demonstrated that the rate (73.2%) of sepsis diagnosis with only CRP positivity would increase to 82.9 and 84.6% following additional analysis of SAA and PCT, respectively. Therefore, it is estimated that the combination of SAA, CRP and PCT for the diagnosis of neonatal sepsis would increase the diagnosis rate from 72 to 76% to 82 to 85%.

There were positive correlations between laboratory values of CRP, PCT and SAA (that is, r: 0.627 for CRP and SAA, $P < 0.001$; r: 0.602 for PCT and SAA, $P < 0.001$ and r: 0.563 for PCT and CRP, $P < 0.001$) at the onset of the study. Mean percent increases in CRP, PCT and SAA compared with their normal laboratory values were 71.4, 75.8 and 70.8%, respectively. There were no statistical differences in terms of mean percent increases between CRP and SAA, or CRP and PCT, whereas mean percent increase in PCT was statistically higher ($P = 0.004$) than that in SAA according to the coefficient of variations (Figure 2). When CRP, PCT, SAA levels and the percentage variations at baseline, 48 h, 7 and 10 days were evaluated in group 1 and group 2 + 3 separately, the results in both groups were similar to the total patient group and there were no significant differences between groups.

The sensitivity of CRP, PCT and SAA at 0 h were 72.3, 74.8 and 76.4%, respectively. Although it was not statistically significant, SAA was found to be more sensitive than CRP and PCT in the diagnosis of neonatal sepsis (Table 3). Initial CRP, PCT and SAA levels were

Table 3 Comparison of CRP, PCT and SAA according to sensitivity, specificity, PPV, NPV and AUC at 0, 48 h and 7 days

Marker	*Time*	*Sensitivity (%; 95% CI)*	*Specificity (%; 95% CI)*	*PPV (%)*	*NPV (%)*	*AUC*	P-*value*
CRP, >0.5 mg per 100 ml	0 h	72.3 (63.6–80)	100 (91.1–100)	100	54	0.870	
	48 h	71.5 (62.4–79.5)	100 (91.1–100)	100	54.8	0.861	
	7 days	50 (40.5–59.5)	100 (91.1–100)	100	41.2	0.758	
PCT, >0.5 mg per 100 ml	0 h	74.8 (66.2–82.2)	100 (91.1–100)	100	56.3	0.870	>0.05
	48 h	57.7 (48.2–66.9)	100 (91.1–100)	100	44.9	0.807	
	7 days	28.4 (20.5–37.6)	100 (91.1–100)	100	32.5	0.657	
SAA, >6.8 mg per 100 ml	0 h	76.4 (67.9–83.6)	100 (91.1–100)	100	58	0.875	
	48 h	66.3 (57–74.9)	100 (91.1–100)	100	50.6	0.837	
	7 days	44.7 (35.4–54.3)	100 (91.1–100)	100	38.8	0.712	

Abbreviations: AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; NPV, negative predictive value; PCT, procalcitonin; PPV, positive predictive value; SAA, serum amyloid A.

Table 4 Table showing the mean CRP, PCT and SAA levels in three groups at 0, 48 h, 7 and 10 days

Time after sepsis onset	*High probably sepsis* (n = *108*)	*Probable sepsis+possible sepsis* (n = *15*)	*No sepsis* (n = *40*)
CRP mg per 100 ml			
0 h	3.9 ± 5.1*	1.7 ± 2.9*	0.3 ± 0.5
48 h	4.8 ± 8.1	1.9 ± 2.9	N/A
7 days	2.3 ± 4.2	0.9 ± 1.0	N/A
10 days	1.1 ± 2.3	0.3 ± 0.6	N/A
PCT mg per 100 ml			
0 h	6.2 ± 15.7*	5.1 ± 7.5*	0.1 ± 0.4
48 h	4.2 ± 9.1	4.0 ± 6.8	N/A
7 days	1.1 ± 3.6	1.1 ± 1.9	N/A
10 days	0.3 ± 1.1	0.1 ± 0.2	N/A
SAA mg per 100 ml			
0 h	44.4 ± 57.3*	37.9 ± 41.7*	3.2 ± 3.4
48 h	39.5 ± 67.1	40.8 ± 34.5	N/A
7 days	15.7 ± 28.7	22.0 ± 41.7	N/A
10 days	5.8 ± 15.0	2.9 ± 5.9	N/A

Abbreviations: CRP, C-reactive protein; N/A, not available; PCT, procalcitonin; SAA, serum amyloid A.
*$P<0.05$.

significantly higher in sepsis group than those in control group (Table 4). Significant differences were observed between sepsis (groups 1 + 2 + 3) and control (group 4) groups with respect to initial CRP, PCT and SAA levels, as expected (Table 5).

