

# C reactive protein rapid assay techniques for monitoring resolution of infection in immunosuppressed patients

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**SUMMARY** Three rapid assay techniques (latex agglutination, laser nephelometry, and EMIT enzyme immunoassay) have been evaluated for serial monitoring of the serum C reactive protein (CRP) concentration in immunosuppressed patients with fever. Radial immunodiffusion assay was used as a reference method. Latex agglutination reliably distinguished between normal and raised serum CRP concentrations. Enzyme immunoassay also provided a result within minutes, showed particularly close correlation ( $r = 0.967$ ) with the reference method, and was free from interference by lipaemic or icteric sera. In 27% of 55 episodes of fever studied serially in immunosuppressed patients, the enzyme immunoassay provided clinically useful information by indicating incomplete resolution of infection despite resolution of fever.

There is increasing awareness of the clinical value of a rapid C reactive protein (CRP) assay service from diagnostic laboratories for monitoring febrile episodes in immunosuppressed patients. This acute phase reactant protein normally has a serum concentration of  $<10$  mg/l, but increased hepatic synthesis may result in a rise to 400 mg/l or more within 6-8 h of an inflammatory stimulus.<sup>1</sup> In immunosuppressed patients, in whom clinical signs of infection are notoriously unreliable, an increase in serum CRP concentration to  $>100$  mg/l is consistent with clinically important bacterial infection.<sup>2</sup> Smaller responses occur in viral and protozoal infection.<sup>3</sup> Serial CRP assay in immunosuppressed patients is not only of value in diagnosing occult infection but may also indicate unresolved infection and thereby provide an objective end point with which to monitor antibiotic efficacy in clinical trials or during the care of individual patients.<sup>4,5</sup>

There is therefore a diagnostic requirement for a CRP assay sufficiently precise to allow serial monitoring of the serum concentration and also sufficiently rapid to provide a result within an hour of the onset of fever in an immunosuppressed patient. We have evaluated three rapid assay techniques (latex agglutination, laser nephelometry, and EMIT enzyme immunoassay) in comparison with

the 48 h CRP radial immunodiffusion assay as a reference method. The clinical usefulness of the enzyme immunoassay was then assessed in a serial study of 55 episodes of fever in patients with haematological disorders.

## Patients and methods

CRP assays were performed by each of the three methods on 120 sera from hospital inpatients; duplicate radial immunodiffusion assays were also performed on these specimens as a reference method.

## CRP REFERENCE MATERIAL

In the absence of an internationally recognised CRP reference preparation, the CRP calibrator supplied with the EMIT kit was used as a standard for both the radial immunodiffusion and enzyme immunoassay methods. When used in the laser nephelometer, however, the EMIT calibrator gave falsely low results. The calibrator was therefore used in conjunction with the radial immunodiffusion reference method to assign a CRP value to pooled human serum for use as a secondary standard in the nephelometric assay. No standard preparation was required for the latex agglutination method.

## LATEX AGGLUTINATION ASSAY

The Rapi Tex CRP Kit (Hoechst UK Ltd, Houns-

low) consists of an aqueous suspension of polystyrene particles sensitised with purified anti-CRP antiserum and formulated to agglutinate in the presence of about 1 mg/l CRP. A 40  $\mu$ l volume of latex reagent was mixed, on the test plate provided, with an equal volume of serum diluted 1/40 in 0.9% wt/vol saline. Positive and negative control sera (provided) were incorporated in each batch of tests. The plate was rocked gently for exactly 2 min and then inspected for agglutination. Agglutination at this dilution corresponds to a CRP value greater than 48 mg/l (manufacturer's instructions); positive specimens were retested at dilutions of 1/80, 1/120, and 1/160 until the reaction became negative. Specimens negative for agglutination at 1/40 were retested at 1/10.

