Plasma Neopterin as an Adjunct to C-Reactive Protein in Assessment of Infection

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C-reactive protein (CRP) concentrations are increased in plasma in people with inflammatory conditions and bacterial infections. Plasma neopterin concentrations are increased in people with bacterial septicemias, viral infections, and graft vs host disease. Plasma concentrations of CRP and neopterin were measured daily in 21 bone-marrow transplant (BMT) patients, 64 patients in intensive-care units (ICU), and 12 patients with squamous cell carcinoma of the head and neck (HN). In the BMT patients, plasma neopterin measurements in addition to CRP measurements allowed infectious episodes to be distinguished from graft vs host disease. In the ICU patients, increased concentrations of CRP were not specific for infection and the additional plasma neopterin measurements did not improve this specificity. In all three patient groups, the derivation of a neopterin/CRP ratio was of no clinical use. These three groups of patients showed patterns of CRP and neopterin concentrations characteristic of their underlying diseases, the BMT patients with the immunological activation of graft vs host disease showed predominantly increased concentrations of plasma neopterin, ICU patients with infectious and inflammatory conditions had increased concentrations of both CRP and neopterin in plasma, and the HN group with localized inflammation showed increased plasma concentrations of CRP without increases in neopterin.

Additional Keyphrases: bone marrow transplants · intensive-care patients · cancer · viruses

C-reactive protein (CRP), an acute-phase reactant, is generally a good indicator of infection (1) and has been shown to distinguish intercurrent infection from episodes of graft vs host disease (GVHD) in bone-marrow-transplant (BMT) patients in the post-graft period (2). Plasma concentrations of CRP are also reportedly independent of rejection episodes in cyclosporine-treated allograft recipients (3-5).

Neopterin, a pteridine intermediate metabolite in the bioppterin synthetic pathway, is synthesized and secreted by monocytes/macrophages upon stimulation, mainly by gamma interferon produced by activated T cells (6). Plasma concentrations of neopterin have been reported to be increased in allograft rejection (7, 8), in GVHD (9, 10), and in infections, particularly viral infections. Plasma neopterin alone, therefore, does not distinguish rejection episodes or GVHD from infections; however, in combination with CRP, at least bacterial infections are distinguishable. Furthermore, the ratio of plasma neopterin to CRP can help detect infection and rejection episodes in heart-transplant recipients (11), with ratios <1.0 indicating bacterial infection and >1.0 indicating rejection or viral infection.

Here we have examined the relationship between simultaneous measurement of CRP and neopterin in 21 BMT patients. Although increased concentrations of neopterin in plasma with low or normal concentrations of CRP were useful indicators of GVHD, and increased plasma CRP indicated bacterial infection, no additional information was derived from a ratio of values and, indeed, contrary to the findings of Myara et al. (11), the ratio could be >1 in bacterial infection. To investigate whether the results obtained were peculiar to BMT patients, we also included in this study two other groups of patients, in whom plasma CRP and neopterin were being routinely measured during other work: patients with squamous cell tumor of the head and neck (HN) and patients in intensive-care units (ICU).

Materials and Methods

Specimen Collection

Serial blood samples were obtained from 21 children, ages one to 13 years, undergoing BMT; 64 adults, ages 23 to 83 years, in the ICU; and 12 adults, ages 31 to 73 years, before treatment for squamous cell tumors of the head and neck. In the BMT group a clinical diagnosis of sepsis was made if the patient's temperature rose above 38 °C (or above 37.5 °C in neutropenic patients); blood culture specimens were taken and the patient was treated with azlocillin and netilmicin until culture and sensitivity results were available. In the ICU group the indications for blood culture analysis were a change in temperature; tachycardia, with or without hypotension; tachypnea, or the necessity of intermittent positive pressure ventilation; or any two of the following five 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 decreas-
ing peripheral vascular resistance. Patients with a clinical diagnosis of infection were treated with either ciprofloxacin or metronidazole until culture and antibiotic-sensitivity results were available. No patient with any evidence of sepsis was included in the HN group.

The specimens were centrifuged for 10 min at 1000 \( \times g \) and the plasma or serum was removed and either analyzed immediately or stored at \(-30^\circ C\) until assay.

Plasma Measurements

CRP was measured by rate nephelometry with an Array nephelometer (Beckman Instruments, High Wycombe, U.K.). Neopterin was measured by a commercially produced radioimmunoassay (Henning Berlin, IDS, Tyne and Wear, U.K.). Between-batch coefficients of variation for both assays at both normal and above-normal concentrations were <7%. The upper limit of the reference range for children older than one year and adults is 10 mg/L for CRP and 10 nmol/L for neopterin.

Statistics

Results are presented as mean values. Mann–Whitney U-test was used to test for statistical significance (differences with \( P < 0.05 \) were considered significant). Ratios were derived from the plasma concentration of neopterin (nmol/L) divided by the concentration of CRP (mg/L).

