Sequential study of C reactive protein in neonatal septicaemia using a latex agglutination test

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SUMMARY The usefulness of serial study of C reactive protein in the early detection of neonatal septicaemia was evaluated in a neonatal unit using a commercially available latex agglutination slide test as a rapid screening method and electroimmunoassay as a reference method for C reactive protein determination. A positive latex test was obtained in 11 infants with verified septicaemia (positive blood culture), two infants with clinically evident infection but without bacteriological confirmation, one infant with recurrent chest infection due to Pseudomonas aeruginosa, and one infant who showed signs of birth asphyxia with meconium aspiration, but was not infected. Positive latex test results correlated with raised concentrations of C reactive protein, measured by immunoassay. In some instances, however, concentrations of C reactive protein in excess of 12 mg/100 ml gave weaker agglutination results in the slide test, which could be interpreted as negative results. In a sequential study of the infected infants, 6.3% of the values recorded on a slide test were false negatives. In contrast, false positive values were observed on a slide test in 1.9% of 27 non-infected infants. The higher percentage of false negative values may be due to the presence of excess antigen in the sera of some infected children. It is suggested that the latex test should be carried out on suitable dilutions of serum.

Although the slide test may reliably indicate infection at an early stage in neonates, the C reactive protein response is non-specific, as seen in a non-infected infant who showed signs of birth asphyxia with meconium aspiration. Provided the non-specific nature of the C reactive protein response is recognised, the latex test may be a useful serum measurement for early diagnosis of neonatal septicaemia of the newborn. The test has the advantage of being performed easily, quickly, and cheaply.

Because of the vague and often misleading clinical signs and symptoms which accompany sepsis neonatorum, attempts have been made to develop methods which would identify infants who are likely to develop sepsis during the early stages of their illness. Unfortunately, little progress has been made, and the diagnosis of neonatal septicaemia remains one of the most difficult tasks in clinical medicine. Recent studies have shown that C reactive protein (CRP) concentration is a sensitive and responsive indicator of neonatal sepsis. As a monitor of neonatal infection, CRP has been considered to be more reliable than the classic indicators of leucocyte count, erythrocyte sedimentation rate, and fever. CRP is synthesised in the liver, but the nature of the stimulus to the liver from a site of infection is unknown. The principal drawback of using CRP as an indicator of neonatal sepsis has been finding a suitable assay. Diagnostic tests that might influence therapeutic intervention must be readily available, require a minimum of time to process, and be highly dependable. This report shows the usefulness of a latex agglutination test for detecting CRP in the serum of newborn infants as a rapid and helpful adjunct to the diagnosis of infection.

Patients and methods

All the babies admitted to the neonatal intensive care unit of The London Hospital over a three month period were studied. Only 43 of the original 63 infants in the prospective study could be followed serially because the rest did not fulfil the following criteria: (a) had been in hospital for more than two days; (b) had samples taken for blood culture and CRP on same day; (c) had samples taken before antibiotics had been given.

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When a newborn infant with suspected septicemia was identified investigations included the following: a smear of gastric aspirate for Gram stain and measurement of pus cells; white blood cell count and differential, platelet estimate; and blood, urine, and cerebrospinal fluid cultures. Blood samples for CRP estimation were collected once a day until convalescence. (Most babies were having blood samples taken more than once a day for other reasons.)

Infants with suspected infection presented with two or more of the following risk factors: (a) maternal fever and mother given antibiotics; (b) culture of β-haemolytic group B streptococcus (Streptococcus agalactiae) from vaginal swab; (c) prolonged rupture of membranes (longer than 24 h); (d) purulent liquor; (e) abnormal delivery; (f) birth asphyxia; (g) clinical signs of sepsis; (h) white blood cell count of less than 10 × 10⁹/l on the first day of life; and (i) small for gestational age. All infants were treated with antibiotics.

The CRP latex agglutination test was performed according to the manufacturer’s instructions (CRP-Wellcotest-Wellcome Reagents, Hither Green, London, England) using undiluted serum. Positive slide results consisted of a pattern with obvious coarse agglutination. The latex CRP estimation was positive when the concentration was about 0-8 mg/100 ml or above, although manufacturers of the latex kit claim the test will detect CRP in excess of 0-6 mg/100 ml.

