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*Original Article*

# The Diagnostic Value of Serum Amyloid A in Early-Onset Neonatal Sepsis in Premature Infants

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**Abstract** ***Purpose:*** In this study, the aim was to determine the distinct effectiveness of serum amyloid A in the early stage of early-onset neonatal sepsis in premature infants. ***Methods:*** Preterm newborns hospitalised between 2014 and 2017 for suspected early-onset neonatal sepsis were included in this prospective study. Patients were evaluated according to clinical and laboratory findings at admission and at the 24th and 48th hours after admission. The serum amyloid A values of the patients with sepsis and a control group were compared, and the blood cultures were evaluated. ***Results:*** A total of 319 premature newborns were included in the study: 150 in the sepsis group and 169 in the control group. Their birth weight ranged between 590 g and 3000g and the gestational age was 24-36 weeks. The serum amyloid A values at admission were significantly higher in the cases diagnosed with sepsis compared to the control group. ***Conclusion:*** Serum amyloid A is a reliable diagnostic marker for the early onset of neonatal sepsis, and it has a higher sensitivity at symptom onset or in the first hours after birth in premature infants.

**Key words** Early-onset neonatal sepsis; Premature; Serum amyloid A

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## Introduction

Despite the advances in prenatal health care, neonatal sepsis remains one of the biggest causes of neonatal deaths.[1] Suspected neonatal sepsis is amongst the diagnoses that neonatal specialists consider the most because of a lack of specific signs and symptoms and the absence of a precise marker to distinguish from non-infectious settings with the same manifestations.[2] Symptoms that are miscible with sepsis are frequently encountered, especially in premature infants.

Early diagnosis and early antibiotherapy are of critical importance to reduce mortality and morbidity related to neonatal sepsis. Therefore, antibiotic therapy should be started immediately in any newborn presenting with the signs of sepsis. It takes time to obtain the results of the blood culture, which is the gold standard in diagnosis.[3] However, the blood culture may be negative, despite a generalised bacterial infection, or false positive due to

contamination.[3] Besides, there is evidence that unnecessarily prolonged antibiotic use in premature newborns is associated with an increased risk of late-onset neonatal sepsis and necrotising enterocolitis and increased mortality.[4]

For this reason, numerous laboratory parameters, including white blood cell count (WBC), the ratio of immature neutrophil count to total neutrophil count, platelet count (PLT), C-reactive protein (CRP), serum amyloid A (SAA), procalcitonin (PCT) and different cytokines, are used for the early diagnosis of neonatal sepsis.[5-8] Amongst these, CRP and PCT are widely used. The sensitivity of CRP is low in the early period of early-onset sepsis and is remarkably increased after 10-12 hours.[8] PCT can physiologically increase immediately after birth, which limits its usage in diagnosing early-onset sepsis.[9]

SAA defines a family of polymorphic apolipoproteins released mainly from the hepatocytes.[10] Its release is regulated by proinflammatory cytokines.[11] It increases tenfold in infectious and non-infectious conditions[12] and can also be used as an acute phase reactant in the diagnosis of neonatal sepsis.[12] Several studies reported that SAA increases in the earlier phases of inflammation and has more superior diagnostic value compared to CRP.[13] Further studies are needed to determine and strengthen the role of SAA in the diagnosis and monitoring of neonatal sepsis.

The current study aimed to identify the role of SAA in the early diagnosing of early-onset neonatal sepsis (EOS) in premature newborns. Moreover, it aimed to determine the cut-off value for SAA in predicting early-onset neonatal sepsis with high specificity and sensitivity.

## Methods

This study was carried out prospectively in a tertiary hospital's neonatal intensive care unit. Preterm newborns who were hospitalised between 2014 and 2017 for suspected early-onset neonatal sepsis were included in the study. The study was approved by the ethics committee of our university (201813/21). EOS was defined as sepsis occurring in the first 72 hours of life. Newborns with a congenital anomaly, chromosomal anomaly, history of hypoxic birth and suspected necrotising enterocolitis, and infants whose parents did not give consent were excluded. The gestational age, birth weight, route of delivery, Apgar scores and prenatal demographics of the participants, as well as the history of the early rupture of the membranes and chorioamnionitis, were retrieved from the electronic patient files. The WBC, absolute neutrophil count (ANC), PLT, CRP, PCT and SAA values studied at three different time points – at admission and at the 24th and 48th hours of admission – were recorded. The results of the overall blood cultures taken in the first 72 hours were evaluated.