Results

We analyzed 732 samples collected from the 21 BMT patients, 359 from the 64 ICU patients, and 30 from the 12 HN patients. The BMT group included 283 samples and the HN group five samples with both CRP and neopterin concentrations within the reference range. All ICU samples had at least one analyte concentration above the reference range. The number of plasma samples in each group in which CRP or neopterin alone or both CRP and neopterin exceeded the reference range is shown in Table 1, together with the mean values for each analyte.

In 144 BMT samples, CRP was significantly increased (>20 mg/L); 66 of these had a neopterin concentration <20 nmol/L (mean CRP 40.4 mg/L), and 78 samples had a neopterin concentration >20 nmol/L (mean CRP 50.7 mg/L). Similarly, of the 338 ICU samples with a significant increase in CRP (>20 mg/L), 117 had a neopterin concentration of <20 nmol/L (mean CRP of 129 mg/L); the 221 samples with neopterin >20 nmol/L had a mean CRP concentration of 118 mg/L. In the BMT group, the samples with higher neopterin showed significantly higher CRP concentrations; however, in the ICU group, the converse was true, the samples with higher neopterin having a significantly lower CRP (\( P = 0.006 \)). No HN patient had plasma neopterin >20 nmol/L.

For 625 BMT specimens, clinical information was adequate to classify the subjects as those with infection, both suspected and microbiologically proven; those with GVHD, both suspected and histologically proven; and normal, when patients had no evidence of infection or GVHD. The number of samples in each of these groups, together with mean plasma CRP and neopterin concentrations, is shown in Table 2. The temporal responses of CRP and neopterin during episodes of infection and GVHD followed the characteristic pattern shown for a representative patient in Figure 1. The ICU patients were classified as having suspected infection any time when blood cultures were requested but no organism was isolated, or as having proven infection when an organism was isolated. Of the 359 specimens collected, blood cultures were requested for 52, 13 of which gave positive results. The mean plasma CRP and neopterin concentrations in the ICU groups are shown in Table 3.

We calculated the ratio of neopterin to CRP for samples in which the type of inflammatory, infectious, or immune response was clearly identifiable: proven infection in ICU and BMT patients, proven GVHD in BMT patients, and localized tumor necrosis in the HN patients (Table 4).

Discussion

The patient groups in the present study exhibit characteristic inflammatory or immune responses of distinct but different types. In the BMT group the major problems in the post-graft period are infection and (or) GVHD, whereas the ICU group is subject to multisystem involvement, often with intercurrent clinical infection; in the HN group the inflammatory or immune responses are localized. These various responses were reflected to some degree by the responses seen in CRP and neopterin.

Table 2. Mean Plasma CRP and Neopterin Concentration in Subgroups of BMT Patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>CRP, mg/L</th>
<th>Neopterin, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>No clinical symptoms</td>
<td>414</td>
<td>9.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td>12</td>
<td>35.9*</td>
<td>18.3</td>
</tr>
<tr>
<td>Suspected + proven</td>
<td>90</td>
<td>33.2*</td>
<td>28.7*</td>
</tr>
<tr>
<td>GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td>29</td>
<td>19.1*+b</td>
<td>26.8*</td>
</tr>
<tr>
<td>Suspected + proven</td>
<td>111</td>
<td>21.5*+b</td>
<td>49.0*</td>
</tr>
</tbody>
</table>

* Significantly higher than in no clinical symptoms (\( P = 0.000 \)).
+ Significantly lower than in proven in suspected + proven infection (\( P = 0.002 \)).
In 35.6% of the abnormal BMT samples, the CRP concentration was increased, as was that of neopterin. Previous studies have indicated that a significant increase in CRP is associated with infection, particularly bacterial (2); apparently, therefore, infection in BMT is associated with increases in both CRP and neopterin. In the present study, the mean CRP concentrations during infection were somewhat lower than those reported in our previous study (2). We attribute this to the prompt use of antibiotic therapy when plasma concentrations of CRP are increased, a policy adopted as a result of the previous study. BMT represents an unusual set of circumstances, in that patients are profoundly immuno-suppressed and infection may predispose to GVHD (12). Our data showed significantly higher CRP associated with the higher concentrations of neopterin, suggesting that infectious episodes may precipitate GVHD.

In the ICU patients, 83.3% of samples showed increases in both CRP and neopterin. Apparently, inflammatory and immune responses frequently occur concurrently; however, the significantly lower concentrations of CRP in the samples with the higher concentrations of neopterin suggest that inflammatory episodes do not automatically trigger a specific immune response that results in neopterin production. The lack of correlation in any group between the plasma concentration of CRP and neopterin (data not presented) indicates that independent mechanisms control the synthesis of these two markers. Neopterin also is reportedly a sensitive marker of viral infections and, throughout the present studies, it has been impossible to exclude implicating viruses for the increased values seen.

In the HN group, a quite different pattern of response was seen, 84% of abnormal samples showing an increase in CRP alone; in only four samples, two with increased CRP also, was the neopterin concentration increased and then only marginally. In only 13.4% and 15.9%, respectively, of BMT and ICU abnormal samples was the CRP alone raised.