Quantitative estimation of CRP was carried out according to the method of Laurell. Results were available within 3-4 h of an electrophoretic run. A reference curve was prepared by serial dilution of the standard serum. All the assays were batched and assayed blind without knowledge of the clinical condition of the infant. Results did not therefore influence treatment decisions during the study. Data were analysed and matched retrospectively to the clinical, haematological, and bacteriological findings.

Results

In 27 infants (four term and 23 preterm) the clinical course was without signs of infection; the CRP concentrations in these infants are shown in Fig. 1. Most CRP concentrations in the 27 infants who were not infected were less than 0-3 mg/100 ml, which is the lower limit of detection of CRP by immunoturbidimetric assay. There were a few instances, however, where these values were above 0-3 mg/100 ml. There was a good correlation with the slide test, with only 1-9% false positive values. At the time of diagnosis, of 12 infants (11 preterm and one term) with proved sepsis (positive blood culture) all except one showed appreciably raised CRP concentrations (Table), which decreased in many cases after treatment was started. A positive latex test result was obtained with raised concentrations of CRP (Table), although on serial sampling of these patients a weaker agglutination on a slide test was seen in some instances. This occurred with serum CRP concentrations above 12 mg/100 ml.

Bacteriological confirmation was not obtained in four episodes of clinically evident infection in two preterm infants (gestational ages 26 to 30 weeks), but the CRP concentration was raised during each episode, which lasted a few days (Fig. 2). The infant of 26 weeks' gestation presented with hypothermia, blood pH of 6-9, and increasing jaundice from the second to the fourth day of life; apnoea and bradycardia were evident on days 5, 6, 8, and 9 and again on days 19 and 20. Antibiotics were given during both episodes. The infant of 30 weeks' gestation had recurrent apnoea on days 2, 10, and 22 of life, together with bradycardia on days 10 and 22. In both cases CRP concentrations returned to normal after the start of antimicrobial treatment (Fig. 2). Here again there were instances when latex agglutination test was not conclusive (weakly positive), particularly when the CRP concentration was substantially increased. Fig. 3 shows CRP values and latex results designated as positive or negative in a preterm infant with recurrent chest infections due to Pseudomonas aeruginosa (isolated on several occasions from the thick purulent secretions in a tracheal aspirate). The administration of antibiotics before

![Fig. 1. Serum C reactive protein concentrations in 27 newborn babies (23 preterm and four term) without proved or suspected infection. Most values are below the lower limit of detection (0.3 mg/100 ml). Most of the sera showed no agglutination on a slide test.](http://jcp.bmj.com/doi/10.1136/jcp.37.9.1014)
Details of infants with positive blood cultures

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Age at sampling (days)</th>
<th>CRP (mg/100 ml)</th>
<th>CRP latex agglutination test</th>
<th>Cultured agent designation</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>&gt;12</td>
<td>+</td>
<td>β-haemolytic group B streptococcus (also isolated from respiratory tract)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>&gt;12</td>
<td>+</td>
<td>Enterococcus</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>7</td>
<td>+</td>
<td>(1) = β-haemolytic group B streptococcus</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>12</td>
<td>+</td>
<td>(2) = Staph epidermidis</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>&gt;12</td>
<td>+</td>
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</tr>
<tr>
<td>4</td>
<td>8</td>
<td>12</td>
<td>+</td>
<td>(1) = P aeruginosa</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>10</td>
<td>±</td>
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<tr>
<td>5</td>
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<td>&gt;12</td>
<td>+</td>
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<tr>
<td></td>
<td>14</td>
<td>12</td>
<td>+</td>
<td>(2) = Staph epidermidis</td>
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<tr>
<td></td>
<td>31</td>
<td>10</td>
<td>+</td>
<td>(3) = β-haemolytic group B streptococcus</td>
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<tr>
<td>6</td>
<td>1</td>
<td>&gt;12</td>
<td>+</td>
<td>Klebsiella spp</td>
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<td>7</td>
<td>16</td>
<td>&gt;12</td>
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<td>26</td>
<td>10</td>
<td>+</td>
<td>bacteriological confirmation not obtained</td>
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<td></td>
<td>35</td>
<td>12</td>
<td>+</td>
<td>Streptococcus mutans</td>
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<tr>
<td>8</td>
<td>1(T)</td>
<td>8</td>
<td>+</td>
<td>Staph epidermidis</td>
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<tr>
<td>9</td>
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<td>±</td>
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<tr>
<td>12</td>
<td>9</td>
<td>&lt;0.3</td>
<td>-</td>
<td>Staph epidermidis</td>
</tr>
</tbody>
</table>

CRP = C reactive protein.
+ = positive slide test—that is, CRP concentration >0-8 mg/100 ml.
= negative slide test—that is, CRP concentration <0-8 mg/100 ml.
± = weak positive slide test.
Numbers in parentheses refer to three positive blood cultures on the same patient on three different occasions at the ages stated.

