Septicemia is an important cause of neonatal morbidity and mortality but diagnosis of this eminently treatable condition is often delayed due to its non-specific clinical features. Laboratory detection and quantification of some of the acute phase reactants may serve as a useful diagnostic aid early in the course of this illness.

By definition, acute phase reactants are proteins, whose concentration increase or decrease in association with an inflammatory stimulus. They subserve roles in inflammation as mediators and also as inhibitors, both of mediator pathways and of proteolytic enzymes released from phagocytosing leukocytes. The acute phase response thus represents a physiological mechanism providing increased concentrations of certain proteins in tissue fluid at the site of inflammation. This may modulate the nature of the inflammatory lesion(1).

Of these numerous proteins only C-reactive protein (CRP) has been extensively evaluated in neonatal sepsis(2-9). Alpha-1-antitrypsin (α-1-AT) and alpha-2-macroglobulin (α-2-MG) have been evaluated and had a higher index than both α-1-AT and α-2-MG for predicting outcome in septicemia. Serial use of CRP alone is, therefore, recommended for both purposes.

Key words: C-reactive protein, Alpha-1-antitrypsin, Alpha-2-macroglobulin, Evaluation, Neonatal sepsicemia.
for the same purpose, in a few studies from Europe(4,10,11) with conflicting results. Most of these studies were single estimation studies, and the \( \alpha \)-1-AT and \( \alpha \)-2-MG profiles were not followed sequentially during the course of illness. The present study was, therefore, undertaken to evaluate serially, \( \alpha \)-2-MG and \( \alpha \)-1-AT as early diagnostic aids and prognostic indicators in neonatal sepsis *vis-a-vis* CRP.

**Material and Methods**

This prospective study was done on babies delivered in the Obstetric unit of Lok Nayak Jai Prakash Narayan Hospital during the period between September, 1985 and February, 1986.

Babies were divided into two groups based on certain selection criteria:

1. The control group comprised 25 normal newborns of which 18 were full term (\( \geq 37 \) week of gestation) and 7 preterm (\(< 37 \) weeks of gestation); 11 male and 14 female. All were delivered after an uneventful prenatal and natal course and subsequently remained healthy during the first month of life.

2. The study group comprised 20 ill newborns (8 full term/12 preterm; 11 male/9 female) with septicemia documented by isolation of an organism by blood culture. Cases of both early and late onset septicemia(12) were included in the study. A preliminary selection into the study group was based on clinical findings(12). However, those who had a negative blood culture were subsequently excluded from the study. Also excluded from the study were neonates with birth asphyxia (5-minute Apgar \( \leq 5 \)) and whose mothers had any antenatal problems.

Serial samples were taken from every healthy (control) neonate for estimation of CRP, \( \alpha \)-1-AT and \( \alpha \)-2-MG at delivery (cord-blood); at 12-24 hours of life; at 2 week (14 \pm 4 days) of life; and at 4 weeks (28 \pm 4 days) of life.

The values thus obtained for each of the three acute phase proteins were utilized to compute a single mean value of the acute phase protein being estimated, for the entire period from birth to one month of age.

Serial samples from every neonate with sepsis were taken at initial clinical suspicion of sepsis (labelled S1); 12-24 hours after first sample (S2); and one week (7 \pm 3 days) after first sample (S3).

All neonates with septicemia were given appropriate antibiotics and supportive care and followed up till death or complete recovery. Of the neonates with septicemia, 15 died as a result of their illness. Of these, the third sample (S3) could only be obtained in 5.

CRP levels were estimated by a modified latex particle agglutination technique using a kit (Rapi-Tex CRP, Hoechst Pharmaceuticals Ltd., Bombay). Both alpha-1-antitrypsin and alpha-2-macroglobulin levels were estimated by single radial immunodiffusion technique of Mancini *et al.*(13), for limit diffusion in agar gel. Monospecific antisera for both were obtained commercially (Moloy Laboratory Inc., Springfield, USA) as were the necessary reference standards (Kallestad Laboratories Inc., Austin, USA).

Population means were estimated for each of the proteins for individual periods of sampling and significance of difference between means of control and study group estimated using Student's t-test. Cut-off
values for each of the variables for diagnostic and prognostic purposes were those that had the highest sensitivity and specificity. The Youden index(14) was used to rate the efficacy of each of the acute phase proteins for early diagnosis and prognosis. The Youden indices were then compared using the Student’s t-test(14).

Results

Both the control and study groups were comparable in weight. Mean weight of the former was 2.5 kg (±0.5 kg) and of the latter 2.2 kg (±0.5 kg).

Of the 20 septicemic neonates, 18 had Gram-negative organisms cultured from their blood (Klebsiella 8; Escherichia coli 6; Pseudomonas aeruginosa 2; Acinetobacter 2), while 2 had Gram-positive organisms (both Staphylococcus aureus). The commonest presenting features were, respiratory distress (35%), refusal of feeds (25%) and icterus (25%).

C-reactive protein (CRP)

Mean serum CRP levels in healthy neonates were 2.34 mg/L, in the first month of life. Comparative levels in septicemic neonates were significantly higher at onset of illness, 12-24 hours, and even 7±3 days after disease onset (Fig. 1).

Within the infected group, higher mean CRP levels were observed in neonates recovering from their illness as compared to the group dying as a result of their illness (Table I).