Although the area under the curve (AUC) for SAA at 0 h was higher than that in PCT and CRP, the difference was not statistically significant. There were no statistical differences between CRP, SAA and PCT according to PPV, NPV, AUC at 0, 48 h and day 7. Table 3 shows the sensitivity, specificity, PPV, NPV and AUC values for CRP, PCT and SAA at 0, 48 h and day 7. Figure 3 shows the comparison of AUC for CRP, PCT and SAA at 0, 48 h and day 7.

Discussion

Systemic infection is a devastating and important cause of morbidity and mortality in both term and preterm infants. Although the initial clinical signs and symptoms are subtle, the clinical course can rapidly progress and worsen, and may lead to disseminated intravascular coagulation and death within hours.[2,20] Also, noninfected infants such as those with transient tachypnea of the newborn, meconium aspiration syndrome, respiratory distress syndrome, apnea of prematurity and acute exacerbation of chronic lung disease are often clinically indistinguishable from infants who are really in the initial stages of bacterial infection.[21] If the absence

of systemic infection can be detected early, then the number of infants started on antibiotics could be reduced, the length of hospitalization could be shortened, and the potential for emergence of resistant organisms could be lessened. However, to date, although infection markers might help to diagnosis, no single laboratory test has provided rapid and reliable identification of early infected neonates.[22] Hence, it is important to diagnose neonatal sepsis in a rapid and accurate way especially in preterm infants. Therefore, we performed this prospective study to determine possible diagnostic value of SAA in neonatal sepsis and also compared its efficiency with more widely used markers CRP and PCT.

CRP is the most commonly used acute-phase reactant in neonates.[23] As CRP increases 12 to 24 h after the onset of infection, it is usually used in combination with other markers. PCT is another marker, which has been used recently in combination with CRP in the diagnosis of neonatal sepsis. High PCT levels were reported in neonates with early- or late-onset neonatal sepsis.[24]

There are also studies comparing CRP and PCT in diagnosis and follow-up of neonatal sepsis. There are conflicting results about their superiority to each other in diagnosis of neonatal sepsis in different studies. In our previous study, serum PCT levels seemed to be superior to serum CRP levels in terms of early diagnosis of neonatal sepsis, in detecting the severity of the illness, and in evaluation of the response to antibiotic treatment.[25] This was similar to findings of some,[26,27] but not all[28,29] studies. Therefore, in some studies multiple comparisons were made between CRP, PCT and other cytokines such as IL-6, IL-8 and tumor necrosis factor-α. As a result of these conflicting results, we aimed to compare SAA with PCT and CRP in the diagnosis and follow-up of neonatal sepsis.

SAA has inhibitory effects on inflammation by reducing the production of prostaglandin E2 and oxidative respiration of neutrophils, counteracting the pyrogenic effect of a number of cytokines, inhibiting platelet activation, negatively controlling the production of antibodies and inducing the secretion of collagenase by fibroblasts.[3] Therefore, it has been started to use in diagnosis of neonatal sepsis with other laboratory tests.

Arnon *et al*.[30] reported that SAA could be used as a reliable marker for early detection of LOS in preterm infants. These authors also stated that SAA levels had prognostic value in the first 24 h after the onset of neonatal sepsis.[31] They established that SAA had higher levels, and rose earlier and sharper than CRP.[10] In our study, we found that SAA had higher levels during the initial and 48-h evaluation of infants with sepsis. We have also shown that it declined faster than CRP and PCT during the follow-up of sepsis. These findings are all concordant with literature and we suggest that it can be used safely in diagnosis of neonatal sepsis.

