Rapid Immunometric Measurement of C-Reactive Protein in Whole Blood

Petter Urdal,1 Stig M. Borch,2 Sverre Landaas,1 May B. Krutnes,2 Geir Gogstad,2 and Per Hjortdahl3

We examined an instrument-free test for C-reactive protein (CRP) in whole blood. The NycoCard CRP Whole Blood test uses a cell-solubilizing dilution liquid, a membrane-bound antibody that binds CRP, and a gold-conjugated antibody for making visible the bound CRP. We obtained essentially identical dose–response curves in citrate-, heparin-, and EDTA-treated blood. CVs were 6.7–12.5% within series and 10.1–14.7% between series. The detection limit was 12 mg/L. Intralipid added to blood increased measured CRP by 10–20%, whereas no change was seen with added bilirubin, added serum amyloid P component, or the presence of rheumatoid factor. In 234 patients’ blood samples the results of the NycoCard Whole Blood test correlated well (r = 0.96) with those of a turbidimetric serum method. The test allows reliable measurement of CRP from a small volume of whole blood (25 μL) without using expensive equipment; it should be useful for decentralized testing in hospital departments, emergency units, and primary health care centers.

Additional Keyphrases: intermethod comparison • gold-conjugated antibody

C-reactive protein (CRP) is one of the more characteristic acute-phase proteins and is considered a reliable indicator of disease activity in various clinical conditions (1). Its concentration in blood increases rapidly by as much as 1000-fold upon exposure to various inflammatory stimuli, decreasing rapidly when the stimulus declines, e.g., after effective antibacterial treatment (2).

CRP has been used successfully for clinical diagnosis and monitoring of a variety of infections and diseases, including infections caused by bacteria, fungi, and viruses (3–7); intercurrent infections in leukemia (8, 9) and systemic lupus erythematosus (10); noninfectious inflammatory diseases such as rheumatoid arthritis (11); and diseases with cellular necrosis such as myocardial infarction (12, 13). Measurements of CRP are especially useful in distinguishing viral from bacterial infections (3, 5, 14).

Among the methods used to measure CRP in serum are radioimmunoassay (15); radial immunodiffusion (16); latex agglutination (17), which also is available in a quantitative microtiter version (18); lipid agglutination (19); turbidimetry (16, 20, 21); nephelometry (16); particle-enhanced turbidimetry (22); enzyme immunoassays (23, 24); and fluorescence polarimetry (25). These methods assay CRP in serum, either as instrument-based quantities or as instrument-free, qualitative to semiquantitative agglutination tests. The acute nature of many diseases in which CRP is relevant for diagnosis and monitoring requires a rapid, easily interpreted, instrument-free, quantitative test that may be applied directly to whole blood. We present here a test with such characteristics.

Materials and Methods

Materials

Bilirubin was obtained from Sigma Chemical Co. (St. Louis, MO), serum amyloid P component from Calbiochem Corp. (San Diego, CA), and Intralipid from Kabi-Vitrum (Stockholm, Sweden).

Reference Preparations

We used the 1st International Standard (World Health Organization) for human CRP (National Institute for Biological Standards and Controls, London, U.K.) to establish the concentration of CRP in the reference preparations. These preparations contained CRP at <5 to 1000 mg/L of plasma: purified CRP (Scipac Ltd., Kent, U.K.) added to whole blood (anticoagulated with citrate, heparin, or EDTA) obtained from healthy donors. We similarly prepared controls containing CRP concentrations of <5, 30, and 125 mg/L in plasma, using blood from one donor, and stored these at 4°C. The controls were used for estimating within- and between-
assay coefficients of variation (CVs) by analyzing duplicates on nine separate days during two weeks. To estimate sensitivity, we fortified EDTA-treated whole blood from a healthy individual with the purified CRP and measured CRP repeatedly in both unfortified and fortified blood. The technician estimating the final color intensity did not know which samples had been fortified.