Alpha-2-macroglobulin (α-2-MG)

In the first month of life, healthy neonates had mean α-2-MG levels of 492.4 mg/dl. Septicemic neonates, in comparison, had higher mean α-2-MG levels at disease onset, but showed lower levels 12-24 h and 7±3 days after onset of disease (Fig. 3). Septicemic neonates surviving their illness had lower mean α-2-MG levels than their counterparts dying as a result of septicemia, both at onset of illness and 12-24 hours later, but had higher levels than the latter, 7±3 days after disease onset (Table I).
### TABLE I—CRP, α-1-AT and α-2-MG Levels in Septicemic Neonates Related to Outcome (Mean ± SD)

<table>
<thead>
<tr>
<th>Sample</th>
<th>CRP (mg/L)</th>
<th>P</th>
<th>α-1-AT (mg/dl)</th>
<th>P</th>
<th>α-2-MG (mg/dl)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovered (n = 5)</td>
<td>Died  (n = 15)</td>
<td></td>
<td>Recovered (n = 5)</td>
<td>Died (n = 15)</td>
<td></td>
</tr>
<tr>
<td>S₁</td>
<td>23.4 ± 19.5</td>
<td>8.2 ± 09.8</td>
<td>&gt;0.05</td>
<td>596.0 ± 175.7</td>
<td>567.7 ± 148.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S₂</td>
<td>33.6 ± 21.5</td>
<td>12.0 ± 10.1</td>
<td>&lt;0.05</td>
<td>602.0 ± 087.3</td>
<td>599.3 ± 134.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>S₃*</td>
<td>21.6 ± 21.5</td>
<td>12.6 ± 05.4</td>
<td>&lt;0.05</td>
<td>711.0 ± 156.2</td>
<td>565.0 ± 175.4</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* Recovered n = 5; Died n = 5

### TABLE II—Comparative Utility of CRP, α-1-AT and α-2-MG in Neonatal Septicemia, for Early Diagnosis and Prognosticating Survival (Figures represent Youden Index)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Early diagnosis</th>
<th>Prognosis</th>
<th>7±3 days after S₁ (S₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At disease onset (S₁)</td>
<td>12-24 hours later (S₂)</td>
<td>At disease onset (S₁)</td>
</tr>
<tr>
<td>CRP</td>
<td>0.22</td>
<td>0.32</td>
<td>0.40</td>
</tr>
<tr>
<td>α-1-AT</td>
<td>—</td>
<td>—</td>
<td>0.40</td>
</tr>
<tr>
<td>α-2-MG</td>
<td>0.20</td>
<td>0.18</td>
<td>0.47</td>
</tr>
</tbody>
</table>

p value for differences in Youden indices for individual periods of sampling between CRP, α-1-AT and α-2-MG for both diagnosis and prognosis >0.05
Comparative utility for early diagnosis and prognosticating survival in neonatal septicemia

Cut-off values used for early diagnosis of septicemia, at disease onset and 12-24 h later, respectively were: ≥24 mg/L for CRP and ≥1000 mg/dl and < 270 mg/dl for α-2-MG. Alpha-1-antitrypsin could not be used as an early diagnostic aid, because the range of values obtained in both healthy and septicemic neonates was very wide, with considerable overlap. Table II shows comparative utility of CRP and α-2-MG for early diagnosis of neonatal septicemia. Although CRP had a higher Youden index for this purpose than α-2-MG, both figures were very low and not significantly different, either at disease onset or 12-24 hours later.

Cut-off values used for prognosticating survival, at disease onset, 12-24 hours later and 7±3 days after disease onset respectively were: ≥36 mg/L, ≥36 mg/L and ≥24 mg/L for CRP; ≥600 mg/dl, ≥500 mg/dl and ≥600 mg/dl for α-1-AT; ≤600 mg/dl, ≤400 mg/dl and ≥350 mg/dl for α-2-MG. Table II also shows comparative efficacy of the three acute phase proteins for prognosticating survival in neonatal septicemia. Although CRP had the highest Youden index, when used for this purpose, the figures were not significantly better than either α-1-AT or α-2-MG.

Discussion

Like several other studies(2-9) this study also demonstrates that CRP shows an early increase in neonatal septicemia, and that higher CRP levels, early in the course of infection, exert a protective influence(3,4). Unlike other studies(2-9) however CRP used as a screening test for early
detection of septicemia, had a very low Youden index. On the contrary, CRP estimation had considerable utility in predicting outcome, in terms of survival. It is, therefore, suggested that this may be the most important clinical use of CRP in neonatal septicemia.

Alpha-1-antitrypsin was ineffective in early diagnosis of neonatal sepsis and had a lower Youden index for predicting survival than CRP, used for the same purpose. Similarly, α-2-MG too, did not possess any advantage over CRP for both purposes.

CRP is easily quantitated at the bedside, using a freely available kit. Alpha-1-antitrypsin and α-2-MG estimation requires access to an immunological laboratory, and takes 48 hours, unless laser nephelometry facilities are available. Further every additional screening test adds to the cost of patient management. In addition, this study clearly shows, that CRP estimation by itself is as effective, if not better, than α-1-AT and α-2-MG, both as an early diagnostic aid and prognostic indicator in neonatal septicemia. We, therefore, recommend that CRP while continuing to remain a part of the neonatal sepsis screen, also be serially estimated 12-24 hours and 7±3 days after onset of septicemia to predict outcome.

REFERENCES


