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Immunologic Markers of Progression to Acquired Immunodeficiency Syndrome are Time-Dependent and Illness-Specific

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Since prevalent cohorts may be biased by the duration of human immunodeficiency virus (HIV) infection (onset bias), it is useful to assess the potential predictive value of markers in incident cohorts of HIV-positive subjects for whom the date of seroconversion is known or can reliably be estimated. Of 131 homosexual men with HIV-1 seroconversion from New York City and Washington, DC, who were evaluated annually beginning in 1982, 60 developed acquired immunodeficiency syndrome (AIDS) by the end of 1989. The prognostic significance of immunologic markers (proportion of $CD4^+$ T-lymphocytes, neopterin, β_2-microglobulin, serum interferon, and anti-p24 antibody) and of a virologic marker (HIV p24 antigen) was determined using measurements made at defined time intervals after the known or estimated date of HIV seroconversion. When measurements made 3 years after seroconversion were used, all markers except anti-p24 antibody were found to be significant estimators of AIDS risk in univariate analyses. In multivariate Cox regression modeling, the maximum information was obtained by including neopterin, interferon, and the $CD4^+$ T-lymphocyte proportion. The predictive value of markers after HIV seroconversion could change considerably from one interval to another. Elevated levels of β_2-microglobulin and neopterin significantly predicted the development of Kaposi's sarcoma. These two markers were highly correlated ($r = 0.74$). The authors conclude that immunologic markers can be important for an HIV staging system for estimating prognosis and facilitating early therapeutic intervention in HIV-positive patients. *Am J Epidemiol* 1992;136:71–80.

beta 2-microglobulin; HIV antibodies; HIV antigens; HIV-1; interferons; proportional hazards models; pteridines; T4 lymphocytes

The risk of developing acquired immunodeficiency syndrome (AIDS) among persons infected by the human immunodeficiency virus (HIV) can be estimated by a variety of immunologic and virologic markers, such as the $CD4^+$ T-lymphocyte count, urinary or serum neopterin, β_2-microglobulin, interferon, soluble interleukin-2 recep-

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Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; IU, international units; β, regression coefficient.

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tors, presence of HIV p24 antigen, and antibodies to p24 or gp120 (1–7). We have recently shown that the levels of some immunologic markers may significantly predict the risk of developing AIDS as early as 5 years before diagnosis (8). To assess the absolute risk of the development of AIDS in an HIV-1 seropositive individual, we prospectively evaluated immunologic and virologic markers of AIDS in HIV-positive homosexual men using actuarial methods. As studies of prevalently seropositive subjects are potentially biased because the duration of HIV-infection is unknown (onset bias) (9), markers were analyzed in relation to the known or estimated time of HIV seroconversion.

MATERIALS AND METHODS

Subjects and study design

Of 131 HIV-1 seropositive homosexual men evaluated approximately annually since 1982 (1), 60 had developed AIDS by the end of 1989. A seroconversion date, defined as the midpoint between the last negative and first positive sample, was available for 47 of the 131 infected men. For the 84 men already infected at the onset of the study, seroconversion dates were estimated using the method of backcalculation to develop regional norms. The average date of seroconversion for men in New York who were seropositive in 1982 was probably June 1980; for those in Washington, DC, it was probably June 1981 (10). To check the reliability of this approach, the annual and cumulative AIDS incidence rates using these imputed seroconversion dates were examined and found to be very similar to those of subjects in the same cohort with midpoint seroconversion dates (8).