The BMT group was unusual in that 51% of the abnormal samples showed an increase in plasma neopterin alone, compared with 0.8% and 8.0%, respectively, for ICU and HN samples. These data confirm our previous preliminary findings (9), and that of other groups (10), that high neopterin concentrations are associated with GVHD. In addition, this study indicates that, in GVHD, increased neopterin values are associated with CRP values that are lower than the CRP concentrations in infection. The number of occasions on which we obtained histological confirmation of GVHD or microbiological confirmation of infection was few. The relative speed with which CRP and neopterin can be estimated, together with the higher predictive value, makes them valuable markers in the differential diagnosis of these two complications of BMT.

Microbiological confirmation of infection in the ICU group was rare (13 positive blood cultures out of 52 requests). Although CRP is a sensitive marker of infection, this marker in ICU patients, unlike in the BMT group, showed low specificity, being increased in all but three samples. CRP values were higher in suspected infection than in microbiologically proven infection, but this may reflect the poor isolation of organisms in samples from patient groups in whom antibiotics are frequently and widely applied, often before the blood culture is taken. Despite the low specificity of CRP in ICU patients, it has been suggested that, in the context of proven septicemia, the rate of change rather than isolated values can be helpful and that an increase >25% is indicative of intercurrent infection (13). Neopterin was increased in 84% of all ICU samples. The
measurement of neopterin may be complicated by renal impairment (14), although concurrent measurement of plasma creatinine may overcome this (15). In addition, the present data indicate no clinical value for the measurement of neopterin with CRP in this group of patients, with respect to diagnosis or management of infection. An earlier study (16) suggested that neopterin might be help in early evaluation of the immunologic status of a patient at risk for fatal septic complications but did not consider the use of neopterin measurements in the diagnosis and management of sepsis. A more recent study (17) of serum CRP and neopterin concentrations in patients with viral or bacterial infections concluded that the addition of neopterin values to CRP measurements contributed little to the diagnosis of bacteremia, its only role being to increase the specificity and predictive value in the diagnosis of viral infections and tuberculosis, but at the expense of sensitivity.

The HN group provided a more clinically uniform group of patients. Septicemic patients were excluded from the originating study so that the 12 patients investigated were without the iatrogenic immune suppression of BMT patients and were without acute trauma, either from major surgery or accidental. The predominant pattern seen in the HN group was increased CRP in the absence of an increase in neopterin—which could indicate an inflammatory response in the absence of a specific immune response. However, the HN patients in this study all had advanced disease; because tumor-bearing animals are well known to show many defects of specific immune responses, it is not possible in the HN patient group to exclude an atypical immune response, e.g., reticulo-endothelial block.

The derivation of a ratio of neopterin to CRP confirmed to some extent the findings of Myara et al. (11), who investigated these markers during infection and rejection episodes in heart transplantation. In the present study, we found mean values of the ratio to be lower when there was proven infection than when there was GVHD in BMT patients but, because of the wide overlapping range, we could not place an upper limit (1.0 in the Myara study) to distinguish infection from GVHD. Perhaps the wider range of ratios in the present study is attributable to lower CRP values that may have resulted from many samples having been collected during antibiotic therapy for suspected infection in BMT or critically ill patients. Interestingly, the mean value was lowest in the HN group of patients, who showed no systemic infection, only tumor necrosis and possibly localized infection.

The most effective use of both CRP and neopterin measurements was in the BMT group. Temporal relationships seemed more useful than isolated measurements or derivations of ratios, increasing CRP and neopterin being characteristic for infection, whereas an increased neopterin in the absence of increasing CRP was characteristic for GVHD.

In summary, three predominant patterns of response were identified in the present study: neopterin alone in the BMT group, CRP and neopterin in the ICU group, and CRP alone in the tumor group. Because plasma markers of inflammatory and immune responses are synthesized in response to cytokine mediators, the patterns observed probably indicate the involvement of different cytokines. CRP is synthesized by hepatocytes on stimulation by inflammatory cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor (18); these cytokines may be induced by nonspecific inflammatory response mediators, i.e., lipopolysaccharide, or during specific immune responses. Neopterin is synthesized by monocytes (6) on stimulation predominantly by interferon-gamma, which is synthesized by T lymphocytes (19); this synthesis requires specific antigen recognition. In further studies the plasma concentrations of the cytokine mediators of CRP and neopterin will be investigated together with the concentrations of soluble interleukin-2 receptors, which provide a sensitive indicator of immune responses. A second acute-phase protein, α1-antichymotrypsin, behaves similarly to CRP in infection but has also been reported to be associated with GVHD (20, 21) and should also be investigated.

In conclusion, only in BMT and ICU patients are measurements of CRP and (or) neopterin clinically indicated. CRP and neopterin measurements are suitable for monitoring infection and GVHD in BMT patients. CRP can be used to monitor infections in ICU patients, but neopterin measurements in this group of clinically diverse patients are of no additional clinical use.

References
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