The Accuflex decreased the hands-on time by 60% and the analysis time by about 3%. The Hamilton decreased the hands-on time by 80% and the analysis time by 40%. The Primus decreased the hands-on time by 75%, but increased the total analysis time by 50%. (The Primus CLC 330 now handles samples at a rate of 30/h compared with the 15/h reported here.) The advantage that the Primus gave was excellent precision.

We thank Micromedics for assistance in the design and building of the modified Accuflex robot, Hamilton Co. for the use of the Hamilton Microlab 2200 robot, and Primus Corp. for use of the CLC 330. C.D.H. thanks the Juvenile Diabetes Foundation for a summer research award.

References

**C-REACTIVE PROTEIN AND ITS CYTOKINE MEDIATORS IN INTENSIVE-CARE PATIENTS**

Joanna Sheldon,† Pamela Riches,† Roger Gooding,† Neil Soni,‡ and J. R. Hobbs†

C-reactive protein (CRP) is an acute-phase protein produced by the liver during bacterial infections and inflammation. The cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) are widely reported to induce synthesis of CRP by hepatocytes both in vitro and in vivo. We investigated the relation between CRP and its cytokine mediators in 64 critically ill patients during their treatment in the intensive-care unit. Plasma CRP and IL-6 concentrations were significantly lower in patients without any evidence of infection than in those with clinical infection; plasma IL-1β concentrations showed no significant difference between any of the groups, but plasma TNF concentrations were lower in patients with evidence of infection. Significant correlation was seen between plasma concentrations of CRP and IL-6 when the latter was measured by bioassay; however, IL-6 showed, at best, only a 50% predictive value for a change in CRP concentration.

**Additional Keyphrases:** acute-phase response, bacterial infection, interleukin, tumor necrosis factor, sepsis

C-reactive protein (CRP) is an acute-phase protein synthesized by hepatocytes in response to tissue injury and inflammation. It is a good indicator of bacterial infection (1), showing a rapid (6–10 h) and marked increase in plasma concentration after hepatic induction and a rapid decrease when the inducing signal is removed. We reported that an increase in plasma CRP concentration of ≥25% within a 24-h period is indicative of intercurrent infection in critically ill patients (2), although we found that increased plasma CRP concentration was not in itself specific for bacterial infections (3).

Critically ill patients show rapid and massive increases in plasma concentrations of acute-phase proteins; they are therefore a group of patients in whom cytokines, the inducers of hepatic production of acute-phase protein synthesis, should be active. Furthermore, cytokines should be earlier, more sensitive, and more specific markers of disease activity than are the acute-phase proteins themselves.

Three cytokines—interleukin (IL) 1, IL-6, and tumor necrosis factor (TNF)—are reported to be important in the induction of acute-phase protein production by hepatocytes (4). Although all three are produced by a variety of cells, macrophages are the major production site (5). IL-1 acts on a variety of cells to generate a range of actions, including lymphocyte growth, fibroblast proliferation, muscle proteolysis, fever, leukocytosis, stimulation of IL-6 production, and stimulation of hepatic production of acute-phase proteins, both directly and via IL-6 (5). IL-6 has more limited actions, including promoting B cell differentiation, immunoglobulin synthesis, and IL-2 production by T cells and inducing (as the
major inducer) acute-phase protein synthesis by hepatocytes (6, 7). TNF shares many functions with IL-1, including its ability to induce the production of IL-6 and to directly stimulate hepatocytes to produce acute-phase proteins.

We investigated the relationship between plasma CRP and plasma IL-6, IL-1, and TNF concentrations by simultaneous measurements in 64 patients in the intensive-care unit (ICU). In addition we examined the cytokine and CRP concentrations in these patients during episodes of clinical infection and microbially proven infection.

Materials and Methods

**Specimen collection.** Serial blood samples were collected each morning from 64 patients in the ICU. Whenever the patient had clinical evidence of septicemia, blood cultures, in addition to a specimen for cytokine analysis, were taken. Septicemia was diagnosed by a change in temperature; tachycardia, with or without hypotension; tachypnea or the need for intermittent positive-pressure ventilation; or any two of the general signs of toxicity: metabolic acidosis, arterial hypoxemia, oliguria, coagulopathy and a decrease in platelet count, and deranged hemodynamic data indicating sepsis, i.e., increasing cardiac index and decreasing peripheral vascular resistance. All blood was collected into sterile pyrogen-free plastic tubes (Flow Laboratories, High Wycombe, UK) containing 50 μL of sodium heparin for injection (C P Pharmaceuticals, Wrexham, UK). The specimens were centrifuged for 10 min at 1000 × g, and the plasma was removed and either analyzed immediately or stored at −30 °C until analysis. A total of 358 specimens were collected.