#### LASER NEPHELOMETRIC ASSAY

A Hyland Laser Nephelometer PDQ<sup>TM</sup> (Travenol Laboratories, Thetford) was used. Rabbit antihuman CRP (Dako, Copenhagen) was diluted 1/100 in phosphate buffered saline (PBS)-polyethylene glycol (PEG) 4000<sup>4</sup> and filtered through a 0.4  $\mu$ m pore size polycarbonate membrane (Nuclepore Corporation, Pleasanton, California). Pooled human serum, standardised as described above, was double diluted from 1/20 to 1/640 with filtered 0.9% wt/vol saline. Test sera were diluted 1/20 and 1/100 in saline and 0.1 ml of diluted test or standard added to 1 ml of the antiserum in Hyland nephelometer cuvettes (Travenol Laboratories). Each test or standard was matched with a blank prepared by the addition of diluted serum sample to PBS-PEG 4000 without antibody. All tubes were incubated at room temperature for 1 h before reading in the nephelometer. Results were read from a standard curve prepared by plotting the nephelometer readings of the standards arithmetically against concentration.

#### EMIT ENZYME IMMUNOASSAY

The EMIT enzyme immunoassay kit for CRP (Syva Company, Palo Alto, CA94303) is based on the inhibition of the enzyme activity of a  $\beta$ -galactosidase-CRP conjugate by antibody against CRP. Serum CRP competes with the conjugate for antibody binding sites, thereby increasing enzyme availability for conversion of the synthetic substrate (o-nitrophenyl- $\beta$ -galactoside immobilised on a 40 000 dalton branched chain dextran). Enzyme conjugate and antibody substrate reagents were mixed with diluted test serum and the change in absorbance at 405 nm was measured over 30 s. Sample preparation requires a pipettor dilutor of high precision and the kit must be used in conjunction with a digital reading spectrophotometer

equipped with a thermally regulated flow cell capable of maintaining a temperature of  $37 \pm 0.1^\circ\text{C}$ . In the present study, the Syva CP 5000 EMIT Clinical Processor was used together with the Syva Auto-carousel and the Syva SIII spectrophotometer. The Clinical Processor was programmed to compute a standard curve from the absorbance changes of the calibrators and to reject this if outside machine confidence limits. Specimens with CRP concentrations greater than that of the top calibrator were diluted in the serum matched diluent provided.

#### RADIAL IMMUNODIFFUSION ASSAY

Immunodiffusion plates were prepared from agarose (Mercia Brocades, Weybridge, Surrey) containing 1% rabbit antihuman CRP (Dako, Copenhagen). Duplicate 10  $\mu$ l volumes of diluted test or standard serum were applied to 4 mm diameter wells and the precipitin rings measured after incubation for 48 h at room temperature; the mean result was taken. This technique, used as a reference method, gave a within batch coefficient of variation (CV) of 4.3% for 13 assays performed on one serum (mean 57.5 mg/l CRP, SD 2.5); it also gave a between batch CV of 7.5% for 10 batches each containing the same serum sample (mean 63.1 mg/l CRP, SD 4.7).

#### CLINICAL STUDY

The study population consisted of 32 patients with haematological disorders: acute myeloblastic leukaemia (16); acute lymphoblastic leukaemia (6); myeloma (1); chronic lymphocytic leukaemia (4); chronic granulocytic leukaemia (1); and hypoplastic anaemia (4). Serum for CRP assay was obtained from these patients three times weekly during hospital admissions for a total of 55 episodes of fever. CRP assays were performed by one of us, without knowledge of the clinical state of the patient, using the enzyme immunoassay method described above. The clinical usefulness of serial measurement of CRP concentration was assessed by an independent observer by retrospective study of the medical case record after the patient's discharge from hospital or death.