Patients' Samples

We measured CRP and hematocrit in capillary and venous blood samples (n = 234) drawn from patients, at the Pediatric Department, Ullevål Hospital, during April–October 1990. We used EDTA-treated whole blood for measuring hematocrit and for measuring CRP by the NycoCard test (Nycomed Pharma AS, Oslo, Norway). We used serum for turbidimetric measurement of CRP. All analyses were performed on the day the sample was drawn.

Thirteen serum samples containing increased concentrations of rheumatoid factor (titer ≤4200 by the Waaler test) were obtained from the Institute for General and Rheumatic Immunology, Rikshospitalet, Norway.

Analysis for CRP

NycoCard method: The NycoCard CRP Whole Blood test is an immunometric flow-through test based on (a) a blood-solubilizing system (dilution liquid), (b) test cards with six test holes containing monoclonal antibodies specific for CRP immobilized on a porous membrane positioned over an absorbing paper pad, and (c) CRP-specific monoclonal antibodies conjugated with ultrasmall (3–5 nm) colloid gold. We performed all analytical procedures, storage, and handling of the reagents according to the manufacturer's instructions. The reagents were stored at 4 °C, and the dilution liquid was allowed to reach at least 15 °C before use. The other reagents were used without temperature equilibration.

Whole blood was diluted 41-fold (25 μL:1000 μL), mixed, and allowed to stand for at least 40 s (maximum 60 min), which lysed the cells and allowed their contents to be mixed with the plasma phase. We then applied 25 μL of diluted sample to a test hole, allowed it to soak in, and applied one drop (~45 μL) of conjugate solution and washing solution. A stable purple-reddish color appeared immediately in samples containing >5–10 mg of CRP per liter. We estimated the concentration of the sample by comparing the color intensity of the reaction zone (3.5 mm diameter) with five color zones on a reference color chart. Each reference color zone had a central hole through which the reaction zone could be viewed, thereby allowing direct color comparison. These reference color zones correspond to responses of whole-blood samples containing CRP at 10, 25, 50, 100, and 200 mg/L of plasma (assuming a hematocrit of 0.40).

The test procedure was typically completed within 2–3 min. Eleven experienced technicians performed these analyses. In some experiments we read the absorbance of the color response with a reflectometer (MacBeth Colour Eye; Kollmorgen Corp., Newburgh, NY), and expressed the result either in relative absorbance units or as milligrams of CRP per liter after comparison with responses from the reference preparations. The NycoCard method with reflectometric reading of absorbance was also used to establish the CRP concentrations of the reference preparations with respect to the International Standard.

Routine method: CRP was measured in serum by turbidimetry with a Cobas-Bio centrifugal analyzer (Hoffmann-LaRoche, Basle, Switzerland) and reagents from Orion Diagnostica (Espoo, Finland). In the sera containing rheumatoid factor, CRP was also measured by enzyme-linked immunosorbent assay (ELISA) (23) with reagents from Dakopatts (Glostrup, Denmark).

Hematocrit

Hematocrit was measured in all whole-blood samples (ELT 800WS; Ortho Diagnostic Systems, Westwood, MA). When whole-blood CRP concentrations were corrected for high or low hematocrit values, we used the following equation, which presupposes that CRP is present only in the plasma phase:

\[
\text{CRP}_{\text{040}} = \left[ (1 - 0.40)(1 - \text{hematocrit}) \right] \times \text{CRP (measured)}
\]

Results

The dose–response curves obtained with citrate-, heparin-, and EDTA-treated whole blood enriched with CRP were essentially identical (Figure 1). Within-and between-assay CVs were 6.7–12.5% and 10.1–13.5% at CRP concentrations of 25 and 130 mg/L, respectively (Table 1). For the control <5 mg/L, all results were below that of the lowest reference preparation (10 mg/L). Controls prepared in EDTA-treated blood showed a decrease in CRP of ~1% per day; CRP concentration in the other whole-blood controls was stable for the two weeks of the study. The sensitivity (detection limit) was estimated to be 12 mg/L, because all 21 samples drawn from unfortified whole blood (CRP, 2 mg/L of plasma, estimated by ELISA) were <10 mg/L by NycoCard.
Table 1. Precision (CV, %) at Two CRP Concentrations

