Can the rapid semiquantitative estimation of serum C reactive protein be adapted for the management of bacterial infection?

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SUMMARY Serum C reactive protein concentrations measured by a laboratory based assay were compared with the semiquantitative results obtained with a visual agglutination method (Wellcotest, CRP kit). Using this agglutination kit, diluting serum 1/10 and 1/20 gave C reactive protein results which could be of more clinical value than those obtained using the 1/2, 1/4, 1/8, and 1/16 dilutions recommended by the manufacturers. The kit was also used on the ward by junior medical staff, who showed that after minimal training reproducible serum C reactive protein results could be obtained.

Measurement of C reactive protein (CRP) concentration is useful in diagnosing and monitoring bacterial infections.1–6 Serial measurements of serum CRP values have shown that they rise considerably within the first 24 h of an illness and a concentration of CRP of 100 mg/l or more has been suggested as being indicative of significant bacterial infection. In particular, patients with acute leukaemia or other malignancies, including those who are neutropenic,7,8 rarely have CRP values greater than 50 mg/l as a result of the disease process alone, and therefore in these patients CRP estimation may also be of value in the early diagnosis of bacterial infection.

The clinical decision to start antibiotic treatment is often made without the benefit of the CRP result because rapid estimations are not readily available. We have therefore investigated the use of a rapid, yet inexpensive, semiquantitative CRP test which could be performed on the ward by medical personnel. The results were compared with those obtained using a laboratory based immunoassay.

**Material and methods**

CRP concentrations were determined in serum samples obtained from 150 patients using a laboratory based latex enhanced immunoassay* and also semiquantitatively by the CRP Wellcotest rapid latex test kit (Wellcome Diagnostics, Dartford, England). In this kit polystyrene latex particles coated with antibodies to human CRP are used to detect the presence of CRP in serum by agglutination.

Initially, 28 samples were diluted 1/2, 1/4, 1/8, and 1/16 in normal saline for semiquantitative esti-
samples were serum tested and assessed the effect of antigen
ations was procedure microlitres Fifty
formation, as recommended by the manufacturers. Fifty
microlitres of the diluted serum was then added to one drop of CRP latex from the Wellcotest
kit and these were mixed on a glass slide. After 2
min the presence of any agglutination was recorded and was interpreted as a positive result (Fig. 1). This
procedure was repeated using 1/10 and 1/20 dilu-
tions of the serum samples. A further 122 serum
samples were tested at 1/10 and 1/20 dilutions only.
A serum sample with a high CRP value of 484 mg/l
was tested at dilutions of 1/2, 1/10, and 1/20 to
assess the effect of antigen excess.

Ten resident doctors from the Department of
Paediatrics in Nottingham performed Wellcotest kit
assays on five different serum samples. After only
basic instruction concerning the use of a bench cen-
trifuge and variable pipette (5–200 µl, Gilson), they
tested the serum samples at dilutions of 1/10 and
1/20 without prior knowledge of the laboratory
result. Control positive and negative serum samples
are supplied with the kit for reference.

Results

Fig. 2 shows the distribution of the serum CRP con-
centrations of the 150 samples determined by the
laboratory immunoassay, with an overall range of 1
to 295 mg/l.

In Fig. 3 the CRP kit results are recorded as either
positive or negative agglutination and the range of
results obtained using the manufacturer’s recom-
ended serum dilutions of 1/2, 1/4, 1/8, and 1/16
are shown in Fig. 3 compared with the CRP value
in mg/l. At a 1/2 dilution all 28 sera gave positive
agglutination whereas at a 1/16 dilution only 23
gave positive agglutination. Further dilutions were
then made to try to obtain positive or negative
agglutinations around the 100 mg/l value. Fig. 3
shows the results using 1/10 and 1/20 serum dilu-
tions, compared with serum CRP values in mg/l. At
a dilution of 1/10 all the CRP values gave a positive
reaction whereas at 1/20 only those samples with
CRP concentrations >100 mg/l were positive.

Fig. 2 Distribution of C reactive protein concentra-
tions in serum samples obtained from 150 patients determined by
latex enhanced immunoassay.

Fig. 3 CRP results for 28 sera determined by a latex enhanced immunoassay and at
various dilutions using the Wellcotest kit. Positive agglutination is denoted by open
circles and negative agglutination by closed circles. (a) dilutions recommended by the
manufacturers (1/2, 1/4, 1/8, 1/16). (b) dilutions recommended by the authors (1/10,
1/20).
Table 1  Comparison of serum C reactive protein (CRP) values determined by latex enhanced immunoassay and the Wellcotest kit*

<table>
<thead>
<tr>
<th>Range of CRP values (mg/l)</th>
<th>No of sera</th>
<th>No of positive kit agglutination results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum dilution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/20</td>
</tr>
<tr>
<td>0-50</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>51-99</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>&gt;100</td>
<td>60</td>
<td>58</td>
</tr>
</tbody>
</table>

*Tests were performed by laboratory staff.

