

Markers solubili di immunoattivazione

Parte 1 e parte 2

Nelle due review seguenti, viene esaminato il valore predittivo dei markers solubili di immunoattivazione. All'inizio sono state inserite alcune conclusioni estrapolate dal testo e tradotte. Ancora una volta il Viral Load, qui posto a confronto ed usato come principale parametro per valutare l'efficacia dei farmaci fino ad oggi, ne esce perdente.

"I tre markers di attivazione del sistema immune qui considerati sono la β 2-microglobulina, la neopterina ed il TNF (Tumor necrosis factor)".

"I ricercatori dello studio (L Ashton et al., "Predictors ...," *AIDS Research and Human Retroviruses* 14, no. 2 (1998): 117-121.) conclusero che nei sieropositivi asintomatici a lungo termine (LTNP), la Beta-2 microglobulina ha una più forte capacità di predizione del declino dei CD4 rispetto il Viral Load".

"I ricercatori dello studio (R Zangerle et al., "Serum HIV-1 RNA Levels ..." *International Archives of Allergy and Immunology* 116 (1998): 228-239.) conclusero che i markers di immunoattivazione correlavano meglio sia con il declino dei CD4 che con la progressione di malattia rispetto al Viral Load".

Sebbene il valore prognostico di una singola, precoce misurazione è limitata, misure seriali dei markers di immunoattivazione come la β -2Microglobulina si sono dimostrate essere molto utili nel monitorare il corso della malattia negli individui HIV infetti. Paragonati al Viral Load, i markers solubili di immunoattivazione mostrano una migliore correlazione con il declino dei CD4 e progressione della malattia.

<http://www.devicelink.com/ivdt/archive/99/01/009.html>

Soluble markers of immune system activation

Part 1: The correlation between HIV-1 infection and β 2-microglobulin

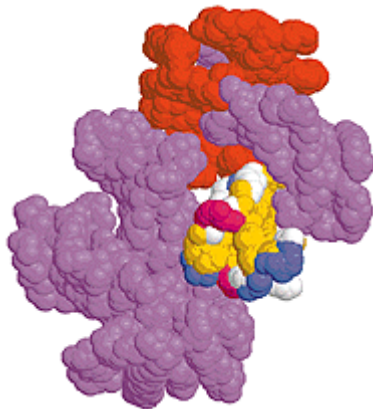
IVD Technology Magazine 1999

Keeping up with the rapid progress of clinical research is essential for manufacturers seeking to create therapy-guiding AIDS assays.

David A. George

Predicting the clinical progression of disease in individuals infected with the human immunodeficiency virus-1 (HIV-1) is often difficult with the routinely used markers of disease progression.¹ (continua)

Historically, CD4 T-cell lymphocyte (CD4) count has been used to monitor the clinical progression of disease, and a strong correlation exists between a low CD4 count and the development of acquired immunodeficiency syndrome (AIDS) and death.²⁻⁶ CD4 count has also been routinely used to classify and assess the risk of HIV-infected patients. In general, patients who maintain a CD4 count of at least 500 cells/ μ l for a prolonged period of time (8 years or longer) are deemed long-term nonprogressors (LTNPs) and are at a reduced risk for the development of AIDS. Those with a count of 200 cells/ μ l or less are at increased risk and are candidates for aggressive prophylactic or antiretroviral therapy.⁷⁻⁹ CD4 count, however, does not always correlate with disease stage—especially in early-stage disease—or with progression to AIDS. Frequently, patients with similar CD4 counts do not follow the same clinical course.^{1,7}



Three-dimensional structure of the class I major histocompatibility complex (purple), β 2-microglobulin (red), and the alpha-3 domain from human histocompatibility leukocyte antigen B2705 (other colors). Illustration Courtesy Glaxo Wellcome Experimental Research

