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SERUM NEOPTERIN AS A MARKER FOR REACTIONAL STATES IN LEPROSY

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SUMMARY

Reactions, a relatively common phenomenon among leprosy patients in treatment, require early detection and proper management to prevent serious sequelae. It is generally accepted that reactional states are immunologically mediated, and as such, usually improve with immunomodulatory treatments such as corticosteroids or thalidomide. Neopterin, a product of interferon-γ activated macrophages, is a marker for CMI activation and may be useful to detect reactional states in leprosy. Here, we compared neopterin levels in single serum samples from leprosy patients with and without reaction, with untreated controls, and when available, serial samples among patients with and without reaction. Levels in the single sample measurements, conducted in 22 patients with reversal reaction (RR; mean 14.5 nmol/L, SD 8.7) and 13 with erythema nodosum leprosum (ENL; mean 16.9 nmol/L, SD 13.6), were significantly higher ($p = 0.02$ and $p = 0.001$, respectively) than levels in 26 untreated patients (mean 9.1 nmol/L, SD 7.3). Values above the upper limit of normal (10 nmol/L) were found in 7 of 26 untreated patients, 14 of the 22 RR patients ($p = 0.01$) and 10 of the 13 ENL patients ($p = 0.003$). Serial serum samples, obtained from 6 patients that developed reactions and 14 that remained free of reaction, indicated that RR or ENL paralleled a concomitant increase in the serum neopterin level. Neopterin levels generally declined upon corticosteroid therapy. Neopterin may be a useful marker for reactional states in leprosy by providing a laboratory parameter to assess onset, progression, response to therapy, and resolution.
INTRODUCTION

Leprosy is an unstable disease characterized by immunologically mediated reactional states such as reversal reaction (RR), occurring mainly in borderline lepromatous leprosy (BL), and erythema nodosum leprosum (ENL), occurring in lepromatous leprosy (LL) and BL [1,2]. ENL and RR may develop rapidly and can be associated with sequelae, most importantly permanent nerve damage (3). Although ENL is responsive to corticosteroids or thalidomide and RR improves with corticosteroids, there is no currently accepted laboratory parameter to identify patients at high risk of developing reactional states, their associated sequelae, or for monitoring the response to therapy (4,5). Neopterin, a pteridine compound synthesized from guanosine triphosphate (GTP) via GTP cyclohydrolase I in activated macrophages, is considered an early, specific, and sensitive marker of cell-mediated immune (CMI) activation (6). In vitro studies show that human monocytes (macrophages) produce neopterin when stimulated by interferon-γ from activated T cells (7,8,9). Other cell types do not produce measurable amounts of neopterin (10,11). Neopterin has been used to monitor CMI responses in acute allograft rejections, viral infections, intracellular infections, autoimmune diseases, and some malignancies (12). The value of obtaining neopterin levels to monitor for reactional states in leprosy patients is unclear. In East Africa, urine neopterin was increased in most LL as well as tuberculoid leprosy patients (13). However, there was no distinction between patients with and without reactions. Here, we studied the relationship between the occurrence of leprosy reactions and serum neopterin concentrations, as well as the influence of treatment with corticosteroids. To accomplish this, we used banked sera obtained from leprosy patients before, during, and after reaction.

MATERIAL AND METHODS

Patients and controls
The sera for this study were obtained from serum banks at Leonard Wood Memorial Center for Leprosy Research, Cebu, Philippines and the Department of Dermatology of the Academic Medical Center in Amsterdam, the Netherlands. The patients were classified according to the Ridley-Jopling scale (14). All clinical diagnoses were histologically confirmed. Multibacillary (MB) leprosy patients included all borderline and lepromatous patients with a bacterial index (BI) of
at least 2+ on the Ridley scale at any one site. Paucibacillary (PB) leprosy patients included indeterminate, tuberculoid (TT) and borderline tuberculoid (BT) with BIs < 2+ at any one site. Single serum samples were available from 26 untreated leprosy patients not in reaction, from 22 patients during RR before treatment and from 13 patients during ENL reaction before treatment. In addition, serial samples were obtained at fixed intervals during MDT from 14 patients who remained free of reactions within the period of follow-up (7 BL/LL; 7 TT/BT) and from 6 patients who developed a reaction. Of these 6, 4 patients developed RR (single episodes) and 2 patients developed multiple episodes of ENL (one with 4 episodes, the other 2 episodes). All 6 patients with reactional episodes received conventional doses of oral Prednison as therapy. Sera from 10 healthy Dutch subjects were used as controls.

Laboratory investigation
Serum neopterin levels of patients and controls were determined using a commercially available radioimmunoassay kit (Henning, Berlin, FRG). This radioimmunoassay is based on the competition of unlabelled neopterin of the serum samples or standards and radiolabelled neopterin for the binding sites of a neopterin-specific antibody. The radioactivity of the neopterin-antibody complex is reversibly proportional to the concentration of unlabelled neopterin in the sample. The upper limit of the normal range is approximately 10 nmol/L serum (= 2.5 ng/ml) (15).

Statistical Analysis.
The differences observed between the controls and the different patient groups were assessed by one-way analysis of variance (ANOVA).