Shortland *et al*.[32] reported that CRP remained normal in 54% of very low-weight premature infants during culture positive neonatal sepsis. No differences between septic infants with positive or

Table 5 Table showing the comparison of mean CRP, PCT and SAA levels in sepsis (groups 1+2+3) and control (group 4) groups at 0, 48 h, 7 and 10 days

Time after sepsis onset	*Sepsis group (groups 1+2+3,* n = *123)*	*Control group (group 4,* n = *40)*
CRP mg per 100 ml		
0 h	3.6 ± 4.9*	0.3 ± 0.5
48 h	4.4 ± 7.6	N/A
7 days	2.1 ± 3.9	N/A
10 days	1.0 ± 2.1	N/A
PCT mg per 100 ml		
0 h	6.0 ± 14.9*	0.1 ± 0.4
48 h	4.1 ± 8.8	N/A
7 days	1.1 ± 3.4	N/A
10 days	0.2 ± 1.0	N/A
SAA mg per 100 ml		
0 h	43.6 ± 55.5*	3.2 ± 3.4
48 h	39.6 ± 64.2	N/A
7 days	16.5 ± 30.5	N/A
10 days	5.3 ± 14.1	N/A

Abbreviations: CRP, C-reactive protein; N/A, not available; PCT, procalcitonin; SAA, serum amyloid A.

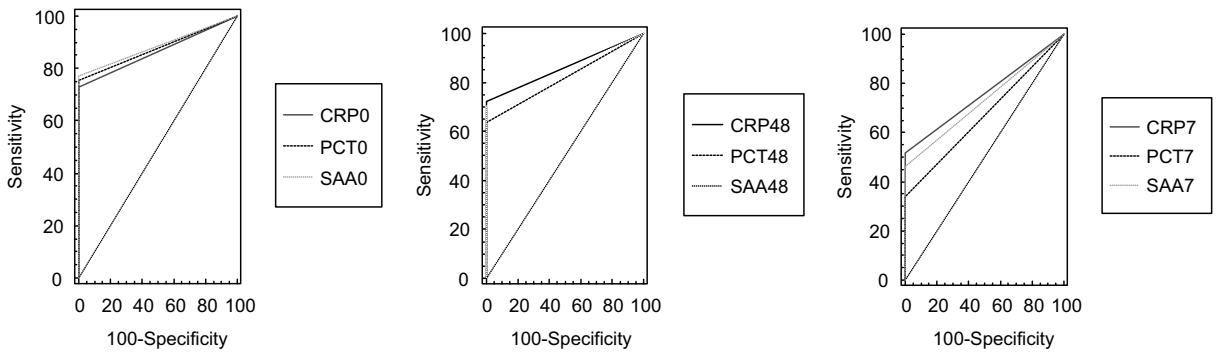

Figure 3 Graphics showing the comparison of the area under curve (AUC) for C-reactive protein (CRP), procalcitonin (PCT) and serum amyloid A (SAA) at onset, 48 h and day 7.

*$P < 0.05$

negative cultures with respect to CRP, PCT and SAA levels were found in our study. These findings may suggest that culture positivity does not increase CRP, PCT or SAA levels in infants with neonatal sepsis.

Arnon *et al*.[11] found that SAA had significantly largest AUC compared with CRP at 0 h of sepsis evaluation. Enguix *et al*.[22] reported that SAA, CRP and PCT had the same diagnostic efficiency and the same area under the ROC curve in neonates with bacterial sepsis. In our study, we also found no significant differences between CRP, PCT and SAA in terms of AUC, PPV, NPV and specificity.

In our hospital, the cost of measuring each CRP or SAA ($4) is one-fourth that of PCT. Therefore, although combining SAA with other two tests for the diagnosis of sepsis may not be considered cost-effective, our finding that combination of the three methods could increase the rate of sepsis diagnosis by about 10% suggests that measuring all three parameters is reasonable to consider.

The limitation of our study is the small number of patients in the control group. We believe that the power of the study would be increased considerably under such circumstance that we were able to recruit adequate number of infants in the control group.

As a result, SAA is an accurate and reliable marker for diagnosis and follow-up of neonatal sepsis. It is especially useful at the onset of inflammation with rapid diagnosis of neonatal sepsis. It can also help clinicians to follow the clinical course of neonatal sepsis up. The rapid diagnosis of neonatal sepsis will reduce the morbidity and mortality of sepsis by starting the antibiotic therapy as soon as possible. Also, it can be used to establish the duration of treatment and response to treatment. Therefore, SAA can be safely and accurately used either alone or in combination with other sepsis markers such as CRP and PCT in diagnosis and follow-up of neonatal sepsis in preterm infants. Future studies with larger number of patients are warranted for determining the best marker for the diagnosis of neonatal sepsis.

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