The advent and refinement of assays for HIV-1 viral load (the number of copies of HIV-1 RNA found per milliliter of plasma or serum) have substantially improved the ability to stage HIV-infected individuals and monitor the clinical course of disease. In certain patient populations, however, HIV-1 viral load determinations have not correlated well with the course of disease. Two such populations are the LTNPs described above, which represent approximately 5–8% of HIV-infected patients, and those patients with advanced disease and very low CD4 counts.^{9,10}

As a result, researchers and clinicians are looking to soluble markers of immune system activation to augment the data provided by CD4 and HIV-1 viral load determinations. They hope to find additional prognostic data by which to determine the best course of treatment for an individual—for example, whether or not the patient should begin receiving antiretroviral therapeutic agents. Three immune system activation markers under consideration are β 2-microglobulin (β 2M), neopterin, and tumor necrosis factor receptor type II (TNFR-II). β 2M is the light chain moiety of the class I histocompatibility leukocyte antigen (HLA) complex; neopterin is a product of guanosine triphosphate catabolism and is primarily accounted for in serum by interferon- Γ -

stimulated macrophages; and TNFR-II is a cell-membrane-bound receptor specific for the cytokines TNF- α and lymphotoxin- α and is released into circulation upon proteolytic cleavage of its extracellular component. In many of the studies discussed here the data provided by β 2M, neopterin, and TNFR-II were of approximately equivalent clinical value, and although neopterin and TNFR-II deserve consideration and will be briefly discussed, the remainder of this article will focus on β 2M, for which clinical immunoassays are currently available.

β 2M belongs to the β -globulin family of human plasma proteins. It is bound noncovalently to the heavy chain subunit of the class I HLA complex, and is found on the cell surface of all nucleated cells.¹¹ Overproduction of the HLA complex can cause β 2M to be released into circulation. The lymphocytes are largely responsible for the overproduction of this solubilized β 2M, which is normally filtered out of serum through renal glomeruli and ultimately reabsorbed and catabolized by epithelial cells. Serum elevations of β 2M occur, however, in several clinical conditions such as multiple myeloma, nasopharyngeal carcinoma, and a variety of lymphoproliferative disorders.¹²⁻¹⁵ In addition, β 2M has been studied extensively as a marker of immune system activation in HIV-infected individuals in an effort to identify and monitor those patients at high risk for progressing to AIDS.¹⁶⁻¹⁸

β 2M Correlation with HIV-1 Infection

The association between β 2M and HIV-1 infection is well documented, with β 2M serum levels rising in correlation with disease progression and reaching a peak just before death.¹⁸⁻²² Some early investigations suggested a prognostic role for β 2M, as patients with higher β 2M serum levels tended to develop AIDS sooner than patients with lower levels.^{23,24} Recent studies have confirmed the strong correlation among β 2M, CD4 count, and HIV-1 infection (see Table I).²⁵

CD4 Count (cells/μl)	β2M Serum Level μg/L (mean \pmSD)
<50	4.55, 1.24
50-199	3.82, 0.75
200-500	3.75, 1.10
Healthy controls (>500)	1.31, 0.22

Table I. The strong correlation between β 2M serum levels and CD4 counts in HIV-infected individuals (n = 200).²⁵

However, conflicting results have been reported regarding the prognostic value of β 2M, that is, whether the determination of β 2M serum levels early in the course of HIV-1 infection can be used to predict the time-course of progression to AIDS or death. A recent prospective investigation involving 34 asymptomatic HIV-infected individuals evaluated the prognostic value of β 2M and several viral

markers, including HIV-1 viral load.²⁶ For each subject, all markers were measured upon study entry and every eight weeks thereafter for a period of three years. Those subjects who progressed to AIDS or to an HIV-related disease were placed into the progressor (P) group, and those who remained asymptomatic were placed into the nonprogressor (NP) group. Table II shows that at study entry, β 2M serum levels were higher in the P group than in the NP group, but the difference was not significant enough to predict clinical progression in asymptomatic individuals. Only HIV-1 viral load at study entry showed a significant correlation between the NP and P groups ($p < 0.003$, two-sided Mann-Whitney test).