#### Results

##### ASSAY PRECISION

Within batch reproducibility of the latex agglutination method was assessed by testing 20 aliquots from each of two sera, containing similar (85 mg/l and 110 mg/l) concentrations of CRP, that were randomly distributed throughout a larger batch containing other samples of unknown CRP concentration. Identical results were obtained for 16

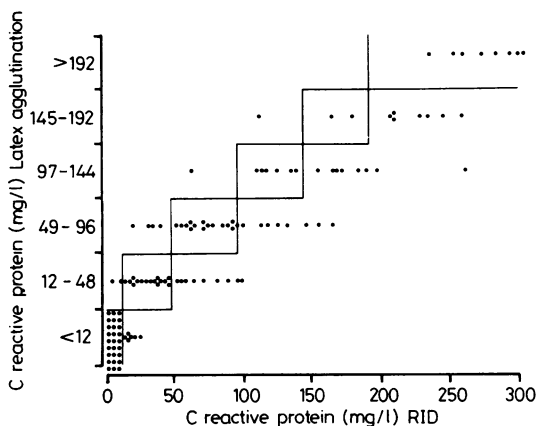


Fig. 1 Correlation between latex agglutination and radial immunodiffusion (RID) assay results for C reactive protein (mg/l) in 120 sera. Boxes show C reactive protein range for each dilution of the latex assay. Points to the right of the boxes represent sera for which latex agglutination gave lower results than radial immunodiffusion.

assays using the 85 mg/l serum and for 19 assays using the 110 mg/l serum; no results differed by more than one dilution. Between batch precision was estimated by the duplicate assay, using two operators, of 105 specimens distributed randomly within larger batches. Identical values were obtained for 67 of 105 samples (64%); no duplicates differed by more than one dilution.

For the nephelometric assay a within batch CV of 3.5% was obtained for 20 assays performed on one serum (mean 75.5 mg/l CRP, SD 2.7); a between batch CV of 4.3% was also obtained for 10 batches

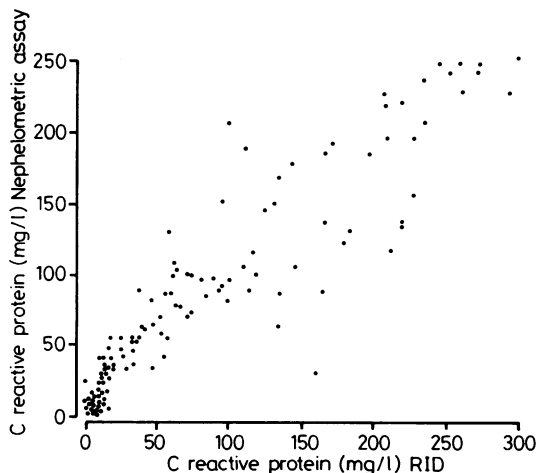


Fig. 2 Correlation between laser nephelometric and radial immunodiffusion (RID) assay results for C reactive protein (mg/l) in 120 sera.

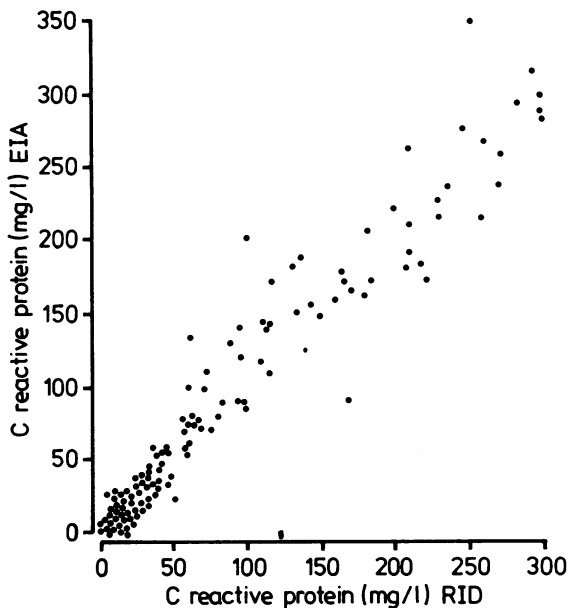


Fig. 3 Correlation between EMIT enzyme immunoassay (EIA) and radial immunodiffusion (RID) assay results for C reactive protein (mg/l) in 120 sera.