<table>
<thead>
<tr>
<th>CRP, mg/L</th>
<th>Citrate</th>
<th>Heparin</th>
<th>EDTA</th>
<th>Citrate</th>
<th>Heparin</th>
<th>EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>9.8</td>
<td>6.7</td>
<td>12.5</td>
<td>13.7</td>
<td>10.9</td>
<td>14.7</td>
</tr>
<tr>
<td>130</td>
<td>7.3</td>
<td>7.3</td>
<td>8.2</td>
<td>10.1</td>
<td>11.1</td>
<td>13.6</td>
</tr>
</tbody>
</table>

* Concentrations added to whole blood treated with citrate, heparin, or EDTA. Results are from nine duplicate analyses performed during two weeks.

whereas all 21 samples from fortified whole blood (CRP, 12 mg/L) were ≥10 mg/L by NycoCard.

When Intralipid (3–10 g/L) was added to blood having various CRP concentrations, no false-positive results were obtained in CRP-negative (i.e., <10 mg/L) samples. In samples with CRP >10 mg/L, there was a tendency to overestimate CRP by 10–20%, as compared with controls to which the corresponding volume of distilled water was added (results not shown). No effect of bilirubin (85–680 μmol/L) and serum amyloid P component (500 mg/L) was found in similar experiments. In the 13 serum samples containing rheumatoid factor, CRP was measured by turbidimetry, ELISA, and NycoCard CRP, the NycoCard result being corrected for the zero hematocrit. The eight serum samples containing CRP <10 mg/L by turbidimetry all contained <10 mg/L by NycoCard CRP. In the remaining five samples, the NycoCard CRP and ELISA results agreed well (mean 11 vs 13 mg/L, range 4–26 vs 2–33 mg/L), whereas the turbidimetric results were higher (mean 24 mg/L, range 14–44 mg/L). By artificially varying the hematocrit, we found a linear relationship between hematocrit and measured CRP (Figure 2) and a close agreement between calculated and measured CRP.

The 234 patients' blood samples were analyzed by NycoCard CRP (visually) and by turbidimetry, with no correction for variations in hematocrit. The two sets of results were closely correlated (Figure 3, r = 0.96). When we grouped the results obtained with the two methods according to the color chart, 79.5% of the results of both methods were in the same group and 99.6% were within the same group or an adjacent group.

![Fig. 2. CRP measured in whole blood by NycoCard CRP at various hematocrits.](image1)

Plasma enriched with two concentrations of CRP (20 and 57 mg/L) was mixed with blood cells to give hematocrits of 0.0–0.84. CRP was measured (closed symbols) and calculated (open symbols) from the results for CRP at hematocrit 0.40.

![Fig. 3. Correlation between CRP measured in whole blood by NycoCard CRP and in serum by turbidimetry.](image2)

For the samples with CRP <200 mg/L (n = 230), the regression equation was  

\[ y = 0.91x + 1.1 \] (r = 0.96)

(Table 2). Retesting 186 of the samples and reading the color responses with a reflectometer did not improve the correlation to the turbidimetric results (r = 0.93 read reflectometrically and r = 0.96 read visually).

Of 15 samples with hematocrit ≥0.50, 9 (hematocrits 0.50–0.82) had CRP concentrations <10 mg/L by both NycoCard and turbidimetry. In the remaining six samples, the CRP concentration was underestimated by NycoCard CRP (Table 3); the degree of underestimation increased with increasing hematocrit. This discrepancy was reduced when we corrected for the displacement effect of the cells (Table 3).