The values reported for β 2M in this study are discrepant from those reported in Table I, in which the β 2M serum levels of all HIV-infected groups were significantly elevated above normal. Further investigation would likely reveal factors that might have contributed to this discrepancy, such as the small size of the recent study's population, differences in data interpretation, or differences in the β 2M assay methodology (e.g., selection of an appropriate upper reference limit).

A similar but retrospective study evaluated HIV-1 viral load, β 2M, neopterin, and TNFR-II in HIV-infected individuals who were matched by baseline CD4 count, rate of CD4-count decline, and other demographic factors such as age and ethnic origin.¹ The 90 study subjects were categorized into one of three groups—slow, moderate, and rapid progressors—and were evaluated for up to a 10-year period. Samples were obtained and assayed upon study entry (baseline), at six months, and again at the time closest to AIDS onset, death, or at the end of the follow-up period; a total of three samples were evaluated from each subject. For slow and moderate progressors, the third sample was obtained at a visit chronologically closest to that of the clinical progression in the rapid progressor group. At baseline, levels of all markers increased incrementally from the slowest to the most rapid progressor groups, but only HIV-1 viral load and TNFR-II showed a statistically significant difference between each group (see Table III). In addition, univariate and multivariate matched conditional regression models of the baseline data indicated that only HIV-1 RNA and TNFR-II were significant predictors of progression to AIDS in the rapid progressor group.

Marker	Nonprogressors	Progressors
Log HIV-1 viral load	4.30	4.94 ($p < 0.003$)
CD4 count	490	500 (p value not reported)
β 2M	2.55	3.45 (p value not reported)

Table II. Marker values for nonprogressors ($n = 25$) and progressors ($n = 9$) at study entry.²⁴

Marker	Slow	Moderate	Rapid
HIV-1 RNA* (copies/ml)	1683	6816	17,830
TNFR-II* (ng/ml)	3.13	3.64	4.11
CD4 count (cells/ μ l)	625.5	590.5	534
Neopterin (nmol/L)	10.29	10.35	11.17
β 2M (μ g/L)	1.9	2.09	2.52
*Denotes a statistically significant trend across the three progression groups ($p < 0.001$, Friedman's test).			

Table III. Baseline marker values for three progression groups (n = 30 for each group).¹

It is interesting to note that in both of the studies discussed above, β 2M tended to correlate with disease progression, although the increases were not statistically significant. In the prospective study, β 2M was more elevated in the progressor group than in the nonprogressor group. In the retrospective study, in which HIV-1 viral load was significantly predictive of clinical disease progression, β 2M increased across all three progressor groups and correlated significantly with HIV-1 viral load. The authors of the latter study offered no hypothesis to explain why β 2M levels would correlate with HIV-1 viral load but lack statistical significance as a predictor of disease progression. However, it could be that the preclassification of their study subjects by CD4 count and CD4-count decline biased the interpretation of β 2M measurements.

Conclusion

The studies discussed here reveal a distinct correlation between HIV-1 infection and elevated serum levels of β 2M. The question remains, however, just how β 2M and the other markers of immune system activation can best be used in monitoring disease progression or in predicting the prognosis of HIV-infected individuals. These and other issues will be addressed in the [second installment](#) of this article, which appears in the March/April 1999 issue of *IVD Technology*.

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Soluble markers of immune system activation

Part 2: AIDS prognosis and the clinical utility of β 2-microglobulin

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Studies suggest that serial measurements of immune system activation markers could have value for monitoring the progress of HIV disease.

To help determine the best course of treatment for individuals infected with the human immunodeficiency virus (HIV), researchers and clinicians are increasingly looking to soluble markers of immune system activation. The hope is that such markers will provide useful prognostic data to supplement the markers of disease progression already in routine use.

