SOLUBLE INTERCELLULAR ADHESION MOLECULE-1
AND HIGHLY SENSITIVE CRP AS DIAGNOSTIC MARKERS
OF NEONATAL SEPSIS

Prof. Dr. Mona A. Albishry, MD., Prof. Dr. Osama Z.Ahmed, MD., Dr. Elham A. Mohamady, MD., Dr. Hisham A. Eissa *, MD and Eman R. Abd-Almonaem, M.B., B.CH.

Departments of Pediatrics, Clinical Pathology*, Faculty of Medicine – Benha University.

Abstract

Aim of the study: Was to examine the potential diagnostic value of serum soluble intercellular adhesion molecule-1 (s.ICAM-1) and highly sensitive CRP (hs-CRP) in the setting of neonatal intensive care unit.

Methods: 50 consecutive serum samples were taken from 50 infants undergoing sepsis work up in a neonatal intensive care unit (30 neonates as study group and 20 neonates as control group) clinical diagnosis was established in prospective manner, blind to the result of study measurement. Infants were classified by an experienced paediatrician as infected or not infected. Classification was based on clinical presentation, routine laboratory and radiological investigations. The infected group were sub-classified as (a) culture positive infection (b) culture negative infection. s.ICAM-1 and hs-CRP levels were determined from stored serum samples after diagnosis was established and after 2-3 weeks of antimicrobial therapy. Further subgroup analysis of results was undertaken according to early or late onset of infection and preterm or term status. Statistical analysis utilized Mann Whitney U test and ROC curve analysis.

Results: There were significantly increased serum levels of s.ICAM-1 and hs-CRP in infected infants compared with non infected infants (p=0.001) and infected infants after 2-3 weeks of antimicrobial therapy (p=0.001). ROC curve analysis indicated area under the curve values of 0.86 (s.ICAM-1) and 0.63 (hs-CRP) .ROC curve analysis also defined optimum diagnostic cut off level for each measurement, however further studies are required to elucidate this finding.
**Introduction:**

Neonatal sepsis is a leading cause of neonatal morbidity and mortality in both full term and preterm neonates world wide, premature neonates are particularly susceptible to infection because of physiologic immaturity, co-morbidity and extraneous medical interventions. Institution of emperic therapy usually starts after initial diagnosis & before confirmation by positive microbiological culture [5].

There exists a significant need for rapid, objective in vitro tests for diagnosis of infection in neonates who are experiencing clinical instability [6].

CRP is a globulin produced by the liver during any generalized inflammatory process, but there is a delay of about 10-12 hours between the onset of infection and CRP increase, which limit usefulness of CRP in the initial evaluation of the septic infants. Serial measurements of CRP are useful in monitoring the progress of infection [6].

Highly sensitive CRP (hs–CRP) analysis has been developed for the detection of low levels of CRP and has been applied to monitor neonatal infection [3].

Serum CRP analysis in neonates could have profound effects on diagnosis and subsequent treatment, Neonatal levels of CRP are lower than adults, and the increase in the pathological states is not so pronounced, therefore hs-CRP test shows great potential for CRP analysis in neonates. Measurement of neonatal hs-CRP in the 6-12 hours following infection may be crucial for effective and optimal treatment [4].

Soluble intercellular adhesion molecule-1 (s.ICAM-1) participates in the adhesion of leukocytes to the endothelium and may be crucial in regulating leukocytes activation at a very early inflammatory response [3].

Soluble ICAM-1 (s.ICAM-1) is normally present in the serum of healthy adults, and recent studies examined the role of s.ICAM-1 in neonatal infections but with controversial results [3].

**Methods:**

Infants (mature and premature) attending Neonatal Intensive Care Unite in the Department of Pediatric, Banha University Hospital, Egypt were studied prospectively. This is a regional referral centre based in a University Teaching Hospital which receives infants both from the hospital’s obstetric unit and surrounding district general hospitals. Infants were recruited consecutively, as they developed acute clinical deterioration. At the time of enrolment into the study, an enrolment form was completed indicating the infant’s age, underlying diagnosis, symptoms indicative of acute deterioration, investigations undertaken and therapy (if any) commenced.

A peripheral venous blood sample (0.5-1ml) was taken into plain bottle prior to the administration of antibiotics and transported routinely to the laboratory. Serum was then separated and stored at -30 °C until analysis. There were no special storage / handing requirements. Venous blood samples were also taken at the time of routine venepuncture
from a number of infants attending intensive care with no acute signs of deterioration, as controls.

