Rapid, Semi-Quantitative Assay of C-Reactive Protein Evaluated

Hilary Vallance and Gillian Lockitch

We evaluated a new rapid semi-quantitative immunometric assay of C-reactive protein (CRP) as a screening test for sepsis by comparison with an automated nephelometric method. Plasma samples (n = 101) from preterm infants during the first week of life were saved for CRP analyses. We measured CRP by the Nyocard semi-quantitative method and compared the results with those obtained with a Behring Nephelometer. A CRP value <10 mg/L was considered to be negative for infection. All CRP results read as <10 mg/L (negative) by the Nyocard method were also <10 mg/L by the comparison method, and all CRP values found to be >20 mg/L (positive) by the Nyocard method were also positive by the comparison method. Results in the 10-20 mg/L range were considered equivocal. We conclude that the Nyocard CRP semi-quantitative method is a rapid and useful screening test for sepsis in preterm infants.

Additional Keyphrases: screening · sepsis · immunoassay · pediatric chemistry · newborns · nephelometry compared

Measurement of C-reactive protein (CRP) in neonates has been found to be a reliable indicator of serious infection (1–3). In recent years, CRP assays have become increasingly sensitive, precise, and accurate (4). However, cost constraints force many diagnostic laboratories to batch CRP requests, thus delaying turnaround time. The Nyocard rapid semi-quantitative assay of CRP (NYC; NYCOMED Pharma AS, Oslo, Norway) has been designed as a single-assay system. We compared this new CRP method with a quantitative nephelometric method used to screen for sepsis in a group of preterm infants.

Materials and Methods

Leukocyte plasma samples collected from preterm infants during the first week postpartum were saved and frozen. We assayed CRP in batches, using a nephelometer analyzer (Behring Diagnostics, Montreal, Quebec). The detection limit for this nephelometric comparison method, which requires ~60 µL for analysis, is 2.3 mg/L (CV 3.7%). We then measured CRP in the same samples by the NYC semi-quantitative immunometric method, which requires only 20 µL of plasma. (Note: when all samples for a patient gave CRP results <2.3 mg/L by the comparison method, one of the samples was chosen without conscious bias to be measured by the NYC method.) Color development on test cards was compared with a color chart (a component of the kit) by two independent observers. CRP results were recorded as follows: <10 mg/L, 10–20 mg/L, 21–40 mg/L, 41–60 mg/L, and 61–100 mg/L. A CRP value <10 mg/L was considered negative for infection. The technologists interpreting the CRP analyses were unaware of the results of the comparison method. Inter-observer concordance of results was 91%, with no bias.

Six infants developed blood-culture-proven sepsis during the first week postpartum. All six had a CRP concentration ≥10 mg/L by the comparison method during the first 24 h of clinical deterioration (five infants at the time sepsis was suspected). Fifty-two infants with no indication of sepsis had CRP concentrations <10 mg/L during their first week. Of these infants, 18 had significant hyaline membrane disease requiring intubation. Therefore, both the positive and negative predictive values of the comparison method for culture-proven sepsis were 100%. Using the nephelometric comparison method as the “gold standard,” we evaluated the NYC CRP results.

Results

We performed 101 CRP assays by both the NYC semi-quantitative method and the comparison method (Table 1). Of 65 CRP results that were <5 mg/L by the comparison method, all were read correctly as <10 mg/L, or negative, by the NYC method with no false negatives. For CRP results between 5.1 and 9.9 mg/L (by the comparison method), three of 11 were correctly read as <10 mg/L. The remaining eight were incorrectly read as 10–20 mg/L. With the cutoff point of ≥10 mg/L used to indicate infection, a false-positive diagnosis of infection would have been made in eight of the 101 assays (7.9%).

Of 21 assays giving results between 10.0 and 39.9 mg/L by the comparison method, four would have been placed in a higher category by the NYC method. However, all would still have been interpreted as true positive, and the clinical management would probably not have been altered.

Discussion

CRP measurement in preterm neonates has been shown to be both highly sensitive and specific for serious bacterial infection. CRP determination is most useful in either supporting or refuting the diagnosis of sepsis (5). However, most of the present quantitative assays of CRP, although designed for automated instruments, are not practical or economical to run as single tests. Thus, it is difficult to provide a rapid turnaround time. The NYC single-assay system allows results to be obtained...
within minutes of receiving a specimen, making it ideal as a screening test.

The sensitivity of the NYC CRP method in these samples was 100% at 10 mg/L (with a specificity of 89%). This makes the assay system reliable as a screening test, where a negative result (<10 mg/L) refutes the diagnosis of sepsis. However, it is important to note that, although CRP begins to increase rapidly after the onset of infection, it may not reach 10 mg/L for as long as 24 h (6). Therefore, an isolated low concentration of CRP at the time of clinical deterioration does not rule out sepsis. When two CRP values 24 h apart are <10 mg/L, the diagnosis of sepsis is highly unlikely. Furthermore, even the youngest preterm infants are capable of mounting a CRP response (1). There does not appear to be any loss in sensitivity with decrease in gestational age.

Limitations of this CRP method are important to note. We found that CRP values between 5.1 and 9.9 mg/L by the comparison method were frequently overestimated and read as 10–20 mg/L by the NYC method, thus reducing the latter’s specificity. We suggest that results in the 10–20 mg/L range be interpreted as equivocal and followed up 12–24 h later with repeat testing. In this study, 8% of the results were in the equivocal category. A specificity of 100% (and sensitivity of 80%) will be achieved with results >20 mg/L. This test will then be very useful as a rapid screen.

Correlation between the two methods was good when the CRP concentration was >10 mg/L by the comparison method. Overall, we found a slight tendency to overestimate CRP values by the semi-quantitative method. Because a good response to anti-microbial therapy is associated with a rapid decline in CRP concentrations (2), quantitative CRP assays may therefore be useful in monitoring septic infants during treatment. Although this assay system is able to detect a significant trend in CRP concentration in one direction or another, a quantitative assay system is recommended for serial CRP measurement in septic infants. As a rapid screen that can be used during hours when the quantitative assay is not available, the Nycocard single-assay system should be very useful in the diagnosis of sepsis in preterm neonates.

References

Table 1. Performance of the Semi-Quantitative CRP Method and the Comparison Method

<table>
<thead>
<tr>
<th>CRP concon by comparison method, mg/L</th>
<th>Semi-quantitative CRP method</th>
<th>Comparison method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. in agreement</td>
<td>No. in disagreement</td>
</tr>
<tr>
<td>&lt;2.3</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>2.3–5.0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>5.1–9.9</td>
<td>3</td>
<td>8 (10–20)</td>
</tr>
<tr>
<td>10.0–19.9</td>
<td>5</td>
<td>2 (21–40)</td>
</tr>
<tr>
<td>20.0–39.9</td>
<td>10</td>
<td>2 (41–60)</td>
</tr>
<tr>
<td>40.0–59.9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>60.0–110.0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>12</td>
</tr>
</tbody>
</table>

* With a cutoff of 10 mg/L indicating a positive result, eight samples would have been incorrectly classed as positive by the Nycocard method.

* Although the Nycocard result placed these samples in a higher category, it did not alter the interpretation because all were "true positive" by the comparison method.