**Plasma measurements.** CRP in plasma was measured by rate nephelometry with an Array analyzer (Beckman, High Wycombe, UK) and had a between-batch coefficient of variation (CV) at both normal and above-normal concentrations of <7% and a measuring range of 1–600 mg/L. IL-6 in plasma was measured by radioimmunoassay (RIA) (8) and had a between-batch CV of <7% at both low and high concentrations. IL-6 was also measured in 196 samples by bioassay with the B9 cell line obtained from L. Aarden (Netherlands Blood Transfusion Service, Amsterdam, The Netherlands) and standardized with mixed lymphocyte-conditioned medium cross-calibrated in the RIA. IL-1β in plasma was measured by RIA (9) and had a between-batch CV <7% at both low and high concentrations. Plasma TNF was measured by enzyme-linked immunosorbent assay (10) and had a between-batch CV of <10% at both low and high concentrations.

**Statistics.** All results are presented as mean values. Mann–Whitney U test was used to test for statistical significance, with values <0.05 being considered significant.

**Sample classification.** All samples received in the laboratory were classified into three groups: those taken at the time of microbiologically proven septicemia were designated MS (n=13), samples taken at the time of clinical septicemia but without microbiological confirmation were designated CS (n=39), and samples taken when there was no clinical evidence of septicemia were designated NS (n=306).

**Results**

Median plasma concentrations in addition to the minimum and maximum values for CRP, IL-6, IL-1β, and TNF for all 358 samples are shown in Table 1. Median plasma concentrations and ranges of concentrations for all four analytes for the three sample groups are presented in Table 2. Plasma CRP and IL-6 concentrations were significantly lower in the NS group than in the combined MS and CS group. Plasma IL-1β concentrations were not significantly different between any of the groups. Plasma TNF concentration was significantly higher in the NS group than in the combined MS and CS group. Between the two septicemic groups only IL-6 was statistically different, being significantly higher in CS than in MS.

There was no significant correlation between CRP and IL-1β, TNF, or IL-6 assayed by RIA (r = 0.222 and P = 0.076, r = 0.331 and P = 0.567, and r = 0.085 and P = 0.109, respectively), nor was there any significant correlation between any of the cytokines (data not presented).

In 196 samples, IL-6 concentrations were measured by bioassay (median 0.057 μg/L, range 0.001–46.28 μg/L) and plasma CRP concentrations correlated with these IL-6 concentrations (r = 0.208, P = 0.003). This correlation improved when CRP was compared with the plasma IL-6 concentration from the previous day (r = 0.346, P = <0.001). Concentrations of IL-6 measured by RIA and bioassay were also significantly correlated (r = 0.597, P = <0.001).

Overall, 40 patients stayed in the ICU for ≥2 days, and there were 265 occasions when results were available for the preceding day. These were classified into three groups: specimens showing a >20% increase in CRP, specimens showing a >20% decrease in CRP, and specimens showing a <20% change in CRP. In each of the three groups, the change in each cytokine concentration was calculated; changes >20% were considered significant. There was no consistent pattern of change in the three cytokines that was related to a particular change in CRP concentration (data not presented).

Of the 196 specimens in which IL-6 was measured by bioassay, 169 samples formed part of daily series from individual patients. These specimens were classified into three groups according to the change in CRP, as described above, and analyzed with respect to corre-

| Table 1. Minimum, Maximum, and Median Plasma Concentrations of CRP, IL-6, IL-1β, and TNF |
|---------------------------------------------|-------------|-------------|-------------|
| **Analyte** | **Minimum** | **Maximum** | **Median** |
| CRP, mg/L | 6.0 | 531 | 87.0 |
| IL-6, μg/L | <0.2 | 28.5 | 0.27 |
| IL-1β, μg/L | <0.35 | 6.79 | 1.16 |
| TNF, kU/L | <7.5 | 331.5 | <7.5 |
Table 2. Median (and Range) Plasma Concentrations of CRP, IL-6, IL-1β, and TNF, by Group

<table>
<thead>
<tr>
<th>Sample group</th>
<th>CRP, mg/L</th>
<th>IL-6, μg/L</th>
<th>IL-1β, μg/L</th>
<th>TNF, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>99</td>
<td>0.30</td>
<td>0.92</td>
<td>&lt;7.5</td>
</tr>
<tr>
<td>(37–143)</td>
<td>(0.21–0.37)</td>
<td>(0.58–4.89)</td>
<td>(&lt;7.5)</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>163*</td>
<td>1.76</td>
<td>1.58</td>
<td>&lt;7.5</td>
</tr>
<tr>
<td>(6–406)</td>
<td>(0.1–28.5)</td>
<td>(0.43–6.40)</td>
<td>(&lt;7.5–63.1)</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>84b</td>
<td>0.38c</td>
<td>1.45</td>
<td>&lt;7.5d</td>
</tr>
<tr>
<td>(6–531)</td>
<td>(0.1–9.48)</td>
<td>(0.38–8.79)</td>
<td>(&lt;7.5–331.5)</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly higher than in MS (P = 0.046) and in NS (P = <0.001).

b,c,d Significantly different from the combined CS plus MS groups: bp = 0.001, cp = 0.005, dp = 0.048.

