β₂-Microglobulin and Neopterin: Predictive Markers for Human Immunodeficiency Virus Type 1 Infection in Children?

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The value of β₂-microglobulin and neopterin concentrations in serum for early diagnosis of infants born to human immunodeficiency virus type 1 (HIV-1)-infected mothers was assessed. Concentrations of both markers were measured in serum samples from pediatric patients (Centers for Disease Control classifications P0, P1, and P2), as well as in age-matched normal subjects. Both β₂-microglobulin and neopterin were significantly increased in HIV-1-infected symptomatic subjects (P2) compared to controls. Seventy-five percent of asymptomatic patients (P1) also had increased values. On the other hand, a significant overlap in concentrations of both markers in serum was found between controls and P0 patients. Thirty-eight percent of the P0 patients had values comparable to those of the P2 group. Persistently high concentrations of both markers in P0 patients may be indicative of HIV-1 infection.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immunodeficiency syndrome (AIDS). The primary pathogenic process is the progressive depletion of the CD4 (helper-inducer) lymphocyte subset which eventually leads to immune dysfunction (18, 20). Decreased numbers of CD4 T lymphocytes in HIV-1-infected adults are a useful predictor of disease progression to AIDS (6, 10, 11). Other laboratory markers of disease progression include p24 antigenemia (22, 28), increased serum concentrations of β₂-microglobulin (β₂m) (11, 16, 22, 33, 34), and increased neopterin concentrations in serum or urine (3, 11, 14, 15). On the other hand, markers such as soluble interleukin-2 receptors (24, 26), soluble CD8 (1, 27), thymosin (23), and acid-labile interferon (8) are increased in HIV-1-infected subjects but are not useful in differentiating the various stages of the disease.

HIV-1 serology is not a useful test for diagnosis of infection in children who are offspring of infected mothers and younger than 15 months of age, primarily because of passively acquired maternal antibodies (12, 25, 32). The detection rate of p24 antigen in asymptptomatically infected children under 6 months of age was only 25%, and no p24 antigen was found in infected infants tested at birth (4). Formation of immune complexes between p24 antigen and maternally derived antibodies is the probable explanation for the low detection rates. Viral cultures are expensive and laborious and require too much blood to be useful for neonates (17). CD4 T-lymphocyte numbers usually do not decline to abnormally low values in children until advanced stages of the disease are reached (12).

The rate of maternal-child transmission of infection has been estimated at 29 to 73% by various investigators, indicating that 27 to 71% of seropositive infants are not infected (2, 12, 21, 31). To provide early treatment of infection, it is essential to achieve an early diagnosis. In the present study, we investigated the clinical utility of β₂m and neopterin concentrations in serum as early indicators of HIV-1 infection in infants and children born of infected mothers. These two surrogate markers are byproducts of immune activation and cellular activity and have proven predictive value in infected adults.

MATERIALS AND METHODS

Subjects. The study population consisted of 83 HIV-1-seropositive infants and children, aged 2 months to 9 years. Patients were divided into three groups according to the Centers for Disease Control (CDC) surveillance definitions (7). Forty-five patients under the age of 15 months (mean ± standard deviation, 7 ± 3.4 months) with indeterminant HIV-1 status were classified as P0, 16 asymptomatic patients (30.7 ± 20.7 months) were classified as P1, and 22 symptomatic patients (36.3 ± 33.3 months) were classified as P2. Nine of the latter group had Pneumocystis carinii pneumonia, one exhibited progressive neurologic disease, and twelve suffered from lymphocytic interstitial pneumonitis and recurrent bacterial infections. Included in this study were 76 HIV-1-seronegative controls. The control subjects could be subdivided into five age groups: 13 were 2 to 12 months (7 ± 3.4 months), 11 were 13 to 24 months (16.7 ± 3.0 months), 12 were 25 to 36 months (31.6 ± 4.4 months), 20 were 4 to 6 years (62.3 ± 9.1 months), and the remaining 20 were 7 to 9 years (97.1 ± 8.6 months). The mean age of the controls taken as a group was 47.8 ± 34.5 months.