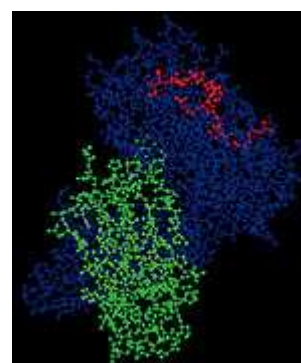
The [first installment](#) of this article outlined the association between β 2-microglobulin (β 2M) and HIV-1 infection, noting that β 2M levels in serum generally increase as the disease progresses. This second installment further investigates the association between β 2M and HIV-1 infection, as well as the relationships among β 2M, the general immune-system response to HIV-1 infection, and HIV-1 viral load.

β 2M versus CD4 Count

Two recent studies investigated β 2M and CD4 T-cell lymphocyte (CD4) count, and explored the relationship between the two and how this relationship may relate to the onset of acquired immunodeficiency syndrome (AIDS) and death.

In a prospective study involving 539 HIV-infected individuals without AIDS, the researchers measured β 2M and CD4 count approximately every 1½ months for a period of 21 months.¹ Data were separately analyzed with respect to disease progression to AIDS or death (see sidebar). Contrary to some of the reports discussed in the first installment of this article, univariate analysis indicated that both β 2M and CD4 count were significantly associated with an increased risk of developing AIDS (see Table I).

Rendering of the class I major histocompatibility complex H-2DD heavy chain (blue), complexed with β 2-microglobulin (green) and an immunodominant peptide, P18-110 (red), from the HIV-1 virus envelope glycoprotein 120.



Furthermore, multivariate analysis—adjusting for β 2M and CD4 count as well as for treatment and demographic factors—demonstrated that both β 2M and CD4 count were independent markers of progression to AIDS. With death as the endpoint, instead of AIDS onset, multivariate analysis adjusted for the same parameters again indicated that both β 2M and CD4 count were strong prognostic markers (see Table I).

When the data were analyzed using only the baseline measurements rather than serial measurements (simulating the availability of only a single patient sample), univariate analysis showed a significant reduction in the predictive value of both β 2M and CD4 count for both

progression to AIDS and death. Similarly, multivariate analysis using only baseline values resulted in altered predictive ability of both β 2M and CD4 count.

Analyzing their data further, the researchers noted that it is the most recent determination of β 2M and CD4 count that provides the most accurate prognostic data with respect to AIDS onset or death, and that, among patients with similar CD4 counts, those with higher β 2M serum levels are at increased risk of disease progression. The authors concluded that β 2M supplied information supplemental to and independent of CD4 count and that serial measurements of β 2M were more valuable than just a single measurement.

In another in-depth study, researchers evaluated CD4 count, β 2M, neopterin, and the relationship among these markers in 198 HIV-infected individuals for a period of approximately 10 years.² This study investigated the relationship between the onset of AIDS and the levels of these markers at various time periods:

- Pre-seroconversion.
- Post-seroconversion 0–12 months.
- Post-seroconversion 13–30 months.

During the last of these periods, the researchers used changes in marker levels for statistical analysis, rather than absolute measurements. The investigators sought to determine the effect of immune system status at the time of HIV-1 infection, as well as the effect of the immune system's response shortly after infection, on the development of AIDS.

<i>Marker</i>	<i>Univariate</i>	<i>Multivariate</i>
Relative Hazard of AIDS		
β 2M (per g/L increase)	1.79 (p < 0.0001)	1.37 (p = 0.0012)
CD4 count (per log increase)	2.5 (p < 0.0001)	2.17 (p < 0.0001)
Relative Hazard of Death		
β 2M (per g/L increase)	2.06 (p < 0.0001)	1.34 (p = 0.0004)
CD4 count (per log increase)	2.99 (p < 0.0001)	1.91 (p < 0.0001)

Table I. Univariate and multivariate relative hazard of AIDS and death according to the Cox proportional hazards model.¹

Pre-seroconversion. The results showed that the general trends for the pre-seroconversion levels of these markers were consistent throughout the follow-up period. If pre-seroconversion CD4 count was high, CD4 count generally remained high. Likewise, if pre-seroconversion β 2M or neopterin levels were low, they remained low throughout. As in the first study discussed above, the markers were independent of one another.

Post-seroconversion 0–12 Months. During the first year post-seroconversion, CD4 count inversely correlated with both β 2M and neopterin levels. If first-year β 2M or neopterin was low, future CD4 count was high. Similarly, if first-year β 2M or neopterin was high, future CD4 count was generally low. As above, general trends were consistent for each marker: low first-year levels remained low, and high first-year levels remained high, throughout the follow-up period.

<i>Subject Groups</i>	<i>Pre-seroconversion</i>	<i>0–12 Months Post-seroconversion</i>	<i>13–30 Months Post-seroconversion</i>
CD4 count—high	1.0	1.0	1.0
CD4 count—medium	1.204	1.656	0.848
CD4 count—low	1.369	2.931*	2.407*
β 2M—low	1.0	1.0	1.0
β 2M—medium	1.014	1.628	1.794
β 2M—high	1.109	2.510*	3.552*
Neopterin—low	1.0	1.0	1.0
Neopterin—medium	0.898	3.645*	1.565
Neopterin—high	1.319	4.235*	2.261*

*Denotes statistically significant increased relative hazard ($p < 0.05$).

Table II. Relative hazard of AIDS according to the Cox proportional hazards model.³

Post-seroconversion 13–30 Months. During this period of monitoring, those patients with the greatest β 2M or neopterin increases had the lowest future CD4 count, and those with the smallest β 2M or neopterin increases had the highest future CD4 count.

Throughout this study, β 2M and neopterin levels correlated closely with one another. Although CD4 count correlated inversely to β 2M and neopterin levels, this association was much weaker. CD4 count had little effect on future β 2M and neopterin levels, but the reverse associations were quite strong: Changes in β 2M and neopterin levels correlated strongly with future CD4 count. In particular, β 2M and neopterin levels in the 0–12-month post-seroconversion period were the best predictors of future CD4 count. The authors thus proposed that immune system activation, marked by significant increases in β 2M and neopterin, is indicative of future CD4 depletion.

Cox proportional hazards models were used to estimate the relative hazard of progression to AIDS for the subjects studied (see Table II). Individuals with the lowest CD4 counts and highest β 2M and neopterin levels during the 0–12-month postseroconversion period were at the greatest risk of developing AIDS, while those with the highest CD4 counts and lowest β 2M and neopterin levels had the least risk of developing AIDS. No statistically significant correlation could be made between the onset of AIDS and any of the pre-seroconversion marker levels.

<i>Marker</i>	<i>p Value</i>	<i>Correlation Coefficient</i>
β 2M	0.0001	0.68
TNFR-II	0.0002	0.66
IL-2R	0.0008	0.62
Neopterin	0.001	0.61
TNF- α	0.01	0.54

Table III. Association between HIV-1 viral load and other markers of immune system activation.⁵

The authors noted that although individuals with higher pre-seroconversion CD4 counts maintained higher CD4 counts throughout the course of infection, those higher CD4 counts did not correlate to a longer period before progression to AIDS. It simply resulted in these patients developing AIDS at a higher CD4 count than most others.

From their data, the investigators concluded that the response of the immune system to HIV-1 infection during the first year post-seroconversion has a significant impact on the course of disease and progression to AIDS, while the state of the immune system at the time of infection has less influence. It appears that during the first year after infection a relationship is established between the virus and the infected individual, and the patients with the best prognosis are those with the least-active immune systems, as indicated by low levels of β 2M and neopterin.

These results were corroborated in a similar study of the hazard of progression to AIDS, in which the researchers reported a similar link between CD4 count and both β 2M and neopterin.⁴ Although the correlation was stronger with β 2M than with neopterin, the investigators reported that hazard estimates made using CD4 count were enhanced by the additional information provided by β 2M and neopterin. This was especially true in the later stages of disease, when the CD4 count was low (100 cells/ μ l or less). The authors concluded that when CD4 count is known, β 2M determinations significantly augment the prediction of the hazard of progression to AIDS in the late stages of disease.