One week after deterioration, the routinely available investigative results and subsequent clinical course were reviewed. Investigations commonly used included e.g. complete blood count, differential white count, C reactive protein, urine, blood, culture and chest or abdominal X-ray. Infants were classified as infected or non-infected. Infants were regarded as not infected if (i) their clinical deterioration was transient (ii) a specific non infective cause for the deterioration was identified and (iii) there was no haematological, microbiological or radiological evidence of infection. The infected group was sub-classified as (a) culture positive infection or (b) culture negative infection, based on the identification of any positive microbiological results from blood, urine, CSF or other cultures. Control samples were taken for comparative purposes; however the data from these samples were not utilized in the calculation of diagnostic performance of the test measurements. S.ICAM-1 and hs-CRP levels were measured by commercial ELISA. Statistical analysis utilized non-parametric tests (Mann-Whitney U) from a computerized database. Receiver operating characteristic curves were plotted and analysed to compare diagnostic performance and optimum diagnostic cut off levels.

**Results:** 80 samples were obtained from 50 infants. 20/80 of these were control samples, 30/80 were taken from infants who had neonatal sepsis (before treatment = Gp Ia), 30/80 were taken from septic infants (after 2-3 weeks of antimicrobial therapy = Gp Ib). 10/30 samples were from preterm infants (<36 weeks gestation) and 20/30 from term infants (>36 weeks gestation), 11/30 were with early onset sepsis and 19/30 were with late onset sepsis. Gestational range was 32-36 weeks. The descriptive data and statistical comparisons of studied cases are illustrated in following tables and figures:

**Table (1):** Comparison between cases and control as regards levels of hs-CRP and s.ICAM-1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean ± SD</th>
<th>Student test</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>Cases</td>
<td>5.37±3.26</td>
<td>7.128</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.146±0.073</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.ICAM-1</td>
<td>Cases</td>
<td>336.0±172.2</td>
<td>4.846</td>
<td>0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>146.5±33.45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (1) shows that highly significant difference between cases and control as regards levels of hs-CRP and s.ICAM-1.
Table (2): Comparison between group Ia and group Ib as regards levels of hs-CRP and s.ICAM-1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Mean± SD</th>
<th>Student t test</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/l)</td>
<td>Gp Ia(before ttt)</td>
<td>5.37±3.26</td>
<td>8.72</td>
<td>0.001</td>
<td>Highly significant</td>
</tr>
<tr>
<td></td>
<td>Gp Ib(after ttt)</td>
<td>0.149±0.144</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s.ICAM-1 (ng/ml)</td>
<td>Gp Ia(before ttt)</td>
<td>336.0±172.2</td>
<td>5.54</td>
<td>0.001</td>
<td>Highly significant</td>
</tr>
<tr>
<td></td>
<td>Gp Ib(after ttt)</td>
<td>164.33±25.82</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2) shows that highly significant difference between group Ia (septicemic group before ttt) and group Ib (septicemic group after ttt) as regards levels of hs-CRP and s.ICAM-1.

Table (3): Comparison between early onset and late onset sepsis as regards level of s.ICAM-1.

<table>
<thead>
<tr>
<th>Onset (N)</th>
<th>s.ICAM-1 (ng/ml)</th>
<th>Mean ± SD</th>
<th>Student t test</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (11)</td>
<td>210.62±67.879</td>
<td>6.86</td>
<td>0.001</td>
<td>HS</td>
<td></td>
</tr>
<tr>
<td>Late (19)</td>
<td>479.3±139.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3) shows highly significant difference between early onset sepsis and late onset sepsis as regards level of s.ICAM-1.

Table (4): Comparison between early onset and late onset sepsis as regards level of hs-CRP

<table>
<thead>
<tr>
<th>Onset (N)</th>
<th>hs-CRP (mg/l)</th>
<th>Mean ± SD</th>
<th>Student t test</th>
<th>P</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early (11)</td>
<td>7.0±3.389</td>
<td>2.223</td>
<td>0.034</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Late (19)</td>
<td>4.42±2.874</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (4) shows a significant difference between early onset sepsis and late onset sepsis as regards level of hs-CRP.
**Figure (1):** ROC curve of s.ICAM-1 at cutoff point

![ROC Curve](image)

Diagonal segments are produced by ties.

s.ICAM-1 (Area under the curve = 0.869, Cutoff point=275, Sensitivity=86%, Specificity =100%, PVP=100% and NPV=74%)

**Figure (2):** ROC curve of hs-CRP at cutoff point

![ROC Curve](image)

Diagonal segments are produced by ties.

Hs-CRP (Area under the curve = 0.636, Cutoff point=2.5, Sensitivity =83%, Specificity =100%, PVP=100% and NPV=80%)
**Discussion:**

This prospective study was designed to confirm the hypothesis that measurement of sICAM-1 and hs-CRP in routinely collected serum was of diagnostic value in neonatal infection.