RESULTS
The serum neopterin levels in each patient group are summarized in Table 1. The distribution of the serum neopterin concentrations are demonstrated in Fig. 1. Levels in patients with RR (mean 14.5 nmol/L, SD 8.7) and with ENL (mean 16.9 nmol/L, SD 13.6) were significantly higher (p = 0.02 and p = 0.001, respectively) than levels in untreated patients (mean 9.1 nmol/L, SD 7.3). No difference was found in neopterin levels between untreated PB and MB patients. Normal controls had a mean value of 5.4 nmol neopterin/L. Values above the upper limit of normal (10 nmol/L) were found in 7 of 26 untreated patients, 14 of
Neopterin, reactional states in leprosy

Fig. 1 Serum neopterin values in nmol/L in 22 patients with a Reversal Reaction (RR) and 13 patients with an Erythema Nodosum Leprosum reaction (ENL) before treatment and 26 untreated leprosy patients (UL) without reaction.

the 22 RR patients (p = 0.01) and 10 of the 13 ENL patients (p = 0.003). Figures 2A, 2B, and 2C show the neopterin levels in serially collected samples from patients with and without development of reactions. Figure 1A shows that the majority of leprosy patients who remained free of reaction during an 18 month follow up period (n = 14) showed little change in neopterin values. Notable exceptions occurred in 2 patients (18.4 and 19.7 nmol/L) at 12 months of follow up. Neither developed clinical evidence of reaction. Figure 2B shows neopterin levels in 4 patients that developed a single episode of RR. In 3 patients, elevated levels correlated with the development of RR. Upon Prednison administration, the levels in 3 patients dropped. In the 4th patient, who had already received Prednison for 3 months at the time of sampling, the levels were within normal limits (9.3 nmol/L). This patient received Prednison for 3 more months. Two months after treatment was stopped, the neopterin value rose to 18.5 nmol/L but by 4 months after treatment has stopped, the value was within normal limits and no further RR was noted. Figure 2C shows levels in 2 patients that developed ENL. One patient developed 4 episodes of ENL. In the period when this patient

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>n</th>
<th>mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>26</td>
<td>9.1</td>
<td>7.3</td>
<td>—</td>
</tr>
<tr>
<td>RR</td>
<td>22</td>
<td>14.5</td>
<td>8.7</td>
<td>0.02*</td>
</tr>
<tr>
<td>ENL</td>
<td>13</td>
<td>16.9</td>
<td>13.6</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*ANOVA test
did not receive Prednison therapy, i.e. from 10 days before the second episode of ENL until the day of blood collection at the third episode of ENL, neopterin levels in serum were above the limit of the normal range of 10 nmol/L. The second patient developed 2 episodes of ENL during follow-up and received Prednison therapy for 10 days in the period between the first and second episode of ENL.

DISCUSSION
It is generally accepted that reactional states in leprosy are strongly associated with CMI activation (16). Neopterin, a product of activated macrophages, may in that respect be expected to correlate with the development of reactional states (12). Here, we have shown that leprosy patients in reaction, either RR or ENL, have significantly elevated serum neopterin levels in comparison with patients not in reaction. A longitudinal assessment of 6 patients showed that an increase in neopterin generally paralleled the occurrence of reactions. Although the mean
Neopterin levels as well as the number of patients above the limit of detection (i.e. 10 nmol/L) were both significantly higher in reactional states, there were also patients in reaction with normal neopterin serum levels. This may have been due to the timing of serum collection in relation to the progression of the reaction as well as medication that had already been administered. Corticosteroid therapy would generally be expected to reduce neopterin production (4). A small number of untreated leprosy patients without clinical signs of a reactional state at the time of blood collection had neopterin serum levels slightly higher than the 10 nmol/L upper limit. This upper limit is based on neopterin levels in populations with less risk for infections with microorganisms. In some patients, there may have been concomitant conditions that influenced the neopterin level. 

Longitudinal measurements in patients with and without reactions provided further insight into the value of neopterin levels. Neopterin levels clearly paralleled the occurrence of RR and ENL. However, neopterin levels in patients already receiving Prednison therapy were, not unexpectedly, relatively low. In the majority of patients not developing a reactional state, the neopterin levels did not increase above the upper limit of 10 nmol/L. As expected, this study showed that serum neopterin levels are generally increased during the development of reactional states and decline during immunosuppressive treatment. This is in agreement with our previous findings in which we demonstrated that neopterin levels were increased in RR and ENL compared to untreated TT/BT and BL/LL patients suggesting that CMI activation plays a large role in reactional states (17). Here we extended the study to confirm our initial results. However, elevated neopterin levels in a few patients not in reaction illustrate heterogeneity in neopterin production, emphasizing the importance of clinical observations. With this baseline data, we believe that a prospective study in which neopterin levels, alone or in combination with other immunologic markers, should be evaluated as a potential tool for the early detection of reactional states. Such a study might also be useful to determine whether neopterin levels discriminate between RR and a relapse, a distinction that is sometimes difficult.
REFERENCES

5. Shannon EJ and Sandoval F. Thalidomide is agonistic to the synthesis of IL-2 and it can be agonistic or antagonistic to the synthesis of TNF-alpha. Int J Lepr 1995, 63: 654-656.

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