In the 46 samples with hematocrit <0.30, CRP was not systematically overestimated with the NycoCard CRP. Correction for the low hematocrit in these samples did not improve the correlation to the turbidimetric results (results not shown).

We used one batch of cards and reagents during the six-month study. When we compared the results from NycoCard and turbidimetry for the first and last three months, we found no systematic change (slopes 0.89 and 0.92, r = 0.96 and 0.97, respectively). Similarly, when we used different batches of reagents, we found the dose–response curves shown in Figure 1 to be essentially unchanged for at least one year (results not shown).

![Table 2. NycoCard Whole Blood CRP vs Serum Turbidimetric CRP Method](image3)

<table>
<thead>
<tr>
<th>Turbidimetric method</th>
<th>NycoCard whole-blood test CRP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, mg/L</td>
<td>1–10</td>
</tr>
<tr>
<td>0–10</td>
<td>107*</td>
</tr>
<tr>
<td>11–25</td>
<td>13</td>
</tr>
<tr>
<td>26–50</td>
<td>7</td>
</tr>
<tr>
<td>51–100</td>
<td>6</td>
</tr>
<tr>
<td>101–200</td>
<td>2</td>
</tr>
<tr>
<td>&gt;200</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number of results in this category.
Comparison of other commonly used CRP assays, the NycoCard CRP has several methodological advantages. CRP can be analyzed directly in a small volume of whole blood, because the sample dilution medium lyases cells and the cell fragments thus obtained soak through the porous membrane to which the sample is applied. The assay allows CRP concentrations as low as 10 mg/L to be measured. Being a solid-phase immunoassay with sequential addition of reagents, the NycoCard test is not subject to prozone effects as are agglutination and turbidimetric assays. Hemolysis is not a problem, because the assay is performed on a mixture of plasma and lysed cells. Lipids, bilirubin, and rheumatoid factor do not markedly interfere with the assay, probably in part because the sample is initially diluted and CRP is subsequently concentrated by the use of solid-phase CRP antibodies. Also, the sample is drawn quickly through the porous membrane, thereby reducing low-affinity nonspecific binding and minimizing chemical interference. In turbidimetric assays, lipids may cause interference (16), as may rheumatoid factor.

CRP measurements are used for diagnosis and monitoring, which require quantitative measurement. The NycoCard method is slightly less precise than some other commonly used methods but is still acceptable. It is well suited for decentralized use because it may be used with capillary whole blood and does not require special equipment. The small volume required makes it especially well suited for pediatric use. In hospital laboratories, it might also be used for rapid analyses or for samples containing large quantities of bilirubin, lipid, or rheumatoid factor, which interfere with other CRP assays.

References

Table 3. CRP Results (mg/L) in Six Whole-Blood Samples with High Hematocrit

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>Uncorr.</th>
<th>Corr.</th>
<th>turbidimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>40</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>0.50</td>
<td>30</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>0.53</td>
<td>40</td>
<td>51</td>
<td>77</td>
</tr>
<tr>
<td>0.57</td>
<td>40</td>
<td>56</td>
<td>63</td>
</tr>
<tr>
<td>0.60</td>
<td>20</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>0.67</td>
<td>20</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Mean</td>
<td>31.7</td>
<td>42.8</td>
<td>49.2</td>
</tr>
</tbody>
</table>

* Uncorr. not corrected for hematocrit; corr., corrected for hematocrit.

Discussion
This immunometric assay of CRP is particularly well suited for use in decentralized testing. It allows direct measurement in capillary whole blood without the use of expensive equipment. The procedure is simple and takes ~2–3 min per sample. Heparin-, citrate-, and EDTA-treated blood all can be used. From experiments not presented here, we know that capillary blood without anticoagulants may also be used if the sample is analyzed before coagulation. The one lot of reagents tested here was stable and suited for storage; we have noted similar stability with other reagent lots.