Patients were evaluated by the European Medicines Agency (EMA) neonatal sepsis criteria at the 24th and 48th hours of admission.[14] The patients were divided into two groups according to the results of two evaluations: the sepsis group (Group 1) and the control group (Group 2). Antibiotic treatment was discontinued in patients who were re-evaluated at the 48th hour of hospitalisation, and they were excluded from the sepsis group according to the EMA criteria.[14] The patients in the sepsis group were evaluated in two groups: proven sepsis (group 1a) and clinical sepsis (group 1b), according to their culture results.

The newborns with a history of premature rupture of membranes (PROM) and chorioamnionitis were managed in accordance with the AAP guidelines.[2] Accordingly, antibiotic therapy was started after the blood culture was taken from all the newborns with chorioamnionitis and from the premature newborns with PROM. The decision for the maintenance of treatment was made based on the results of both the acute phase reactants and the clinical evaluations performed at admission and at the 24th and 48th hours of admission as the routine procedure of our clinic.

All CRP, PCT and SAA levels are studied in same laboratory using the ELISA method; CRP >9 mg/L, PCT > 0.5 µg/L are considered positive according to our laboratory.

In each group, the demographic characteristics of the patients, the prenatal risk factors and the maternal and neonatal characteristics were evaluated. The laboratory findings were compared between each of the three groups as well as between the overall sepsis cases (Group 1 and Group 2) and non-sepsis cases. In the early-onset neonatal sepsis cases, the cut-off values were calculated for CRP, PCT and SAA on different days. The sensitivity and specificity of the cut-off values given by our laboratory for CRP and PCT were evaluated for the patients.

SPSS software (V.23) was used for the comparisons. The demographic data were evaluated by a student t-test and chi-square test. The laboratory data of the groups were evaluated using the Mann Whitney U test. $P<0.05$ was considered significant. The MedCalc (V.13) programme was used to evaluate the cut-off values and to analyse the receiver operating characteristic (ROC).

## Results

There were a total of 420 premature babies admitted to our NICU over a period of four years for early-onset neonatal sepsis suspicion. Of these newborns, 101 were excluded since they met at least one of the exclusion criteria, or their parents did not give consent. Finally, 319 newborns with birth weights between 590 g and 3000 g and gestational ages between 24 and 36 weeks were included in the study – 150 in Group 1 and 169 in Group 2.

No difference was determined between the demographic characteristics of the newborns in both of the two groups (Table 1).

At admission, the most common clinical signs in the suspected sepsis cases were respiratory distress, feeding intolerance and non-specific signs that were mentioned in the EMA neonatal sepsis criteria (Table 2).

Both of the two groups were not different in terms of PCT values at admission. The CRP and SAA values at admission were significantly higher in the cases diagnosed with sepsis compared to the control group. The CRP, PCT and SAA at the 24- and 48- hour samples were significantly higher in the sepsis cases (Table 3). No significant difference was determined between the proven sepsis (n: 40) and clinical sepsis (n: 110) groups in terms of the CRP, PCT and SAA values obtained from any sample.

The cut-off values and ROC analysis for the sepsis prediction of SAA, PCT and CRP in predicting sepsis, and the area under the ROC curve, sensitivity and specificity for these values are demonstrated in Table 4.

The breakdown list of bacterial species causing EOS was shown in Table 5.

No difference was determined between the three groups in terms of the WBC, ANC and platelet count at any time.

## Discussion

Infections in the neonatal period, which is the most sensitive period of life, cause substantial side effects. While early-onset sepsis does not manifest itself with significant clinical symptoms in the first hours, it is associated with high mortality rates unless treated.[15,16] Therefore, it is important to start appropriate antibiotherapy in newborns with any sepsis-related suspected clinical symptom or who are at high risk for early-onset sepsis.[15] However, a clinicians' concern may result in many newborns receiving unnecessary antibiotherapy. In the present study, linking the symptoms to non-sepsis etiologies during monitoring, antibiotherapy was discontinued in 53% of the newborns with suspected early-onset sepsis. Likewise, Murphy et al reported that sepsis was excluded and antibiotherapy was discontinued in the early period in nearly 50% of the patients.[17]

Although numerous acute phase reactants and scoring systems are being used, there is yet no single laboratory test that will rapidly and reliably detect the infection in newborns with suspected sepsis.[8] The current study demonstrated that SAA provides significant information concerning the diagnosis of early-onset sepsis from the first hours of birth.