β 2M and HIV-1 Viral Load

In a study designed to determine the relationship between cytokine expression and HIV-1 viral load (the number of copies of HIV-1 RNA

found per milliliter of plasma or serum), another group of researchers evaluated HIV-1 viral load, tumor necrosis factor- α (TNF- α), TNF receptor type II (TNFR-II), interleukin type 2 receptor (IL-2R), β 2M, and neopterin in 34 asymptomatic HIV-infected individuals with CD4 counts greater than 100 cells/ μ l.⁵ Of all the markers studied, β 2M had the highest correlation with HIV-1 viral load ($p = 0.0001$; see Table III).

In addition, when patients were staged into high and low HIV-1 viral load groups (above and below 40,000 copies/ml), statistically significant differences were noted between the two groups for all markers. On the other hand, when patients were staged according to their CD4 count (above and below 300 cells/ μ l), statistically significant differences were noted only for β 2M, neopterin, and TNFR-II; no significant differences were observed for HIV-1 viral load, TNF- α and IL-2R.

HIV-1 viral load

A patient's HIV-1 viral load—the number of copies of HIV-1 RNA found per milliliter of plasma or serum—is closely associated with progression to AIDS. Several studies have asserted the excellent prognostic value of HIV-1 viral load determinations, noting that high HIV-1 viral load after primary infection is predictive of rapid progression to AIDS, while low HIV-1 viral load is associated with long-term asymptomatic disease.^{5,7-12} Measurements of viral load have rapidly come into routine clinical use for monitoring both disease progression and a patient's response to antiretroviral therapy.^{8,13,14}

Although HIV-1 viral load is currently considered the best single predictor of progression to AIDS and AIDS-related death, its predictive ability can be improved by adding measurements of CD4 count and soluble markers of immune system activation. Refinement of the assay methods for HIV-1 viral load (including reverse transcriptase polymerase chain reaction, branched DNA signal amplification, and nucleic acid sequence-based amplification) make it useful not only to clinicians but also to researchers investigating the dynamics of HIV-1 replication in vivo.¹⁵⁻¹⁷ Using such techniques, Perelson et al. report that a high level of virus replication and CD4 T-cell lymphocyte destruction occurs at all stages of HIV-1 infection.¹⁸ Their results are summarized below.

- * Approximate daily production of virus particles: 10.3 billion
- * Average virus life span: 0.3 days
- * Average life span of infected cell: 2.2 days
- * Approximate virus replication cycles per year: 140
- * Average length of virus generation: 2.6 days

Length of virus generation includes the entire cycle of virus release, infection of a new cell, cell death, and subsequent release of new virus particles.

The researchers concluded that HIV-1 viral load correlated well with levels of both β 2M and neopterin in asymptomatic HIV-infected patients, strengthening the link between these immune system activation markers and HIV-1 disease progression. They also demonstrated the absence of a significant correlation between CD4 count and HIV-1 viral load.

In a recent prospective study, another group of investigators evaluated the ability of HIV-1 viral load, CD4 count, CD8 count, β 2M, and immune complex dissociated (ICD) HIV-1 p24 antigen to predict disease progression in 67 long-term nonprogressors (LTPNs; asymptomatic HIV-infected patients with CD4 count of at least 500 cells/ μ l).⁶ Each marker was measured at study entry and at six-month intervals for a period of two years.

<i>Marker</i>	<i>HIV-1 Viral Load (RNA copies/ml)</i>			<i>p Value</i>
	<i>< 200 (n = 11)</i>	<i>201–10,000 (n = 30)</i>	<i>> 10,000 (n = 26)</i>	
CD4 count (cells/ μ l) median range	819 561– 1375	699 504–1195	616 507–1258	0.077
CD8 count (cells/ μ l) median range	861 580– 1350	1188 432–2368	1091 476–2080	0.425
β 2M (mg/ml) median range	2.0 1.4–2.5	2.5 1.5–3.5	3.0 1.3–5.5	0.0005*
ICD p24 (pg/ml) median range	5.0 5.0–5.0	5.09 5.0–13.9	5.0 5.0–100	0.0014*

*Denotes statistically significant correlation with HIV-1 viral load ($p < 0.05$).

Table IV. Association between HIV-1 viral load and other markers in long-term nonprogressors.⁶ Kruskal-Wallis test used to determine p values for markers across HIV-1 viral load stages.

When study subjects were staged by their baseline HIV-1 viral load levels, a statistically significant and strong correlation was evident between HIV-1 viral load and β 2M ($p = 0.0005$; see Table IV). A significant correlation was also noted between HIV-1 viral load and ICD p24, but it was less strong ($p = 0.0014$). When staged according to the rate of CD4-count decline, baseline elevations of HIV-1 viral load, β 2M, and ICD p24 all correlated with a more rapid decline in CD4 count, but only β 2M significantly predicted CD4 decline in both univariate and

multivariate analyses (see Table V). This was true both for those patients receiving and not receiving antiretroviral therapy.

The researchers concluded that in LTNPs, β 2M is a stronger predictor of CD4 decline than is HIV-1 viral load. They also noted that elevated β 2M may indicate the occurrence of an immunological event before the appearance of clinical disease. For this reason, when used in conjunction with HIV-1 viral load and CD4 measurements, markers of immune system activation such as β 2M provide valuable prognostic information for determining the risk of disease progression in LTNPs.

Marker	Rate of CD4 Decline Per Month		Univariate		Multivariate		
	>1.5	<1.5	Odds Ratio	p Value	Odds Ratio	95% Confidence Interval	p Value
HIV-1 RNA (copies/ml)							
< 200	3	7	1.0		1.0		
200–10,000	9	10	1.91	0.432	1.24	(0.22, 7.04)	0.811
> 10,000	13	8	4.33	0.079	1.56	(0.25, 9.68)	0.636
CD4 count (cells/ μ l)							
\leq 650	13	12	1.0		1.0		
> 650	12	13	0.85	0.777	1.47	(0.41, 5.31)	0.559
CD8 count (cells/ μ l)							
\leq 1000	15	10	1.0		1.0		
> 1000	10	15	0.44	0.160	0.30	(0.08, 1.12)	0.073
β 2M (mg/ml)							
\leq 2.2	5	13	1.0		1.0		
2.3–2.8	8	8	2.60	0.188	2.42	(0.56, 10.39)	0.234
\geq 2.9	12	4	7.80	0.009*	6.51	(1.21, 32.07)	0.029*
ICD p24 (pg/ml)							
Undetectable	19	22	1.0		1.0		
> 5	6	3	2.32	0.278	1.2	(0.22, 6.40)	0.832

*Denotes statistically significant prediction of CD4 decline ($p < 0.05$).

Table V. Association between baseline marker values and faster CD4 decline.⁶

In studying the relationship between percent change in CD4 count over the course of a one-year period and the onset of AIDS, another group evaluated the associations among HIV-1 viral load, β 2M, neopterin, and

TNF receptor 75 (TNFR-75).⁷ Cited as the most significant finding of their work, the researchers reported that soluble immune system activation markers correlated better with both single-year CD4-count decline and disease progression than did HIV-1 viral load. Neopterin was the best predictor of CD4-count decline and death, while in the subgroup of patients with CD4 counts greater than 200 cells/ μ l, TNFR-75 was the strongest predictor of the onset of AIDS. Of note, in patients with a baseline CD4 count of less than 200 cells/ μ l, no correlation was found for HIV-1 viral load. The investigators concluded that HIV-1 viral load determinations and CD4 counts should be combined with a marker of immune system activation to develop an optimal predictive model for the progression of HIV-1 disease (see box, page 44).

Conclusion

It is clear from the studies discussed here that an undeniable link exists between β 2M and HIV-1 disease progression. During the course of HIV-1 infection, β 2M serum levels correlate with CD4 count, AIDS onset, and death. In addition, the link observed between β 2M and HIV-1 RNA plasma load, as well as the apparent effect of immune system response during the first year of infection on subsequent disease progression, suggest that immune system activation may be involved in the amount of viral replication that occurs and, hence, may influence the clinical progression of disease.