The measurement in plasma of cytokine inducers of acute-phase protein synthesis may increase both the specificity and sensitivity with which we can detect an acute-phase reaction. Detection of the acute-phase response has historically been in plasma, and detection and quantification of cytokines in plasma may not reflect either effective synthesis or biologically active protein. In addition, many assays are poorly characterized, being based on antisera raised against recombinant human cytokines and standardized with the same material. Although cytokine assays perform adequately with recombinant material or supernates produced in assay systems in vitro, they are generally poorly characterized for biological material, which may contain a number of undefined interfering substances. Clinical use is also hampered by lack of data concerning the plasma half-life, reference ranges, and magnitude of significant changes during infection and inflammation.

Table 3. Predictive Accuracy of IL-6 Measured by Bioassay in Predicting Changes in CRP Concentrations (n = 169)

<table>
<thead>
<tr>
<th>CRP response</th>
<th>No change</th>
<th>Rise</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>29.5</td>
<td>36.4</td>
<td>42.6</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>74.7</td>
<td>68.0</td>
<td>62.5</td>
</tr>
<tr>
<td>Predictive value, %</td>
<td>50.0</td>
<td>28.6</td>
<td>29.9</td>
</tr>
<tr>
<td>Efficiency, %</td>
<td>53.8</td>
<td>59.8</td>
<td>56.2</td>
</tr>
</tbody>
</table>

It is now well established that IL-6 can stimulate hepatoma cell lines to produce acute-phase proteins; although they are less potent stimulators, both IL-1β and TNF also induce acute-phase proteins in various hepatoma cultures. In primary hepatocyte cultures, IL-6 is the only one of these cytokines that induced the full spectrum of acute-phase proteins. That IL-6 and CRP concentrations were significantly higher in the combined MS and CS group than in the NS group is consistent with IL-6 induction of CRP. However, the lack of correlation between these two markers may reflect insensitivity of the assay or the dynamics of cytokine production in relation to induction of acute-phase proteins and to sampling time. Indeed, sensitivity of assay may be a contributing factor, because use of the sensitive bioassay improved correlation, particularly of CRP with IL-6 concentrations of the previous day. The time taken to do a bioassay precludes its use as a predictor of CRP response, and even if a rapid immunoassay with sensitivity comparable with that of the bioassay were available, at best the predictive value would be only 50% and therefore of no clinical value.

Although IL-1β was not significantly different in any of the groups, individual patients showed grossly increased concentrations throughout their investigation, with changes in concentration having no bearing on CRP concentration. This cytokine may not be biologically active in plasma, and indeed binding factors for it do occur (11). In addition, plasma concentrations may not truly reflect production, because there is evidence that IL-1 is not a conventionally secreted cytokine; rather, it is synthesized as an inactive precursor in the cytosol of activated monocytes and then released via lysosomal vesicles (12).

TNF has been heavily implicated as the mediator of the clinical manifestations of septic shock; however, the literature is conflicting, with reports of both measurable (13) and unmeasurable (14) TNF concentrations. In the one patient in this study with clinical septic shock, no measurable TNF concentrations were ever recorded, nor was there any detectable plasma TNF in the 13 MS samples. We have considered that our immunoassay may not be adequately sensitive or that native TNF is unreactive, but the assay is capable of detecting TNF in plasma separated from whole blood incubated for 2 h with endotoxin (15). Inconsistent detection of TNF in plasma may reflect both a short half-life and local or intracellular actions. In our experience with an isolated case in which locally administered recombinant TNF leaked into the systemic circulation, major hemodynamic changes, similar to those associated with septic shock, occurred when there was no measurable plasma TNF (16). Significant TNF concentrations were recorded only in samples taken from the pulmonary artery, but not in paired peripheral arterial samples, suggesting rapid pulmonary clearance. The half-life of TNF in the mixed venous samples was 17 min. This is not ideal as a model for studying septic shock, because the dynamics of a rapid and isolated release of TNF into the systemic circulation may be significantly different.
from the relatively chronic TNF production of sepsis with associated cytokine and receptor interactions; however, it does show that TNF can be undetectable in plasma at times when systemic effects are present.

In conclusion, there is no evidence in this study that the measurement of plasma cytokine concentrations, by existing methodology, is of clinical value in the early diagnosis of infection. Of the four variables tested in this study, CRP remains the most effective analyte available to support a clinical diagnosis of sepsis.

References