Our study was carried out on 50 newborn infants, 30 septicemic neonates (subjected to full clinical examination and laboratory investigations at start and 2 weeks after treatment) and 20 neonates with no evidence of sepsis serving as a control group.

In the present study, serum levels of hs-CRP were significantly higher in patients than control. This is in agreement with a study which mentioned that serum hs-CRP levels in newborns with septicemia were significantly higher than those of control group [1].

In the current study, there was highly significant difference between cases and control as regards levels of s-ICAM-1. This is in agreement with a study which reported that neonatal sepsis is associated with increased circulating level of s-ICAM-1, s-VCAM and E-selectin and their results were explained by hypothesis that the endothelium is activated in neonatal sepsis [9].

This difference is similar to a study which reported that neonatal sepsis is associated with activation of the endothelium as evidenced by increased levels of circulating biomarkers as s-ICAM-1 and it was a superior marker of sepsis severity compared with other the markers tested [8].

Regarding the onset of sepsis, our study revealed that hs-CRP was higher in early onset sepsis than in late onset sepsis and this is in harmony with a study which reported that CRP using high sensitivity immunoassays at cut off level 1mg/l in diagnosis of early onset sepsis (EOS) was significantly higher than in neonates with late onset sepsis [3].

On the other hand s-ICAM-1 was higher in late onset sepsis than in early onset sepsis, this is in agreement with a study which mentioned that there is progressive significant rise in circulating s-ICAM-1 from birth to 30 days of life. This fact may reflect the expansion in neonatal immune system in response to environmental influences [7].

Our study showed significant difference of hs-CRP and s-ICAM-1 levels between group Ia (septicemic group at start) and group Ib (septicemic group after treatment), our results are similar to a study which reported that there was highly significant elevation of s-ICAM-1, hs-CRP and E-selectin in 80 septicemic newborns at the time of admission to Neonatal Intensive Care Unit (NICU) after examination by an experienced pediatrician, their means ± SD were significantly higher than those of control, while after 2-3 weeks of antibiotic therapy means ± SD of them showed highly significant decline[2].
In the current study CRP using high immunoassays (hs-CRP) at a cut off level 2.5mg/l had sensitivity 83%, specificity 100%, positive predictive value 100% negative predictive value 80% and diagnostic accuracy 63.6%.

Similar study was reported in Egypt, the study included 51 neonates having positive blood culture in the same unit, they found that the sensitivity of hs-CRP in diagnosis of neonatal sepsis at a cut off level 2mg/l was 81.6%, specificity 100%, positive predictive value 100%, negative predictive value 77 % and diagnostic accuracy 72.13% [1].

Regarding s-ICAM-1, its cut off level was 275 ng/ml with sensitivity 86%, specificity 100%, positive predictive value 100%, negative predictive value 74% and diagnostic accuracy 86.9%.

Similar study reported that the sensitivity of s-ICAM-1 in diagnosis of neonatal sepsis at cut off level 228 ng/ml was 82.3%, specificity 95%, positive predictive value 91%, and negative predictive value 71% [3].

The use of combination of highly sensitive C reactive protein, and soluble intercellular adhesion molecule 1(s-ICAM-1) measurement enhanced diagnostic performance, demonstrating sensitivity of 90.3%, specificity 80.2% and negative predictive values of 91.3%. This study suggests that there may be a value in use of several markers individually and in combination to assist diagnosis of neonatal infection.

Finally we concluded that rapid accurate diagnosis of neonatal sepsis is still a major challenge for workers in the field of neonatal care.

The conclusive results of laboratory investigations should be available within few hours after clinical suspicion and no cases of sepsis are lost or misdiagnosed. Combinations of tests are beneficial as they increase the possibility of correct diagnosis and correlation of the test results with the clinical picture of the patient remains a cornerstone in the diagnosis of neonatal sepsis until the definite culture results are obtained.

Hs-CRP and s-ICAM-1 have proven to be good test for diagnosis of neonatal sepsis, however the cost and difficulty in laboratory technique remain a limiting factor in wide scale usage of this test.

According to the forementioned studies, we can suggest that serum levels of hs-CRP and s-ICAM-1 are elevated during neonatal infection and can be used as diagnostic marker for neonatal septicemia.

**Conclusion:**

Hs-CRP and s.ICAM-1 had proven to be sensitive markers in diagnosis of neonatal septicemia and were valuable in differentiation between early onset and late onset sepsis which is a major challenging problem and can be used to differentiate neonatal sepsis from other causes of acute clinical deterioration.
References: