

Neopterin: A Predictive Marker of Acquired Immune Deficiency Syndrome in Human Immunodeficiency Virus Infection

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Summary: In 79 homosexual men infected with human immunodeficiency virus (HIV), urinary neopterin was significantly higher as compared with 70 HIV-seronegative men in the same cohort ($p < 0.0001$). This highly significant association was found both for naturally occurring oxidized (native) neopterin and for total neopterin (native plus chemically oxidized forms). In prospective follow-up for 18 months, the odds that the acquired immune deficiency syndrome (AIDS) would develop were elevated 25-fold among those whose native neopterin levels were in the highest quartile compared with those with lower neopterin levels, and the corresponding odds ratio for total neopterin was 7.8. Logistic regression analyses indicated that neopterin added useful information to T4-cell count in predicting AIDS onset and that both are statistically significant in the multivariate model. A cross-sectional survey revealed that neopterin levels were correlated with number of receptive anal intercourse partners in the year before HIV seroconversion ($r = 0.60$, $p = 0.0005$). Since neopterin may serve as a marker of monocyte/macrophage activation by soluble factors such as γ -interferon, these data support a growing body of virologic and immunologic evidence that highlights the important role of the monocyte/macrophage in the pathogenesis of AIDS. **Key Words:** Neopterin—Human immunodeficiency virus—Odds ratio—Logistic regression—Macrophage.

The acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV), but the stages of disease progression and mechanisms of pathogenesis require further study. The disease clearly hinges on progressive destruction of HIV-infected T4- ("helper") lympho-

cytes. The incubation period from infection to development of clinical AIDS varies from ~2 to >8 years. The total number of circulating T4-cells (T4 count) and the ratio between helper and suppressor cells are markers of this process (1-3), but other markers may help to elucidate the intervening steps between infection and AIDS, including the role of monocytes and macrophages.

One possible candidate marker is neopterin (6-D-erythro-trihydroxypropylpterin), a low molecular weight compound derived from dihydroneopterin triphosphate, an intermediate in the de novo bio-

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synthesis of tetrahydrobiopterin from GTP. Until 1979, high neopterin concentrations in serum or urine had been noted only in patients with atypical phenylketonuria, a congenital deficiency of phenylalanine hydroxylation resulting from a deficiency of tetrahydrobiopterin. More recently, neopterin was found to be present in high concentrations in supernatants of mixed cultures of human peripheral blood mononuclear cells and of monocytes/macrophages stimulated by γ -interferon (4). Highly elevated neopterin levels have also been reported in clinical conditions involving activation of cellular immunity and in patients undergoing immunostimulatory treatment with α -interferon, γ -interferon, interleukin-2, or tumor necrosis factor (for review see 5).

Antigenic stimulation has been linked to replication of HIV in vitro (6). Some studies have already demonstrated elevated levels of neopterin in HIV-infected subjects (5,7,8). The current study was undertaken to evaluate the predictive value of neopterin for progression to AIDS in HIV-infected subjects in relationship to T4 cell count.

PATIENTS AND METHODS

A cohort of homosexual men, previously described in detail (9-12), was enrolled during the summer of 1982 as consecutive patients of three primary care physicians in Washington, D.C., and Manhattan, New York. Identical procedures and data collection instruments were used at all sites. Patients with AIDS were ineligible. Each subject was reevaluated approximately annually with physical examinations, self-administered questionnaires for clinical conditions and current sexual activities, and blood samples for T-cell subsets and (when they became available) HIV antibodies.

The present study is based on two types of data: (a) a cross-sectional survey of individuals whose neopterin levels were measured between April and August of 1986 and (b) prospective follow-up through December 1987 to determine whether neopterin and/or T4 levels predicted which seropositive individuals surveyed from April to August 1986 would subsequently develop clinical AIDS. At the time of the cross-sectional evaluation, 70 subjects were seronegative; 34 were "seroconverters," i.e., individuals whose dates of seroconversion could be estimated as the midpoint between a previous negative HIV antibody assay and a subsequent positive

HIV antibody assay; and 40 were "prevalent positives," i.e., individuals whose first available HIV antibody assay from serum drawn in 1982 was positive. Five of the prevalent positive subjects had clinical AIDS prior to their 1986 evaluation. The mean duration of infection for seroconverters through June 1986 was 3 years, whereas prevalent seropositive subjects had been infected >4 years on average. The prospective follow-up cohort consisted of those 32 seroconverters and 38 prevalent positives on whom T4 measurements and two types of neopterin measurements (native and total neopterin) were available.

Urinary neopterin levels were measured in two different laboratories by two different applications of high performance liquid chromatography as previously described (13,14). One laboratory measured naturally occurring oxidized neopterin directly (Wachter, Innsbruck, Austria), while another laboratory (Milstien, Bethesda, MD, U.S.A.) oxidized the specimens with manganese dioxide and subsequently measured total neopterin as the naturally and chemically oxidized forms. We refer to these two types of measurements as native neopterin and total neopterin, respectively. Neopterin concentrations were expressed as a ratio to concurrent urinary creatinine concentrations in units of micromoles per mole of urinary creatinine.

HIV antibodies were determined by a commercial enzyme-linked immunosorbent assay (Electronucleonics Inc., Columbia, MD, U.S.A.) with Western blot confirmation (Biotech Inc., Rockville, MD, U.S.A.). T-Cell subsets were measured on frozen-thawed lymphocytes with a fluorescence-activated cell sorter using monoclonal antibodies OKT4 and OKT8 (Ortho Diagnostics Co., Raritan, NJ, U.S.A.) as measures of T-helper and T-suppressor subsets, respectively. The T4 and T8 counts were defined as the total peripheral blood lymphocyte count multiplied by the proportion of mononuclear cells stained by OKT4 or OKT8, respectively.

Cross-sectional associations between neopterin levels and clinical status (HIV seronegatives, seroconverters, prevalent seropositives, and AIDS cases) were tested using nonparametric analysis of variance (Kruskal-Wallis test). Associations between neopterin levels and the numbers of sexual acts and partners with whom specific sex acts had been performed in the previous year were assessed with the Spearman rank-order correlation statistic.

Such analyses were carried out separately for HIV seronegatives, seroconverters, and prevalent positives.

For analysis of prospective follow-up through December 1987, the odds of developing AIDS were calculated for the highest neopterin quartile against the three lower neopterin quartiles and for the lowest quartile of T4 counts against the three higher quartiles of T4 counts, respectively. To compare such odds ratios, 0.5 was added to each cell of the twofold dichotomy, and confidence intervals (CIs) were computed by the method of Woolf (15). Logistic regressions were also carried out to determine the joint effects of T4 count, neopterin, and duration of infection (prevalent seropositives versus seroconverters).

RESULTS

Native and total neopterin values were highly correlated ($r = 0.68$, $p < 0.0001$), but total neopterin was about four times higher than native neopterin. Unless stated otherwise, the following results refer only to native neopterin. In the 149 homosexual men, neopterin levels increased directly in relationship to their clinical status (Fig. 1). Mean \pm SEM neopterin level was 127 ± 9 $\mu\text{mol/mol}$ creatinine among 70 HIV antibody-seronegative subjects (median age 37 years, 86% white) compared with 315 ± 52 $\mu\text{mol/mol}$ creatinine among 34 seroconverters (median age 36 years, 88% white), 380 ± 41 $\mu\text{mol/mol}$ creatinine among 40 individuals who were prevalently seropositive since 1982 (median age 38 years, 85% white), and 759 ± 143 $\mu\text{mol/mol}$ creatinine among 5 AIDS patients (median age 35 years, 80% white). A Kruskal-Wallis analysis indicated that the differences in neopterin levels among these groups were statistically significant ($p = 0.0001$).

Among seroconverters, levels of both native and total neopterin were significantly correlated with the number of receptive anal intercourse partners of the previous year who did not use a condom ($r = 0.60$, $p = 0.0005$ and $r = 0.37$, $p = 0.04$, respectively). Those seroconverters with neopterin levels of <200 $\mu\text{mol/mol}$ creatinine had a mean of 0.4 ± 0.2 (SEM) partners compared with 2.6 ± 0.8 partners for those seroconverters with levels of ≥ 200 $\mu\text{mol/mol}$ creatinine. This correlation was not detected in HIV-seronegative or prevalently positive subjects. There was also no statistically significant

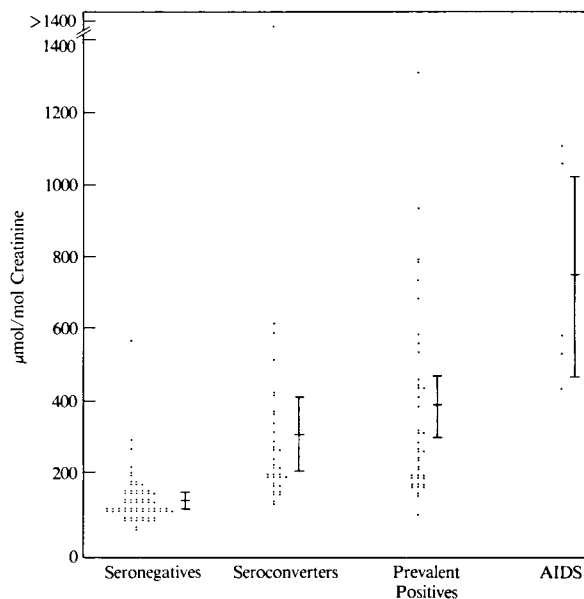


FIG. 1. Urinary native neopterin level by HIV status (70 seronegatives, 34 seropositives, 40 prevalent positives, 5 patients with AIDS) at the time the urine sample was taken. Neopterin values are expressed as $\mu\text{mol/mol}$ creatinine. Vertical bars symbolize means \pm SEM. The null hypothesis that neopterin levels were from identical populations was rejected (Kruskal-Wallis test, $p = 0.0001$).