SAA is mainly secreted from the liver in response to inflammation.[12] Arnon et al demonstrated that SAA is superior to CRP as a marker of early-onset sepsis.[11] Cetinkaya et al found that SAA is an accurate and reliable marker for the diagnosis and monitoring of neonatal sepsis and is beneficial for the rapid diagnosis of neonatal sepsis, particularly in the beginning of an infection. They stated that it can be safely and accurately used together with other sepsis markers, such as CRP and PCT, as well as for

**Table 1** Birth and clinical characteristics of infants included in the study

| | Group 1 (n: 150) | Group 2 (n: 169) | p |
|---|---|---|---|
| Gestational age (week), mean±SD | 31.19±3.0 | 31.55±3.28 | |
| Birth weight (g), mean±SD | 1620±639 | 1594±594 | |
| Admission time (hour), mean±SD | 3.15±2.2 | 2.55±1.9 | |
| Apgar min 5, med (min-max) | 8 (7-10) | 8 (7-10) | >0.05 |
| Cesarean delivery, % | 51.5 | 49.7 | |
| PROM, % | 9.1 | 15.7 | |
| Choriamnionitis, % | 3 | 2.6 | |
| Multiparity, % | 32.4 | 29.4 | |

PROM; premature rupture of membrane

the diagnosis and monitoring of neonatal sepsis in preterm newborns.[10] After this and the similar studies, a meta-analysis performed in 2013 reported that SAA is a valuable marker of neonatal sepsis, and it has proper diagnostic accuracy.[12] Moreover, the literature reports that SAA not only has high accuracy in the early detection of neonatal sepsis, but it also shows the inverse relationship with mortality.[8] In the current study, SAA studied in the early

**Table 2** Clinical findings in suspected sepsis cases

| **Clinical signs** | **N: 319** |
|---|---|
| Respiratory instability, n (%) | 280 (88) |
| Gastrointestinal signs, n (%) | 203 (64) |
| Non-specific signs*, n (%) | 166 (52) |
| Temperature instability, n (%) | 102 (32) |

* Irritability, lethargy and, hypotonia

**Table 3** CRP, PCT, and SAA values of the infants included in the study

| | **Group 1 (n: 150)** | **Group 2 (n: 169)** | **p** |
|---|---|---|---|
| CRP (mg/L), median (min-max) | | | |
| Admission | 5.5 (3.3-96) | 4.5 (3.3-15) | 0.02 |
| 24th hour | 25.5 (3.3-126) | 3.6 (3.3-28) | <0.001 |
| 48th hour | 25 (9-197) | 3.3 (3.3-20) | <0.001 |
| PCT (µg/L), median (min-max) | | | |
| Admission | 0.29 (0.05-79) | 0.31 (0.05-55) | 0.65 |
| 24th hour | 7.9 (1.55-327) | 3.9 (0.05-56) | <0.001 |
| 48th hour | 5.38 (2.05-200) | 1.67 (0.05-36) | <0.001 |
| SAA (mg/L), median (min-max) | | | |
| Admission | 13.62 (4.5-84) | 3.55 (3.3-30) | <0.001 |
| 24th hour | 22.05 (7.3-185) | 3.83 (3.3-31) | <0.001 |
| 48th hour | 23.10 (8.1-207) | 5.22 (3.3-25) | <0.001 |

CRP: C-reactive protein; PCT: procalcitonin; SAA: serum amyloid A

**Table 4** Cut-off values and ROC analysis for sepsis prediction for CRP, PCT, and SAA

| **Variable** | **Cut-off** | **AUC** | **P** | **Sensivity** | **95% CI** | **Specifity** | **95% CI** |
|---|---|---|---|---|---|---|---|
| CRP (mg/L) | | | | | | | |
| Admission | >3 | 0.593 | <0.001 | 72 | 66.1 - 77.9 | 42 | 36.1 - 49.4 |
| | >9 | | | 35 | 30.8 - 40.3 | 94 | 91.8 - 98.9 |
| 24th hour | >4 | 0.716 | <0.001 | 53 | 47.2 - 60.4 | 83 | 77.7 - 88.0 |
| | >9 | | | 75 | 69.2 - 81.9 | 91 | 88.4 - 93.7 |
| 48th hour | >4 | 0.794 | <0.001 | 76 | 70.6 - 81.3 | 76 | 70.3 - 81.5 |
| | >9 | | | 82 | 76.6 - 89.2 | 89 | 85.2 - 91.7 |
| PCT (µg/L) | | | | | | | |
| Admission | >3.25 | 0.542 | 0.12 | 17 | 12.7 - 23.0 | 91 | 87.3 - 95.0 |
| | >0.5 | | | 35 | 29.0 - 41.9 | 68 | 62.2 - 74.9 |
| 24th hour | >7 | 0.626 | <0.001 | 46 | 40.1 - 53.6 | 75 | 68.5 - 80.7 |
| | >0.5 | | | 82 | 77.1 - 87.5 | 28 | 22.8 - 35.5 |
| 48th hour | >1.7 | 0.636 | <0.001 | 62 | 56.4 - 68.8 | 60 | 54.4 - 67.2 |
| | >0.5 | | | 82 | 76.8 - 86.7 | 31 | 25.4 - 37.7 |
| SAA (mg/L) | | | | | | | |
| Admission | >9.5 | 0.706 | <0.001 | 63 | 56.2 - 68.8 | 80 | 73.4 - 84.8 |
| 24th hour | >10 | 0.779 | <0.001 | 63 | 55.7 - 68.6 | 88 | 82.9 - 92.0 |
| 48th hour | >11 | 0.875 | <0.001 | 80 | 74.3 - 84.5 | 85 | 80.2 - 89.6 |

CRP: C-reactive protein; PCT: procalcitonin; SAA: serum amyloid A: ROC: receiver operating characteristic; AUC: area under the ROC curve; CI: confidence interval

period for the diagnosis of early-onset sepsis was higher in the sepsis cases compared to the control group. There was no difference between the SAA values of the clinical and proven sepsis cases.

Different studies have reported different values for the cut-off value of SAA.[18] These differences have been attributed to the differences in the methods used and in the patients' ages.[12] In the current study, the cut-off value of SAA for early-onset sepsis was 9.5 mg/L for the samples obtained at admission, 10 mg/L for the samples obtained at the 24th hour and 11 mg/L for the samples obtained at the 48th hour of admission.

Mithal et al determined significantly higher CRP and SAA values in the umbilical cords of the cases with proven sepsis compared to the control group.[19] However, they determined no elevation in the suspected sepsis cases but similar PCT values between the three groups.[19] In the current study, both the proven and clinical sepsis cases had significantly higher SAA and CRP values compared to the non-sepsis cases at admission. However, the PCT values at admission showed no difference between the sepsis and non-sepsis cases.

The white blood cell count and absolute neutrophil count have been widely used for years as screening tests for neonatal infections. However, they have very low diagnostic values in early-onset neonatal sepsis. In the first 72 hours, the neutrophil count is influenced by the gestational age, route of delivery and gender.[20] Murphy et al stated that normal WBC values do not exclude EOS.[17] Similarly, the literature reported no significant difference between the WBC and PLT counts of the cases with and without sepsis.[6,10] Furthermore, in the current study, no significant difference was determined between the early-onset sepsis cases and non-sepsis cases in terms of white blood cell count and neutrophil count studied consecutively in the first three days. The ratio of the immature neutrophil count to total neutrophil count (I/T ratio) is considered to be a more sensitive marker.[17] In the current study, the I/T ratio was not evaluated.

Although thrombocytopenia can accompany neonatal infections, it is within the normal limits in the majority of infected newborns. The thrombocyte count has a weak sensitivity in diagnosing early-onset sepsis, monitoring response to treatment and in estimating the efficacy.[8,21] Moreover, in the current study, the number of thrombocytopenic patients and the mean thrombocyte count were similar between the cases with and without sepsis. Likewise, studies investigating early-onset and late-onset sepsis cases determined no significant difference in terms of the thrombocyte count and the number of thrombocytopenia cases compared to the non-sepsis cases.[6,10]

In the current study, there was no significant difference between the acute phase reactants of the proven and clinical sepsis cases. Likewise, the literature determined similar acute phase reactant values between the sepsis cases with and without growth in the blood culture.[10]

The limitations of this study were the low number of cases of proven sepsis and the large distribution of birth weights and gestational ages of the babies.

In conclusion, the present study revealed that SAA is a reliable diagnostic marker for EOS, and it has higher sensitivity at symptom onset or in the first hours after birth. Based on the results of the current study, taking 10 mg/L as the cut-off value for SAA appears to be reasonable.

**Table 5** The breakdown list of bacterial species causing early-onset neonatal sepsis

| | |
|---|---|
| *Staphylococcus epidermidis* | 7 |
| *Group B streptococcus* | 5 |
| *Escherichia coli* | 5 |
| *Enterococcus spp.* | 5 |
| *Enterobacter spp.* | 4 |
| *Klebsiella spp.* | 3 |
| *Staphylococcus aureus* | 3 |
| *Candida spp.* | 2 |
| *Moraxella osloensis* | 1 |
| *Staphylococcus haemolyticus* | 2 |
| *Burkholderia cepacia* | 1 |
| *Ralstonia picketti* | 1 |
| *Cronobacter sakasakii* | 1 |

## Declaration of Interest

The authors have no conflicts of interest to disclose.

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