When the final color was read by reflectometer, we obtained a within-day CV of 8–10% and a between-day CV of 10–15%. This precision is not quite as good as that usually obtained with automated quantitative methods such as turbidimetry but should still be acceptable, given the wide range of CRP concentrations in various diseases.

These results read visually correlated as well with the results of the turbidimetric comparison method as did the results obtained by reflectometry; skilled technicians read the final color well. In a previous investigation (26), the readings by skilled technicians correlated better with the results of a turbidimetric reference method than did readings by untrained personnel (r = 0.95 and 0.85, respectively). Thus, the use of a reflectometer might improve the results obtained with the immunometric assay. However, even with untrained personnel, acceptable results were obtained for a very high percentage of the patients.

CRP is located mainly in the plasma fraction of the blood, and our knowledge about changes in CRP during disease is based on serum and plasma measurements. The NycoCard CRP method allows CRP concentration to be measured in whole blood. To avoid misinterpreting the results, NycoCard presents the values as serum values, assuming a hematocrit of 0.40. Therefore, the test will inevitably overestimate CRP when the hematocrit is low and underestimate it when it is high. Even though a large fraction of our samples were from sick children in whom hematocrits are frequently abnormal, the results improved only marginally when we corrected for hematocrit. We suggest that correction for hematocrit is necessary only for values >0.55 and even then only when CRP is >10 mg/L.
Optimized Spectrophotometric Determination of Aldehyde Dehydrogenase Activity in Erythrocytes

R. D. Johnson, J. Bahnisch, B. Stewart, D. J. C. Shearman, and J. B. Edwards

We describe a reliable and sensitive semiautomated spectrophotometric assay of aldehyde dehydrogenase (ALDH; EC 1.2.1.3) activity in erythrocytes. The hemolysate can be stabilized with sucrose, and the technique involves only microliters of hemolysate on a centrifugal analyzer. The use of microcolumns to remove interfering hemoglobin is avoided, and reproducibility of the assay has been improved by manipulating the inherent lactate dehydrogenase activity of erythrocytes by adding lactate and oxalate to the reaction mixture. These modifications have decreased the analytical imprecision of the assay, allowing a better appraisal of aldehyde dehydrogenase activity in erythrocytes as a biological marker of excess alcohol consumption. Erythrocytic ALDH activity was significantly less in 40 alcoholics than in 145 teetotallers (median activity 128 vs 219 mU/g of hemoglobin, respectively; \( P = 0.0001 \)), indicating the potential of this assay as a useful marker of excess alcohol consumption.

Additional Keyphrases: centrifugal analyzer \cdot lactate dehydrogenase \cdot alcoholism \cdot marker of alcohol abuse

A major need in the management of alcoholism is a biological marker of excess alcohol consumption that would be sensitive, specific, and easy to determine (1). Aldehyde dehydrogenase (ALDH; aldehyde:NAD\(^+\) oxidoreductase, EC 1.2.1.3) activity in erythrocytes is a potentially useful marker (2) and reportedly is less in groups of alcoholics than in nonalcoholic subjects (3–8). Methods used to measure erythrocyte ALDH activity include isoelectric focusing and densitometry (3), headspace gas chromatography (4), and spectrophotometry (5–8). Assessment of the spectrophotometric method used to measure erythrocyte ALDH activity indicates that significant errors could occur at several steps, which collectively could lead to distortion of the data and misinterpretation of a subject's status. Such steps include the need to remove high amounts of interfering hemoglobin and the determination of blank activities (primarily from dehydrogenases other than ALDH). In addition, several aldehyde substrates have been used in this assay, and each provides different results.

We have developed a modified procedure that takes these problems into consideration; the procedure is reliable, precise, and easy to perform. We have also used this method to compare the erythrocyte ALDH activity in teetotallers and alcoholics.

Materials and Methods

Apparatus

ALDH activity and hemoglobin concentration were measured with a centrifugal analyzer (Multistat 111; Instrumentation Laboratory, Lexington, MA).