Methods. Sera were obtained from patients and controls and stored at −70°C until being assayed. Peripheral blood was collected in EDTA-containing tubes. Lymphocytes were selectively recovered by using a Ficoll-Hypaque density gradient (5). Percentages of CD4 and CD8 T-lymphocyte subpopulations were quantitated by indirect immunofluorescence after reaction with mouse monoclonal antibodies to T4 and T8 antigens (Coulter Diagnostics, Hialeah, Fl.), followed by washing and reaction with fluorescein-conjugated anti-mouse immunoglobulin serum (Organon Teknika-Cappel, Malvern, Pa.).

Antibody to HIV-1 was detected by enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill.) with a dilution of 1:500. Neopterin was determined by an automated fluorescent assay (Enzyne) with a dilution of 1:25. Immunoglobulin M and E were determined by a latex agglutination assay (Abbott Laboratories, North Chicago, Ill.) with a dilution of 1:50 and 1:10, respectively. All concentrations were expressed as the mean ± standard deviation. Differences between groups were considered significant when the calculated P value was less than 0.05.

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### TABLE 1. β₂m and neopterin concentrations in serum, percentage of CD4 lymphocytes, CD4/CD8 ratio, and p24 antigenemia in control and study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>HIV-1 antibody status</th>
<th>Conc in serum [mean ± SD (no. tested)]</th>
<th>% CD4 lymphocytes [mean ± SD (no. tested)]</th>
<th>CD4/CD8 ratio [mean ± SD (no. tested)]</th>
<th>No. exhibiting p24 antigenemia/ no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Patients</td>
<td>Negative</td>
<td>1.2 ± 0.5 (76)</td>
<td>47.6 ± 5.4 (20)</td>
<td>2.0 ± 0.3 (20)</td>
<td>0/0</td>
</tr>
<tr>
<td>CDC P0</td>
<td>Positive</td>
<td>2.1 ± 1.4 (45)</td>
<td>47.2 ± 7.5 (45)</td>
<td>1.9 ± 0.6 (45)</td>
<td>0/45</td>
</tr>
<tr>
<td>CDC P1</td>
<td>Positive</td>
<td>2.7 ± 0.9 (16)</td>
<td>37.0 ± 8.1 (16)</td>
<td>1.2 ± 0.4 (16)</td>
<td>6/16</td>
</tr>
<tr>
<td>CDC P2</td>
<td>Positive</td>
<td>4.1 ± 1.8 (22)</td>
<td>30.8 ± 7.1 (22)</td>
<td>0.9 ± 0.4 (22)</td>
<td>15/22</td>
</tr>
</tbody>
</table>

* P < 0.001, compared with normal controls (Student's t test).  
* P < 0.001, compared with CDC P1.  
* P < 0.001, compared with CDC P2.

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### RESULTS

Mean serum β₂m and neopterin results are shown in Table 1. All seronegative control subjects regardless of the age ranges had low concentrations of both serum markers (mean, 1.2 ± 0.5 mg/liter and 1.1 ± 0.4 ng/ml for β₂m and neopterin, respectively). In contrast, significantly higher concentrations of both markers were observed in serum samples from seropositive patients. β₂m concentrations progressively increased from 2.1 ± 1.4 mg/liter to 4.1 ± 1.8 mg/liter for CDC groups P0, P1, and P2. A similar trend was observed for neopterin in serum (mean, 1.9 ± 1.3 ng/ml for P0 and 5.5 ± 2.8 ng/ml for P2). The CD4/CD8 ratio and percentage of CD4 cells were determined for all 83 patients and 20 controls. There was no difference between patients in the P0 group and the control subjects. With advanced disease, the mean CD4/CD8 ratio and CD4 percentage significantly decreased from 1.9 ± 0.6 to 0.9 ± 0.4 and 47.2% ± 7.2% to 30.8% ± 7.1%, respectively. HIV-1 antigen was detected by solid-phase antigen capture enzyme-linked immunosorbent assay (Abbott Laboratories). The specificity of positive results was verified by the manufacturer's antigen neutralization assay. A ratio of <0.5 between the optical densities of neutralized and unneutralized sera was considered confirmatory.