Table 2  Results obtained by junior medical staff using the Wellcotest kit at 1/20 serum dilution

<table>
<thead>
<tr>
<th>No of sera tested</th>
<th>No of positive kit agglutination results at 1/20 dilution</th>
<th>Range of CRP values (mg/l) (laboratory assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>1</td>
<td>0-99</td>
</tr>
<tr>
<td>23</td>
<td>23</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

CRP = C reactive protein.

The results of the 150 serum CRP values determined by trained laboratory staff using both the latex enhanced immunoassay and the Wellcotest kit are given in Table 1. Ninety samples had CRP concentrations <100 mg/l by the standard laboratory assay; of these, 87 gave negative and three gave positive reactions using the Wellcotest kit at a serum dilution of 1/20. The CRP values of the three samples which gave positive agglutination were 99, 99, and 61 mg/l. Of the 60 serum samples with CRP values >100 mg/l, 58 gave positive agglutination and two gave negative results using the kit assay at 1/20 serum dilutions. The CRP values of these two negative samples were 105 and 110 mg/l.

The serum sample with a CRP value of 484 mg/l was negative at a dilution of 1/2 but positive at both 1/10 and 1/20.

Sixty samples of serum were tested by medical staff using the kit method: 23 had CRP values greater than 100 mg/l and 37 had CRP values less than 100 mg/l (Table 2). All of the samples with CRP concentrations >100 mg/l and only one sample with a CRP value of less than 100 mg/l gave positive agglutinations at a dilution of 1/20. The kit test took between 7 and 8 min to perform and no difficulties were encountered when reading the agglutination reaction.

**Discussion**

The results confirm that the Wellcotest kit is simple and easy to perform. It can be carried out rapidly by junior medical staff after minimal training, requires only small samples of serum extracted from either capillary or venous blood, and needs only basic equipment.

Serum CRP concentrations >100 mg/l have been suggested as being indicative of bacterial infection in the appropriate clinical situation. Using the kit with serum dilutions of 1/10, 38 of 40 sera with CRP values of 50 to 99 mg/l gave positive agglutination results, whereas at a 1/20 serum dilution only three of the samples were positive. The combination of CRP agglutination results at a serum dilution of 1/10 and 1/20 therefore helps to select those sera with CRP values >100 mg/l. The manufacturers, however, recommend serum dilutions of 1/2, 1/4, 1/8, and 1/16. The CRP range which is encompassed by the 1/16 dilution is 20-189 mg/l and therefore does not allow further differentiation within this range, particularly those in excess of 100 mg/l which might be indicative of bacterial infection. Our findings suggest that 1/10 and 1/20 serum dilutions are of more clinical value, giving a semiquantitative cut off of CRP around 100 mg/l. According to the manufacturers, low serum dilutions—for example, 1/2—are subject to a prozone effect leading to false negative results. We did not encounter this problem with the 150 samples tested. But we did show this effect when using a serum sample with a particularly high CRP value of 484 mg/l. No prozone effect was seen at 1/10 serum dilution with any samples. There is no precise cut off at 100 mg/l using the kit with 1/10 and 1/20 serum dilutions, but only about 3% of samples would give false negative results at a 1/20 serum dilution.

The three serum samples positive at 1/20 serum dilution with CRP values <100 mg/l were tested for rheumatoid factors by the conventional latex and sheep cell agglutination tests, because previous reports have suggested interference by rheumatoid factors in CRP agglutination tests. All three sera were negative.

The results suggest that the CRP values obtained with the kit are of sufficient accuracy to be of value in the early diagnosis and management of patients with significant bacterial infection. Various rapid assays for determining CRP values have recently been described, but these are laboratory based and require expensive equipment and laboratory trained personnel. Therefore, with the current discussion on the contribution that medical staff can make by performing simple pathology tests on the wards, particularly out of normal laboratory working hours, this rapid CRP test should be of interest. Similarly, small hospital laboratories may find this a useful method when CRP is requested by physicians managing patients with bacterial infections.
Rapid semiquantitative estimation of serum C reactive protein and management of bacterial infection

We thank the Special Trustees for Nottingham University Hospitals for their research grant, the Wellcome Foundation Ltd for supplying the CRP Wellcotest Kits, Professor D Hull for allowing us to perform the survey, and Mrs S Brailsford and Miss E Allen for typing the manuscript.

References


Requests for reprints to: Dr RJ Powell, Consultant Immunologist, University Hospital, Queen’s Medical Centre, Nottingham NG7 2UH, England.
Can the rapid semiquantitative estimation of serum C reactive protein be adapted for the management of bacterial infection?

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doi: 10.1136/jcp.38.4.464

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