Although these infants were followed sequentially, only those results of CRP concentration at the time of infection (positive blood culture) are shown.

day 30 seemed to resolve the infection, as shown by a decreasing CRP concentration. Later episodes, however, remained unresolved despite antibiotics, and CRP concentrations remained increased with a positive latex test. A high CRP concentration

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Fig. 2  Serum C reactive protein concentrations in two preterm infants (26 weeks and 30 weeks, by gestation) with clinically evident infection but without bacteriological confirmation. Arrows indicate the time of appearance of the first symptoms. Subscripts 1 and 2 indicate two episodes of clinically evident infection. Closes circles refer to 26 week old infant and the open circles refer to 30 week old infant. + = positive latex test; - = negative latex test; ± = weakly positive.

Fig. 3  Serum C reactive protein concentrations in preterm infant with recurrent chest infections due to Pseudomonas aeruginosa. Arrows indicate instances when Ps aeruginosa was isolated from the tracheal aspirate. + and - indicate latex results.
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together with a positive latex test result was seen in an infant who showed signs of birth asphyxia and meconium aspiration but without proved sepsis. In the serial study of all the infected patients, false negative values were recorded in 6-3% with a latex slide test; however, many of the results included in this category were weakly positive on slide test rather than being completely negative.

Discussion

This study shows that CRP was detectable in the serum by a latex agglutination method, which is readily available, simple to perform, and easy to interpret. Culture proved Gram positive and Gram negative bacteraemias had demonstrable concentrations of CRP at the time of diagnosis. CRP was also detected in two infants with clinically diagnosed infection and one infant with recurrent chest infection due to *P aeruginosa*. A positive latex test result was also seen in an infant who showed signs of birth asphyxia and meconium aspiration but without proved sepsis. One patient had a positive blood culture with *Staphylococcus epidermidis*, but the latex CRP test was negative and there was no rise in CRP concentration. We believe that blood may have been contaminated with *Staph epidermidis*, thus explaining the negative CRP finding. Use of neat serum with raised concentrations of CRP may, in some cases, give false negative values on a slide test, as seen by a comparatively high rate (6-3%) of false negative values. It is important in such cases that serum is tested in different dilutions.

CRP concentrations may be evaluated accurately and quickly by immunonephelometric procedures. A certain amount of skill and investment are needed to acquire this facility however, and some sensitivity is sacrificed; the lower limit of sensitivity of the immunonephelometric assay is 1-8 mg/100 ml.

While latex agglutination as a screening test shows a good balance between sensitivity and specificity, it cannot be used to detect infection within 10 days of other acute tissue damage, such as surgery, since a high concentration of CRP in proportion to the extent of tissue damage will inevitably be present. Raised concentrations of CRP have been reported at birth and during the first days of life in infants who presented with risk factors such as maternal fever during labour, premature rupture of membranes, asphyxia, shock, fetal distress, and aspiration difficulties. We were unable to detect CRP in infants presenting with these risk factors in the absence of infection, although a high CRP value with a positive latex test was seen in one infant who showed signs of birth asphyxia and meconium aspiration but was not infected.

Provided the non-specific nature of the CRP response is recognised and the CRP results are interpreted in the light of clinical symptoms indicative of infection, the slide test may be a helpful diagnostic tool to verify infection at the bedside and may also be used in the subsequent management of infants with proved or suspected infection. The test is simple to perform and is not expensive. Although measurement of CRP concentration in serum may be diagnostically useful, such measurement in cerebrospinal fluid yields no useful information in neonates with suspected sepsis and meningitis. Measurement of CRP concentrations in cerebrospinal fluid of neonates does not distinguish between infants with meningitis and those with no proved infection. In conclusion, the latex agglutination test shows a good correlation with quantitative estimation. This is in agreement with previously published results, although no attempt has been made in this investigation to study serial dilutions of sera.

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References


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