β₂m in serum was determined by immunoturbidimetry using antisera to β₂m from Boehringer Mannheim Biochemicals (Indianapolis, Ind.) and calibrators from Sigma Chemical Co., (St. Louis, Mo.) on the Cobas-MIRA Analyzer (Roche Analytical Instruments, Nutley, N.J.) (29). Neopterin in serum was measured by radioimmunoassay (Neopterin-RIA; Henning-Berlin, Berlin, Federal Republic of Germany).

**Statistical analysis.** The unpaired Student's t test and linear regression were used in the statistical analysis. The level of significance was established at P < 0.05.

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![FIG. 1](http://jcm.asm.org/)  
Comparison of β₂m and neopterin concentrations in serum samples from controls and HIV-1-seropositive pediatric patients (CDC classifications P0, P1, and P2).
not detected in serum samples from all infected patients. Only 6 of 16 P1 patients and 15 of 22 P2 patients were antigenemic.

Figure 1 shows the relationship between $\beta_2$m and neopterin concentrations in serum samples from the control and patient groups. Both markers were highly correlated ($r = 0.81, P < 0.001$). Concentrations of $\beta_2$m and neopterin in serum from each study subject are depicted in Fig. 2. There was a significant overlap in concentrations of both markers between serum samples from control patients and serum samples from patients in the P0 group. For $\beta_2$m, 62% (28 of 45) of P0 patients had values below 2 mg/liter whereas the remainder had values comparable to those of the P2 group. Surprisingly, 25% (4 of 16) of patients in the P1 group had values similar to those of the control group. Equivalent findings were obtained for concentrations of neopterin in serum. The specificity and sensitivity of both serum markers were calculated on the assumption that control subjects were uninfected and P1 and P2 patients were infected. Both markers revealed similar specificity and sensitivity ($\beta_2$m: specificity, 95%; sensitivity, 89%; neopterin: specificity, 96%; sensitivity, 86%).

**DISCUSSION**

$\beta_2$m is a subunit of the human leukocyte class I antigen and is present on the surface of all nucleated cells. Under unstimulated conditions, the cellular source for most $\beta_2$m in serum is B lymphocytes. Upon immune-system activation, both T and B lymphocytes actively release $\beta_2$m into the circulation. Neopterin, on the other hand, is produced and released predominantly by macrophages after these cells have been stimulated by gamma interferon. B lymphocytes also produce neopterin, but to a lesser extent. Other lymphokines such as tumor necrosis factor and interleukin-2 enhance production of both markers. Recent evidence showed that inactivated HIV-1 alone directly enhances neopterin production but not $\beta_2$m in an in vitro assay system.
Increased concentrations of soluble CD8 (sCD8) in serum from infected infants.

Results of the present study demonstrate that βm and neopterin values are significantly increased in serum samples from children infected with HIV-1. This finding is consistent with observations made for adults. Our main interest lies in newborn children assigned to the P0 group because of maternal seropositivity. Circulating maternal antibodies may persist for 15 months and hamper serologic evaluation of these children. Thus, the HIV-1 status of these children is uncertain and administration of potentially toxic chemotherapy may be difficult to justify. Other adult markers of infection such as p24 antigenemia and CD4 cell counts are not very informative in newborn children, probably because of the presence of maternal p24 antibody, low numbers of infected cells, and high lymphocyte counts.

If we were to categorize our P0 children according to βm and neopterin concentrations in their serum samples, they could be divided into two groups: one with increased concentrations similar to those found for our P1 and P2 subjects and the other with low values similar to those of our controls. Assuming that increased production of both markers is indicative of immune activation by the virus, persistently low values suggest lack of infection. However, increased concentrations are not necessarily caused by HIV-1 because Epstein-Barr virus, hepatitis B virus, and cytomegalovirus infections can also induce increased production of both markers (13; J. L. Sullivan, B. Hofmann, and J. L. Fahey, Abstr. Fourth Annu. Conf. Clin. Immunol. 1989, abstr. no. 30). It is vital to rule out other infections when confronted with increased βm and neopterin values. Both markers should remain elevated during longitudinal studies if a child is infected with HIV-1.