correlation in any of the three groups between neopterin level and number of partners who used condoms.

Twelve (four seroconverters and eight prevalently positives) of 70 seropositive subjects on whom data were complete (neopterin determined in both laboratories and T4 count available) developed AIDS during a median follow-up of 18 months after neopterin testing. Eight had *Pneumocystis carinii* pneumonia (PCP), 3 had other opportunistic infections (OIs), and 1 had both PCP and a later OI. None developed Kaposi's sarcoma during the interval. Ten of 12 developed AIDS >2 months after the neopterin specimen.

The odds ratio of AIDS was markedly elevated (Table 1) for subjects in the highest quartile of neopterin levels compared with the three lower quartiles together (25.0-fold, 95% CI 5.3–118.6 for native neopterin, and 8.7-fold, 95% CI 2.3–32.8 for total neopterin). Likewise, the odds of developing AIDS were 16.1 times higher for those in the lowest quartile of T4 counts compared with the three higher quartiles of T4 counts (95% CI 3.9–67.0; Table 1). Exclusion of two subjects who developed AIDS within 8 weeks after neopterin specimens were ob-

TABLE 1. Neopterin and T4 count as markers of subsequent AIDS in 70 HIV antibody-seropositive subjects

Marker	AIDS cases	Seropositives without AIDS	Odds ratio	95% confidence interval
Native neopterin ^a				
95-188	0	17	1.0	(5.3-118.6)
189-248	2	16		
249-418	0	17		
>418	10	8	25.0	
Total neopterin ^a				
16-773	0	17	1.0	(2.3-32.8)
774-955	2	16		
956-1,423	2	15		
>1,423	8	10	8.7	
T4 count (cell/ μ l)				
>771	1	17	1.0	(3.9-67.0)
594-771	1	16		
409-593	1	17		
76-408	9	8	16.1	

Odds ratios comparing the highest quartile of neopterin with lower quartiles and the lowest quartile of T4 count with higher quartiles were calculated after adding 0.5 to each cell of the twofold dichotomization. Note that owing to small numbers, even adding or subtracting 1 to or from a quartile may result in substantial changes of the corresponding odds ratio, although not in the 95% confidence interval.

^a Micromoles per mole creatinine.

tained produced very similar results (data not shown). Fifty-six percent of persons in the highest quartile of neopterin and 53% of persons in the lowest quartile of T4 counts subsequently developed AIDS. This ratio increased to 89% for subjects with the combination of very high neopterin and very low T4 count.

Logistic regression analyses applied to the 70 members of the follow-up cohort led to the following estimate of the logarithm of the odds of developing AIDS:

$$-1.599 + 0.00708(\text{neopterin}) - 0.00599(\text{T4 count})$$

The standard errors of the coefficients of neopterin and T4 count were 0.00228 and 0.00245, respectively, indicating that both measurements were associated with AIDS outcome. Compared to the model with T4 count alone, the neopterin model added significantly (likelihood ratio test $\chi^2 = 14.7$, $p = 0.001$) to T4 count in predicting AIDS outcome. To control for duration of infection, an indicator variable (1 if prevalent seropositive and 0 if seroconverter) was added to the model, but the estimates above were essentially unchanged.

The logistic model determined a family of parallel lines in the plane of neopterin and T4 measurements. One such line is shown in Fig. 2. The model predicted that subjects with points above this diagonal line would develop AIDS and that subjects with points below this line would not. The line