each containing the same serum (mean 77.7 mg/l CRP, SD 3.3). The enzyme immunoassay gave a within batch CV of 2.9% for 21 assays performed on one serum (mean 127.6 mg/l CRP, SD 3.6) and a between batch CV of 6.1% for 10 batches each containing the same serum (mean 76.9 mg/l CRP, SD 4.7). Carry over<sup>6</sup> for the enzyme immunoassay was 0.5% and there was a total upward drift of 7% for a serum containing 39 mg/l CRP which was assayed hourly for 7 h.

#### COMPARISON WITH RADIAL IMMUNODIFFUSION ASSAY

Figs. 1, 2, and 3 show the results when the same 120 samples were assayed by each of the three methods in comparison with the radial immunodiffusion assay. Latex agglutination showed a trend towards lower assay results than radial immunodiffusion particularly at CRP values >97 mg/l (Fig. 1). Correlation with radial immunodiffusion was less good for the nephelometric assay (Fig. 2) than for the enzyme immunoassay (Fig. 3), the latter giving a correlation coefficient ( $r$ ) of 0.967.

#### COST AND EASE OF ASSAY

Each latex agglutination assay cost £0.25, or £0.75 when the cost of controls and dilutions was included. A standard curve for the nephelometric assay cost £2.30 and each assay £0.50. For the enzyme

immunoassay method the standard curve cost £7.40 and each assay £1.30.

An assay result for the latex agglutination method could be obtained in 5 min. For the nephelometric manual assay a fresh standard was required for each batch of tests and 1½ h was required for an assay. The initial assay time, including calibration, for the enzyme immunoassay method was 30 min but since recalibration was not required throughout the working day, unless the instrument was switched off or the assay mode changed, subsequent assays could be performed in 2 min.

The sensitivity of the laser nephelometer to sample turbidity precluded the testing of lipaemic or icteric sera since they often gave unacceptably high specimen blank readings; such samples did not interfere with the enzyme immunoassay method.

#### CLINICAL STUDY

In 10 of the 55 (18%) episodes of fever studied using the enzyme immunoassay method, the serum CRP concentration failed to reach the level of 100 mg/l previously suggested as indicative of bacterial infection;<sup>2</sup> two of these 10 episodes were microbiologically documented bacterial infections (streptococcal bacteraemia and an *Escherichia coli* urinary tract infection).

Serum CRP concentrations fell in parallel with fever in 35 of the 55 (64%) episodes of fever studied serially; in these circumstances monitoring of CRP yielded little additional information of clinical value. In 15 of the 55 episodes (27%), however, resolution of fever was accompanied either by a continuing rise in CRP concentration or by a rate of decline slower than a half life of three days; in 14 of these 15 episodes (93%) fever then recurred within a few days.

#### Discussion

The methods evaluated in this study were selected from commercially available rapid assay methods and ranged from a latex kit screening method to a manual laser nephelometric assay and a fully automated enzyme immunoassay. Latex agglutination was rapid and simple to use but an experienced operator was required to achieve reasonable precision owing to the subjective nature of the end point. This rapid slide test gave reliable distinction between normal and raised serum CRP concentrations but the results tended to be lower than for the two other methods, particularly at CRP values >97 mg/l. Thus it has value as a relatively low cost rapid screening method but is less satisfactory for serial monitoring of the CRP concentration to assess, for example, the response to antibiotics in febrile

immunosuppressed patients.

Laser nephelometric assay was rapid and precise but the method was sensitive to minor degrees of sample lipaemia or icterus. This can be an appreciable disadvantage in the emergency investigation of ill patients with haematological malignancies. The enzyme immunoassay method, using EMIT equipment, did not have this disadvantage, showed a particularly high correlation with the radial immunodiffusion reference method, and this fully automated system was rapid and simple to use. Instrument stability was particularly impressive so that, once calibrated, individual assays could be performed within 2 min during the subsequent working day. Assay costs were relatively high, however, and the kits supplied lacked sufficient serum matched diluent for assay of sera with a high (>200 mg/l) concentration of CRP. We found that dilution of test sera in 0.9% wt/vol saline or in normal serum containing no detectable CRP were satisfactory alternatives. Flushing of the aspiration probe and flow through cuvette with buffer between each batch of assays was necessary to prevent false low results, possibly due to accumulated debris.