Although the prognostic value of a single, early measurement is limited, serial measurements of immune activation markers such as β -2M have been shown to be very useful in monitoring the course of disease in HIV-infected individuals. Compared to HIV-1 viral load, soluble markers of immune system activation show a better correlation with CD4-count decline, disease progression, and death in LTNPs and in late-stage disease.

Future studies should include more in-depth evaluations of serial measurements of β 2M. Although immunoassays for neopterin, TNFR-II, and TNFR-75 are not available as readily as those for β 2M, similar time-course studies of those markers should also be considered.

Study methods and statistics

Correlation coefficient. The correlation coefficient is the linear association between two random variables, x and y . Correlation coefficients range from -1 to $+1$. A positive value indicates a positive association: as x increases, y also increases; and as x decreases, y also decreases. A negative value indicates a negative association: as x increases, y decreases; and as x decreases, y increases. The larger the correlation coefficient, the stronger the association between x and y .

Cox proportional hazards model. This statistical model is a logarithmic presentation of a set of data, such as β 2M serum levels, and its relationship to a progression from a specific starting point (e.g., the diagnosis of HIV-1

infection) to a defined endpoint (e.g., the onset of AIDS or death). In effect, this is a numerical presentation of the relative risk or likelihood of an event, with larger numbers indicating a higher risk of the event being evaluated.

Friedman's test, Kruskal-Wallis test, Two-sided Mann-Whitney test, and Wilcoxon's test. These are examples of nonparametric rank tests. In rank tests, obtained data are replaced by values representing their relative rank. Nonparametric tests do not make distributional assumptions, meaning they do not involve population parameters. The tests do not assume that the data come from normally distributed populations, nor does their validity rely on the population distribution from which the data have been sampled. They do make some assumptions, however, such as equality of population variances. Tests such as these are typically used to compare data from two unpaired groups, with the data being ranked for analysis from low to high, regardless of the population from which they are derived.

Least squares linear regression. A statistical method used to fit a straight line to a series of data.

Multivariate longitudinal studies. In multivariate longitudinal studies, serial measurements of two or more variables are obtained from an individual over a period of time (e.g., CD4+ T-cell lymphocyte count, β 2M, and neopterin measured at three-month intervals). In analyzing the relationships among the variables, researchers must consider both the correlations among the variables taken at the same time and at different times.

95% confidence interval. For a marker under investigation, such as β 2M, the 95% confidence interval is that range of values within which 95% of the study population will fall. For instance, a 95% confidence interval of 1.21–32.07 for β 2M indicates that 95% of the study group will have β 2M values between 1.21 and 32.07 mg/ml. To avoid inadvertently biased results, confidence interval calculations should be used only in a random sampling of a population.

Odds ratio. In an effort to identify factors that may cause harm to a study population, epidemiologists calculate the odds ratio for each of the factors involved. Values for odds ratios are between zero and infinity. An odds ratio of 1 is the neutral value, indicating that the factor being studied shows no difference between the control group and the study group. Harmful factors have odds ratio values greater than 1; higher values are associated with factors that are more harmful. Odds ratios are typically used in studies in which disease prevalence is not known.

p value. The outcome of statistical testing is often expressed as a p value. This value is an indicator of the risk of a Type I statistical error, or the rejection of the null hypothesis. In effect, the p value describes the probability or risk of false-positive statistical error. As this type of error is undesirable, statistical significance is said to be achieved for a set of data when the risk of a false-positive error is low, at the 5% level or less ($p \leq 0.05$).

0.05).

Univariate longitudinal studies. In univariate longitudinal studies, a single variable from one individual is measured over a period of time (e.g., CD4+ T-cell lymphocyte count measured at three-month intervals). In analyzing such data, correlations among the serial measurements are considered.

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