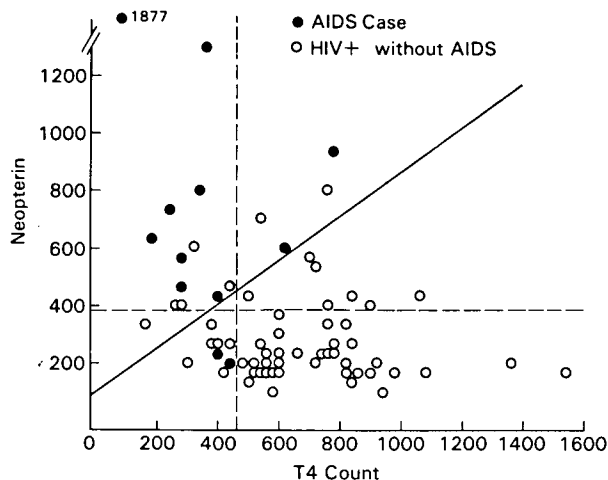


FIG. 2. Filled circles indicate AIDS cases and open circles indicate those who did not develop AIDS among the cohort of 70 seropositives followed prospectively. The linear discriminant given by the equation native neopterin = 100 + 0.846 (T4 count) was obtained by adjusting the intercept in the logistic regression equation in the text. Points above this diagonal line are positive and points below this line are negative. The dashed vertical and horizontal lines separate those subjects who developed AIDS from those who did not, utilizing only data on T4 count and only data on neopterin, respectively, at the same level of sensitivity. Thereby, specificity decreases from 88 to 81 and 78%, respectively.

shown correctly predicted 10/12, or 83%, of the AIDS cases (sensitivity), with a false-positive rate of 7/58, or 12% (specificity = 88%). To estimate the value of the combined information, we evaluated the specificity of each assay alone at the same sensitivity level, i.e., 10/12, or 83%. Utilizing only data on T4 count and a vertical (dashed) line to separate those who developed AIDS from those who did not, the false-positive rate increased to 11/58, or 19% (specificity = 81%). Likewise, using only data on neopterin and a horizontal (dashed) line, the false-positive rate was 13/58, or 22% (specificity 78%). Though the data are sparse, these graphical procedures confirm that a combination of T4 count and neopterin offered better discrimination than either test alone.

DISCUSSION

HIV infects not only T4-lymphocytes, but also cells of monocyte/macrophage lineage that express the CD4 surface molecule (16-19). Moreover, monocytes may be initial targets of HIV (16,20) and may serve as an HIV reservoir that is relatively resistant to HIV-induced cytotoxicity. Antigens ap-

pear to increase the susceptibility to productive HIV infection and to stimulate HIV replication in vitro (6,21,22). In addition, macrophages activated by antigen-stimulated T-lymphocytes release large amounts of neopterin as a product of GTP (4).

In this study, there was a close correlation between neopterin levels measured before and after oxidation of reduced forms, indicating an almost constant relationship between these derivatives. Cross-sectional data demonstrated increasing levels of neopterin with increasing duration of infection or degree of immune impairment (Fig. 1). Furthermore, subjects with very high levels of neopterin were at increased risk of developing AIDS prospectively (Table 1). Moreover, although the data are sparse, the combination of measurements of neopterin and T4 counts predicted AIDS better than either neopterin or T4 count alone (Fig. 2). Exclusion of the two subjects with early AIDS onset after neopterin specimens from prospective analysis revealed similar results. Therefore, high neopterin levels in HIV-seropositive subjects could not be explained by the assumption that these subjects had already developed AIDS.

It has been suggested that exposure to foreign antigens from receptive anal intercourse may result in increased neopterin production (23). Although we confirmed this association among seroconverters, no such association was found among HIV-seronegative or prevalent seropositive members of the cohort. It is possible that this reflects the less frequent use of condoms by HIV seroconverters. Neopterin level was unrelated to anal intercourse with condoms, suggesting that the association was between neopterin level and actual insemination by multiple partners. The difference between seroconverters and prevalent positives remains unexplained but may reflect pathogenetic mechanisms in HIV infection. Further long-term follow-up studies are needed to elucidate factors that promote neopterin formation and to control more precisely for duration of infection and clinical status.

AIDS risk was associated independently with number of T4-lymphocytes and neopterin level, suggesting that neopterin is not simply a marker of dying T4-cells. Based on our own and others' data, we postulate the following model: Neopterin is a marker of specific monocyte/macrophage activation. Antigenic stimulation may cause release of γ -interferon and/or other activators of monocytes. The recent finding of an HIV-1-specific production of γ -interferon by mononuclear leukocyte cultures

during early HIV-1 infection is consistent with this assumption (24). In addition, HLA-associated semen in receptive anal intercourse contains foreign antigens that may lead to a powerful immune stimulation in the HIV-infected host. HIV replication in the monocyte reservoir may promote the further spread of the virus, progressive infection of T-lymphocytes, and destruction of cellular immunity that results in AIDS. Thus, the epidemiologic observations of this study support a growing body of virologic and immunologic laboratory data that highlight the important role of the monocyte/macrophage in the pathogenesis of AIDS. Further study focused on the in vitro effects of specific antigens on neopterin production and intermediate steps may help to clarify the pathogenetic mechanisms and provide guidance for intervention.

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