This study underlines the need for an international CRP standard reference preparation. Reference materials provided by manufacturers for use as calibrators in their individual assay systems may not give comparable results by other assay methods. For this study, it was necessary to use the EMIT calibrator as the primary standard for the nephelometric and radial immunodiffusion methods, but there is a clear need for an independent standard to provide within department quality assurance and to allow between centre standardisation of results. The latter is particularly important when a specified serum CRP assay concentration is recommended for diagnostic purposes. A concentration of above 100 mg/l, for example, was originally proposed as indicative of clinically important bacterial infection in neutropenic patients.<sup>2</sup> Centres using a different reference standard may find this value to be inappropriate. A CRP reference standard is therefore a prerequisite for further clinical studies of this type.

Serial monitoring of CRP concentration yielded clinically useful information, additional to that provided by the patient's temperature chart, in 27% of the febrile episodes studied. Irrespective of resolution of fever, failure of the CRP concentration to fall at a rate approximating to a serum half life of 3 days strongly suggested new or unresolved infection. In these circumstances, antibiotic treatment should clearly be reviewed.

The CRP concentration failed to rise to >100 mg/l in 10 of the 55 febrile episodes (18%) in

this series. Some of these 10 episodes may have been undiagnosed viral infections, in which smaller rises in CRP occur,<sup>3</sup> but in two cases there was microbiological confirmation of clinically important bacterial infection. Six of the 10 patients were being treated with prophylactic co-trimoxazole and two were still receiving intravenous antibiotics after an earlier infection; under these circumstances newly acquired bacterial infection, and the associated rises in serum CRP concentrations, may be partially suppressed or evolve more slowly.

This study has demonstrated the clinical usefulness of the enzyme immunoassay assay for serial monitoring of the serum CRP concentration as a guide to the resolution of infection during antibiotic treatment in immunosuppressed patients. A falling serum CRP concentration can therefore be used as an objective end point in clinical trials of new antibiotic combinations, but multicentre clinical studies of this type would be greatly facilitated by development of an international CRP reference standard.

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#### References

- <sup>1</sup> Crockson RA, Payne CJ, Ratcliff AP, Soothill JF. Time sequence of acute phase reactive proteins following surgical trauma. *Clin Chim Acta* 1966; **66**:435-41.
- <sup>2</sup> Mackie PH, Crockson RA, Stuart J. C-reactive protein for rapid diagnosis of infection in leukaemia. *J Clin Pathol* 1979; **32**:1253-6.
- <sup>3</sup> Voulgari F, Cummins P, Gardecki TIM, Beeching NJ, Stone PCW, Stuart J. Serum level of acute phase and cardiac proteins after myocardial infarction, surgery, and infection. *Br Heart J* 1982; **48**:352-6.
- <sup>4</sup> Rose PE, Johnson SA, Meakin M, Mackie PH, Stuart J. Serial study of C-reactive protein during infection in leukaemia. *J Clin Pathol* 1981; **34**:263-6.
- <sup>5</sup> Schofield KP, Voulgari F, Gozzard DI, Leyland MJ, Beeching NJ, Stuart J. C-reactive protein concentration as a guide to antibiotic therapy in acute leukaemia. *J Clin Pathol* 1982; **35**:866-9.
- <sup>6</sup> Boughton PMG, Gowenlock AH, McCormack JJ, Neill DW. A revised scheme for the evaluation of automatic instruments for use in clinical chemistry. *Ann Clin Biochem* 1974; **11**:207-18.

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