Recently, polymerase chain reaction (PCR) amplification of HIV-1 proviral DNA in infected lymphocytes was used by several investigators for early diagnosis of infection in infants and children. The sensitivity and specificity of PCR were >90 and 100%, respectively, for infants older than 2 months. However, the sensitivity was only 54% for infants aged 1 to 16 days (9, 19, 32). Since PCR is a relatively new test and cross-contamination has been reported as a problem, it is important to confirm PCR-diagnosed infections with other testing modalities (30). An attractive combination that should improve the efficiency of early diagnosis in infants would be PCR testing of lymphocytes followed by confirmatory testing of serum samples of positive patients for concentrations of βm and/or neopterin.

On the basis of our present findings, βm and neopterin concentrations in serum help discriminate between HIV-1-infected and uninfected children. Determining the clinical usefulness of these markers for early diagnosis of infection in P0 infants will require longitudinal studies of this patient population.

ACKNOWLEDGMENTS

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LITERATURE CITED


Letter to the Editor

Neopterin in Diagnosis of Human Immunodeficiency Virus Infection in Infants

In a recent issue of the Journal (October 1990), Chan et al. estimated that the sensitivity and specificity of neopterin determination in identifying children infected with human immunodeficiency virus (HIV) are 86 and 96%, respectively (1). However, in younger infants neopterin does not appear to be as sensitive an indicator of HIV infection as in older children with more advanced disease.

We determined serum neopterin concentrations by a commercially available radioimmunoassay, IMMUnetest Neopterin (Henning-Berlin GmbH, Berlin, Germany). Sequential samples were obtained from 11 HIV-infected infants aged 0 to 31 months (27 samples) and 11 uninfected infants aged 0 to 30 months born either to HIV-infected mothers (16 samples from 7 infants, all currently older than 15 months) or to HIV-negative mothers (8 samples from 4 infants).

Neopterin concentration was inversely correlated with the age of the patient in infected ($r = -0.38, P < 0.05$) and uninfected and control ($r = -0.53, P < 0.01$) infants (Fig. 1).

The usefulness of neopterin determination in the diagnosis of HIV infection in infants is limited by its poor sensitivity and difficulties in interpreting the results: elevated concentrations might be secondary to any other viral or bacterial infection (2). However, in the absence of another plausible explanation high concentrations should be considered suspicious of HIV infection, whereas concentrations within normal range, below 2.5 to 3 ng/ml (10 to 12 nmol/liter), are noninformative.

REFERENCES


Author’s Reply

In principle, I agree with Rautonen et al. that in very young infants (aged 0 to 3 months) the sensitivity of $\beta_2$-microglobulin and neopterin as predictive markers for human immunodeficiency virus infection and progression might be different from that reported for older children. I cannot, however, draw any conclusion based on the data of Rautonen et al. because of insufficient information provided and the small sample size. In order to adequately address this question, a significantly larger population is needed, especially for the human immunodeficiency virus-negative control group. Contrary to what was reported by Rautonen et al., we did not observe great fluctuations in serum $\beta_2$-microglobulin and neopterin concentrations when sequential samples were tested unless the patient had other opportunistic infections (unpublished data). Infected patients in the Centers for Disease Control P2 category with rapidly progressive disease had increasing concentrations, whereas those with stable disease maintained the same initial levels. In three patients, these markers returned to normal levels shortly before death. As shown in our article, some Centers for Disease Control P1 patients have levels in the normal range, and if the levels remain stable, these patients usually have good prognoses. The spurious results obtained by Rautonen et al. might be due to other infections. It is important to evaluate test results in conjunction with the clinical status of patients at the time the test specimen was obtained. The findings that cord blood samples had high levels of neopterin and that there was no
difference between the study groups were not surprising. Neopterin, being a small-molecular-weight compound, can easily pass through the placenta, and what was detected in cord blood samples could be of maternal origin. Therefore, cord blood might not be the appropriate test specimen for this marker.

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