Laboratory assays

HIV-1 antibodies were determined by a commercial enzyme-linked immunosorbent assay (Electronucleonics Inc., Columbia, MD), and reactive samples were confirmed by Western blot analysis (Biotech Inc., Rockville, MD). The relative proportion of $CD4^+$ T-lymphocytes was measured in lymphocytes that had been frozen and thawed using a fluorescence-activated cell sorter and OKT4 monoclonal antibodies (Ortho Diagnostics Co., Raritan, NJ). Serum neopterin was retrospectively measured by radioimmunoassay (Neopterin RIAcid, Henning-Berlin, Berlin, Germany), and β_2-microglobulin was measured in serum using double-antibody radioimmunoassay (β_2-micro RIA, Pharmacia, Uppsala, Sweden). Serum interferon was measured in 96-well microtiter plates with the use of human FS-4 fibroblasts; a challenge was performed with the encephalomyocarditis virus, as described elsewhere (11). A level of >4 international units (IU) was considered positive. HIV-1 p24 antigen was determined with a commercial enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). For confirmation of antigen positivity, an HIV-1 p24 neutralization test was performed. Anti-p24 antibodies were also determined using an enzyme-linked immunosorbent assay (Abbott Laboratories). For the determination of anti-p24 antibody titers, an anti-p24 quantitation assay (Abbott Laboratories) was used in accordance with the manufacturer's specifications. Potentially prognostic laboratory markers were measured sequentially at defined time intervals after HIV seroconversion. These intervals were defined in years (±6 months) from the date of seroconversion.

Statistical analysis

The method of Kaplan and Meier (12) was used to estimate AIDS-free survival. For this analysis, the markers were divided into approximate terciles, or analyzed in a dichotomous fashion with the categories absent or present. For comparison of Kaplan-Meier curves, log-rank statistics were calculated. Cox's proportional hazards model (13) was used to assess the predictive value of the covariates singly and in combination. Generally, the covariates were treated as continuous variables—$CD4^+$ T-lymphocyte proportion, neopterin, β_2-microglobulin, and anti-p24 antibodies—or as categorical vari-

ables—HIV p24 antigen (absent/present) and interferon (absent/present). Interferon was coded as follows: code 0 (absent), <4 IU; codes 1–5 (present)—code 1, 4 to <8 IU; code 2, 8 to <12 IU; code 3, 12 to <20 IU; code 4, 20 to <50 IU; and code 5, >50 IU. A stepwise procedure was performed to determine the relative importance of the covariates in predicting the progression to AIDS. Since the proportion of $CD4^+$ T-lymphocytes has been well established as a predictive marker of AIDS, all bivariate and multivariate models included this variable.

Comparison and choice of models were based on their 2 log-likelihood estimates. For example, first, all possible univariate models were analyzed; second, all bivariate combinations including the proportion of $CD4^+$ T-lymphocytes at any rate were analyzed; then, third, the best bivariate model according to the 2 log-likelihood estimates was used as the basis for multivariate models with three combinations of markers. More elaborate models were not used, because the full model containing all possible variables did not improve the 2 log-likelihood estimate substantially above the level of three variables. These analyses were performed prospectively to estimate the relative hazard of AIDS following measurements taken at defined time intervals after HIV seroconversion. The time intervals 3 years after seroconversion, 4 years after seroconversion, 5 years after seroconversion, and after the level of $CD4^+$ lymphocytes had dropped below 500/ml were chosen because there were sufficient complete sets of marker measurements for these intervals. Spearman rank-order correlation was used for calculation of correlation coefficients between markers.

RESULTS

AIDS-free survival

Complete sets of marker data were available for 81 of the 131 HIV-seropositive homosexual men; 36 of these 81 men developed AIDS during the observation period. Only data for the 81 subjects with complete sets of marker data were used in the Kaplan-Meier analysis. As illustrated in Kaplan-Meier plots (figures 1-6), AIDS-free survival 3 years after HIV seroconversion differed at various levels for immunologic and virologic markers. This was significant for the $CD4^+$ T-lymphocyte proportion, neopterin, β_2-microglobulin, interferon, and HIV p24 antigen, but not for anti-p24 antibody ($p = 0.06$). For example, at 1,525 days (4.18 years), the AIDS-free survival in subjects with a $CD4^+$ T-lymphocyte proportion of <25 percent was zero; it was approximately 60 percent in subjects whose $CD4^+$ T-lymphocyte proportion was between 25 and 35 percent, and 70 percent for those whose $CD4^+$ T-lymphocyte proportion was >35 percent. At this same time interval, 77 percent of the subjects with neopterin levels of <10 nmol/liter were AIDS-free, compared with 56 percent of those with neopterin lev-

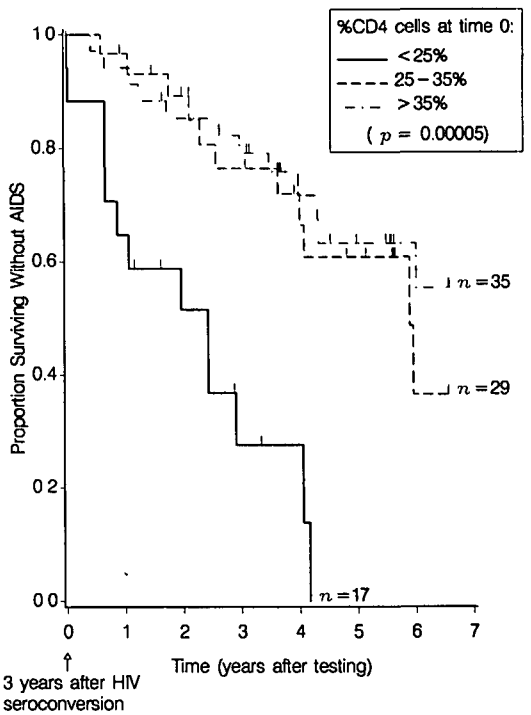

FIGURE 1. Kaplan-Meier plots of the proportion of subjects without AIDS by approximate tercile of the $CD4^+$ T-lymphocyte proportion 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

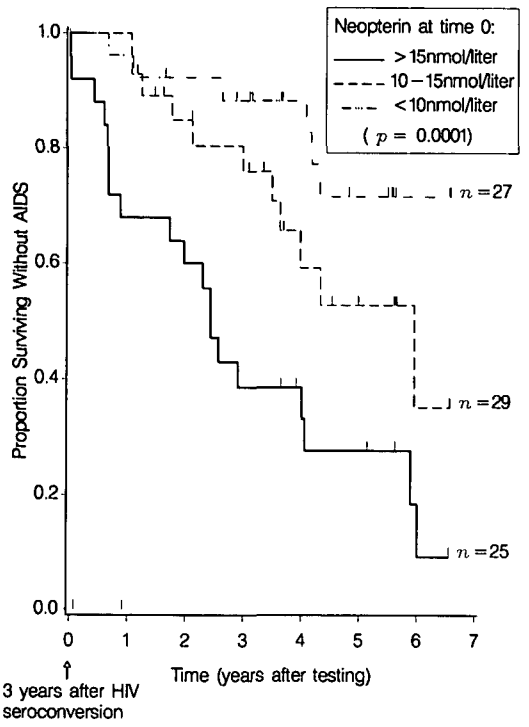

FIGURE 2. Kaplan-Meier plots of the proportion of subjects without AIDS by approximate tercile of serum neopterin 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

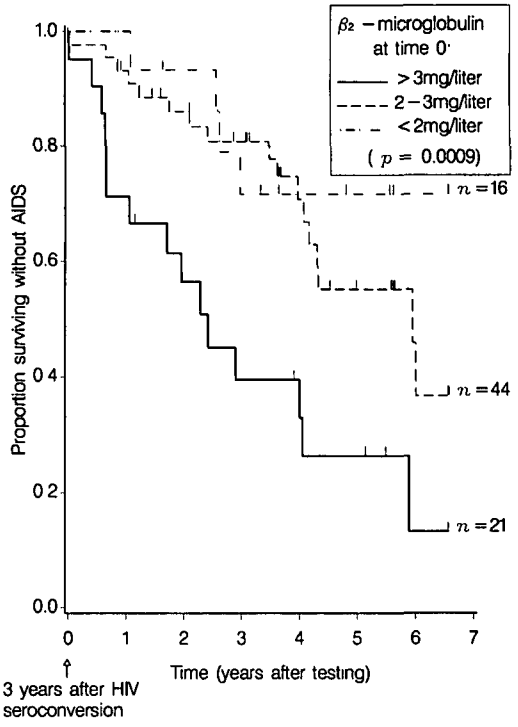

FIGURE 3. Kaplan-Meier plots of the proportion of subjects without AIDS by approximate tercile of β_2-microglobulin 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

els of 10–15 nmol/liter and only 26 percent of those with levels of >15 nmol/liter. With respect to serum interferon, AIDS-free survival was 0 after 2,154 days (5.9 years) when interferon was present, compared with about 53 percent when interferon had been absent. The interval of 3 years after seroconversion was chosen because the marker measurements were most complete at this interval.

Proportional hazards models for AIDS

Table 1 shows the results from univariate, bivariate, and multivariate analyses using proportional hazards models to assess the predictive value of the markers singly and in combination. Again, all markers except anti-p24 antibody proved to be single significant AIDS predictors. Bivariate and multivariate combinations are only displayed for models that include the proportion of $CD4^+$ T-lymphocytes, because the predictive potential of this marker is beyond doubt. As illustrated by the 2 log-likelihood estimates, the model incorporating neopterin, interferon, and the $CD4^+$ T-lymphocyte proportion (2 log-likelihood = −239.65) was the best predictive model, resulting in the following hazard function equation:

$$\lambda(t) = \lambda_0(t) \exp(0.0900 \text{ neopterin} + 0.4462 \text{ interferon} - 0.0221 \text{ CD4\%}),$$

where CD4% equals the relative proportion of $CD4^+$ T-lymphocytes. Compared with this model, the full model with all markers included did not add significant information: (difference in 2 log-likelihood: −238.45 + 239.65 = 1.2, $p > 0.2$).

In addition, we evaluated the prognostic potential of the markers as time-dependent variables. In other words, did the prognostic value of a given marker vary with measurements of this marker at different times in

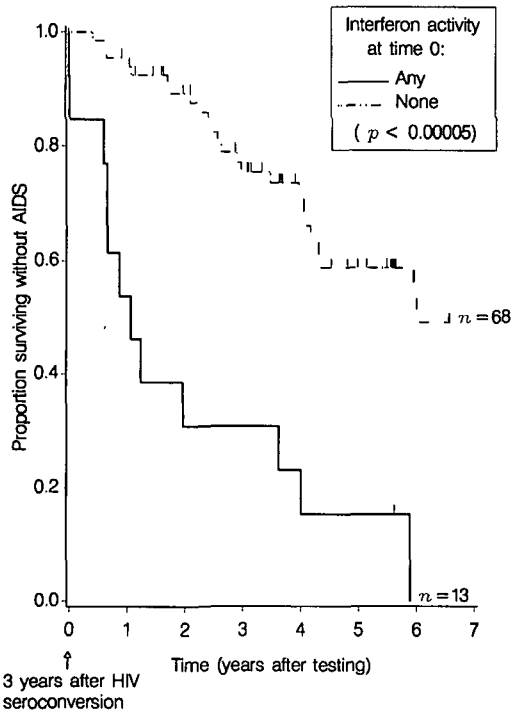

FIGURE 4. Kaplan-Meier plots of the proportion of subjects without AIDS by the presence or absence of interferon 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

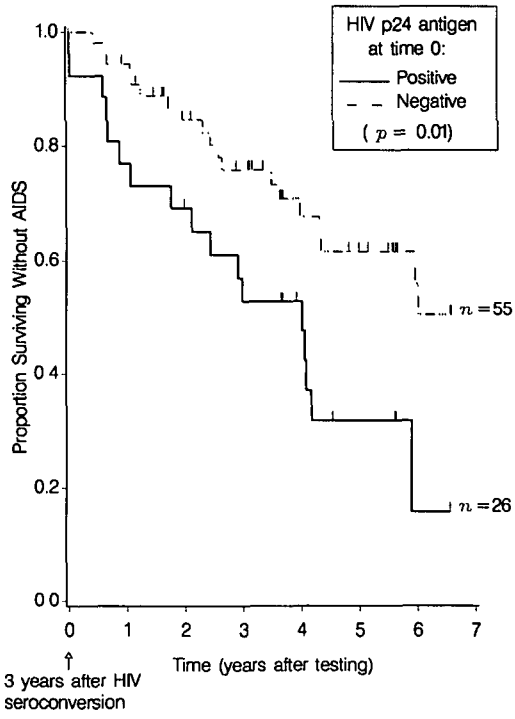

FIGURE 5. Kaplan-Meier plots of the proportion of subjects without AIDS by the presence or absence of HIV p24 antigen 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

the course of HIV disease? Therefore, we analyzed proportional hazards models for AIDS using as starting points measurements made at different times after HIV seroconversion and after $CD4^+$ T-lymphocyte counts had dropped below 500/ml. Table 2 displays beta coefficients and standard errors for the model incorporating the $CD4^+$ T-lymphocyte proportion, neopterin, and interferon at these various other intervals. One sees that the predictive value of markers can change considerably from one time interval to another. Prognostic models at different times after HIV seroconversion could, therefore, contain different combinations of markers (data not shown).

Prognostic markers for the development of Kaposi's sarcoma

In order to be specific for a given category of AIDS illness, we analyzed prognostic markers in the subgroup of subjects whose only initial manifestation of AIDS was Kaposi's sarcoma as compared with markers in HIV-positive controls who did not develop AIDS during the study period. Since the data were sparse (11 cases), this analysis was performed in a univariate fashion only (table 3). Despite the small number of subjects, neopterin and β_2-microglobulin were both found to be significant predictors of Kaposi's sarcoma ($p = 0.0009$ and $p = 0.044$, respectively). Since opportunistic infections were the most frequent initial manifestation of AIDS (43 cases), the predictive markers for this illness category were very similar to those presented above for the entire group of AIDS patients.

Correlations between markers

Table 4 shows the correlations among the six markers from all measurements taken 3

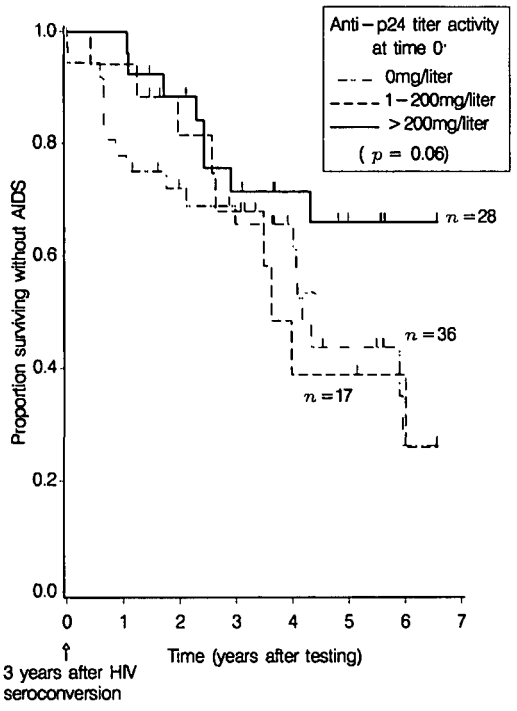

FIGURE 6. Kaplan-Meier plots of the proportion of subjects without AIDS by approximate tercile of anti-HIV p24 antibody 3 years after seroconversion. (Key: *n*, number of HIV-seropositive subjects without AIDS at time 0; vertical tick marks, subjects without AIDS who were censored; the *p* value for the difference in disease-free survival was derived from the log-rank test).

years after HIV seroconversion (Spearman rank-order correlation coefficients). In addition to anti-p24 antibody and p24 antigen ($r = -0.59$, $p = 0.0001$), β_2-microglobulin and neopterin were also very highly correlated ($r = 0.74$, $p = 0.0001$).

DISCUSSION

We analyzed the potential of immunologic and virologic markers to predict AIDS in relation to HIV seroconversion, with measurements taken at different time intervals after seroconversion and before the onset of AIDS. When measurements made 3 years after HIV seroconversion were used, various immunologic markers (the proportion of $CD4^+$ T-lymphocytes, neopterin, β_2-microglobulin, and interferon) and p24 antigen were significant predictors of AIDS for the next 6 years, that is, up to the ninth year after HIV infection (figures 1–6). A proportional hazards model incorporating neopterin, interferon and the $CD4^+$ T-lymphocyte proportion had the best predictive value for progression of HIV-positive homosexual men to AIDS (table 1).

It was interesting that the prognostic value of markers could vary considerably during HIV disease (table 2). A marker may thus be a good predictor shortly after seroconversion but fail to estimate the subsequent risk of AIDS accurately when measured late in the course of HIV infection, or vice versa. Since the variation of estimates may partly be due to the sparse number of subjects, further studies with larger numbers are needed to obtain more precise estimates of the subsequent risk of AIDS for different intervals, and to characterize the exact pattern over time. Nonetheless, our findings clearly indicate that the predictive value of the markers is time-dependent. The following hazard function equation takes this time-dependence into account:

$$\lambda(t) = \lambda_0(t) \exp[\beta_1(t)X_1 + \beta_2(t)X_2 + \ldots + \beta_i(t)X_i].$$

It should also be noted that longer time since HIV seroconversion was itself predictive of AIDS (data not shown), even in the full model.

We are aware that the date of HIV seroconversion of an HIV-seropositive individual is usually not known in the clinical setting. Therefore, one may conclude that the time-dependence with respect to the predictive value of AIDS progression markers is only of theoretical interest and of no practical importance. However, an approximate seroconversion date can be estimated for many persons with prevalent infections based on the history of specific exposures (e.g., rare anal intercourse, contact with a prostitute, or condom failure) or symptoms compatible with the HIV seroconversion syndrome that may occur in one quarter of all incident HIV infections.

In addition, one must be specific about the AIDS illness category: Markers of im-

TABLE 1. Analyses of potential markers of progression to AIDS* using proportional hazards models† with measurements made 3 years after HIV* seroconversion

Proportional hazards model	β‡	SE§	p value	2 Log-likelihood
Null model				−277.16
Univariate models				
CD4+ T-lymphocytes (%)	−0.0519	0.0195	0.008	−268.23
Neopterin (nmol/liter)	0.1145	0.0240	0.0001	−250.01
β_2-Microglobulin (mg/liter)	0.7225	0.1687	0.0001	−262.11
Interferon (codes 0–5)	0.8172	0.1453	0.0001	−253.86
HIV p24 antigen (0–1)	0.8672	0.3384	0.01	−270.85
Anti-p24 antibody (mg/liter)	−0.00002	0.00002	0.39	−274.31
Bivariate models				
CD4+ T-lymphocytes (%)	−0.0268	0.0137	0.05	−244.25
Neopterin (nmol/liter)	0.1185	0.0278	0.0001	
CD4+ T-lymphocytes (%)	−0.0304	0.0185	0.099	−258.49
β_2-Microglobulin (mg/liter)	0.6259	0.1827	0.0006	
CD4+ T-lymphocytes (%)	−0.0313	0.0184	0.089	−250.28
Interferon (codes 0–5)	0.7214	0.1536	0.0001	
CD4+ T-lymphocytes (%)	−0.0399	0.0210	0.057	−266.42
HIV p24 antigen (0–1)	0.5077	0.3757	0.18	
CD4+ T-lymphocytes (%)	−0.0487	0.0194	0.012	−266.30
Anti-p24 antibody (mg/liter)	−0.00002	0.00002	0.42	
Multivariate models				
CD4+ T-lymphocytes (%)	−0.0250	0.0139	0.072	−243.83
Neopterin (nmol/liter)	0.1053	0.0317	0.0009	
β_2-Microglobulin (mg/liter)	0.1584	0.2368	0.50	
CD4+ T-lymphocytes (%)	−0.0221	0.0142	0.12	−239.65
Neopterin (nmol/liter)	0.0900	0.0314	0.004	
Interferon (codes 0–5)	0.4462	0.1902	0.019	
CD4+ T-lymphocytes (%)	−0.0261	0.0146	0.073	−244.24
Neopterin (nmol/liter)	0.1173	0.0293	0.0001	
HIV p24 antigen (0–1)	0.0512	0.4019	0.90	
CD4+ T-lymphocytes (%)	−0.0258	0.0135	0.055	−243.01
Neopterin (nmol/liter)	0.1157	0.0274	0.0001	
Anti-p24 antibody (mg/liter)	−0.00001	0.00002	0.49	
Full model				
CD4+ T-lymphocytes (%)	−0.0214	0.0150	0.15	−238.45
Neopterin (nmol/liter)	0.0843	0.0365	0.021	
β_2-Microglobulin (mg/liter)	0.0615	0.2595	0.81	
Interferon (codes 0–5)	0.4295	0.1989	0.031	
HIV p24 antigen (0–1)	0.0674	0.4213	0.87	
Anti-p24 antibody (mg/liter)	−0.00001	0.00002	0.50	

* AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

† Note that models were built hierarchically upon the CD4+ T-lymphocyte proportion because the predictive value of this marker is well established.

‡ The regression coefficients refer to the units in parentheses next to the variables. For example, for the univariate model "CD4+ T-lymphocytes," the coefficient represents a negative change of 0.0519 related to a unit percentage change in CD4+ T-lymphocytes. For "Interferon (codes 0–5)," the coefficient is related to a change of one code, e.g., from 2 to 3, etc.

§ SE, standard error of the regression coefficient.

mune system stimulation, neopterin and β_2-microglobulin, were significant predictors for Kaposi's sarcoma in our univariate analyses (table 3). This has already been suggested by a descriptive analysis of our cohort (14) and by another study (15). However,

TABLE 2. Results of the proportional hazards model incorporating the CD4⁺ T-lymphocyte proportion, neopterin, and interferon at different times of HIV† disease

Time of HIV disease	β‡	SE§	p value
3 years after seroconversion (n = 81)			
CD4⁺ T-lymphocytes (%)	−0.0221	0.0142	0.12
Neopterin (nmol/liter)	0.0900	0.0314	0.004
Interferon (codes 0–5)	0.4462	0.1902	0.019
4 years after seroconversion (n = 65)			
CD4⁺ T-lymphocytes (%)	−0.0593*	0.0235	0.01
Neopterin (nmol/liter)	0.0539	0.0397	0.17
Interferon (codes 0–5)	0.2457	0.1857	0.19
5 years after seroconversion (n = 49)			
CD4⁺ T-lymphocytes (%)	−0.0594	0.0337	0.078
Neopterin (nmol/liter)	0.0800	0.0313	0.01
Interferon (codes 0–5)	0.5178	0.1914	0.007
<500 CD4⁺ T-lymphocytes/μl (n = 76)			
CD4⁺ T-lymphocytes (%)	−0.0920**	0.0244	0.0002
Neopterin (nmol/liter)	0.0487	0.0249	0.05
Interferon (codes 0–5)	0.0597***	0.1332	0.65

*p = 0.09, **p = 0.01, ***p = 0.05, if compared with the regression coefficient of the marker for the interval 3 years after seroconversion.
† HIV, human immunodeficiency virus.
‡ The regression coefficients refer to the units in parentheses next to the variables.
§ SE, standard error of the regression coefficient.

since the number of subjects was sparse, other markers, such as the CD4⁺ T-lymphocyte proportion, may also become significant predictors of Kaposi's sarcoma with a larger number of subjects.

The results reported here are in agreement with our earlier findings that significant changes in the CD4⁺ T-lymphocyte proportion, neopterin, and β_2-microglobulin occurred within 1–2 years after seroconversion in subjects who developed AIDS during the study period (8). They are also in agreement with our report from early in the HIV epidemic that serum interferon activity predicted AIDS in subjects with hemophilia (16) and with reports from other investigators who found elevated neopterin and β_2-microglobulin levels early in HIV infection (17–19). Recently, we were able to demonstrate that the risk of AIDS can be predicted with immunologic markers as early as even 1 year after HIV seroconversion (20), which supports the observation that events occurring soon after or around seroconversion influence the progression to AIDS (21). Understanding the pathogenesis of immunologically severe HIV seroconversions may provide therapeutic opportunities to reduce the risk of AIDS further.

In our analysis, immunologic markers were superior to HIV p24 antigen in their potential to predict progression to AIDS. Neopterin is a compound of low molecular weight derived from dihydroneopterintriphosphate, an intermediate in the de novo synthesis of tetrahydrobiopterin from guanosine triphosphate. Neopterin was found to be present in high concentrations in supernatants of mixed cultures of mononuclear cells from human peripheral blood and of monocytes and macrophages stimulated by γ-interferon (22). β_2-Microglobulin is a protein of low molecular weight that is present on the surface of all nucleated cells as the constant subunit of class I histocompatibility antigens (23). Most of the interferon activity that we detected was α-interferon, although complete formal characterization has not been done (7). Mononuclear cells, including monocytes, natural killer cells, and B-lymphocytes, produce α-interferon in response to infections with viruses, other intracellular organisms, and other inducers (24). Although the specific inducer or induc-

TABLE 3. Univariate analyses of markers in HIV*-seropositive subjects whose only initial manifestation of AIDS* was Kaposi's sarcoma

	β†	SE‡	p value	2 Log-likelihood
Null model				−64.70
Univariate models				
$CD4^+$ T-lymphocytes (%)	0.0105	0.0404	0.8	−64.63
Neopterin (nmol/liter)	0.2456	0.0738	0.0009	−53.55
β_2-Microglobulin (mg/liter)	1.2035	0.5967	0.044	−60.72
Interferon (codes 0–5)	0.0204	0.4973	0.97	−64.70
HIV p24 antigen (0–1)	−0.6197	1.0698	0.56	−64.31
Anti-p24 antibody (mg/liter)		(no convergence)		

* HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.
† The regression coefficients refer to the units in parentheses next to the variables.
‡ SE, standard error of the regression coefficient.

TABLE 4. Spearman rank order correlations of markers of progression to AIDS* based on measurements made 3 years after HIV* seroconversion

	CD4%†	Neopterin	β_2-Microglobulin	Interferon	p24 Antigen
Neopterin	−0.48				
β_2-Microglobulin	−0.48	0.74			
Interferon	−0.35	0.42	0.38		
p24 Antigen	−0.45	0.41	0.33	0.29	
Anti-p24 antibody	0.28	−0.31	−0.28	−0.23	−0.59

* AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.
† Proportion of $CD4^+$ T-lymphocytes

ers of neopterin, β_2-microglobulin, and interferon have not been defined, these serum proteins are products of immunostimulation. Their roles in elucidating the pathogenesis of AIDS have not yet been precisely defined.

The Centers for Disease Control classification system provides a clinical basis for case definition (25) but does not characterize the degree of illness, pathobiology, or prognosis. On the other hand, the "Walter Reed staging classification for HTLV-III/LAV infection" (26) characterizes the progressive nature of the pathobiology and is prognostic. However, the Walter-Reed system requires not only a physical examination and T-cell testing but also delayed hypersensitivity testing with a battery of skin test antigens that must be read by trained personnel 48 hours after application. Our data suggest that a combination of immunologic markers, including the $CD4^+$ T-lymphocyte proportion, neopterin or β_2-microglobulin, and serum interferon might provide a convenient alternative method of assessing prognosis in HIV-positive individuals. These serological tests are relatively inexpensive by Western medical standards, with costs in the range of a standard HIV antibody test. Since neopterin and β_2-microglobulin are very highly correlated (table 4), it may be sufficient to determine only one of these two markers.

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