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ß2-Microglobulin and AIDS Prognosis

Serial measurements of ß2-microglobulin assist in predicting clinical course of HIV-1 infection

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] Historically, CD4 T cell lymphocyte (CD4) count has been used to monitor the clinical progression of disease in HIV-infected individuals, and a strong correlation exists between a low CD4 count and the development of acquired immunodeficiency syndrome (AIDS) and death.[2,3,4,5,6] In general, patients who maintain a CD4 count of > 500 cells/µl for a prolonged period of time (10 years) are deemed long-term nonprogressors and are at a reduced risk for the development of AIDS, while those with a count of ≤ 200 cells/µl are at increased risk and are candidates for aggressive therapy.[7,8]

CD4 count, however, does not always correlate with disease stage, especially in early-stage disease, or with progression to AIDS. All too frequently, patients with similar CD4 counts do not follow the same clinical course.[1,7] A biochemical marker that could identify asymptomatic HIV-infected individuals at greater risk of progressing to AIDS would, therefore, be quite valuable in the presence of patients with ambiguous CD4 counts.

To augment the data provided by CD4 measurements, researchers have looked to soluble markers of immune system activation, hoping to find additional prognostic data by which HIV-infected individuals could be classified into, for instance, slow, moderate, or rapid progressor groups. Accurate classification of HIV-1 patients would assist physicians in determining the best course of treatment for an individual, i.e., whether or not the patient should receive antiretroviral therapeutic agents. Three immune activation markers under consideration are ß2-microglobulin (ß2M), neopterin, and tumor necrosis factor type II receptor (TNFR-II): ß2M 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. Although neopterin and TNFR-II are deserving of consideration and will be briefly discussed, the remainder of this article will focus on ß2M, for which clinical immunoassays are currently readily available.

ß2M belongs to the ß-globulin family of human plasma proteins and, as mentioned, is the light chain moiety bound non-covalently to the heavy chain subunit of the class I HLA complex and is found on the cell surface of all nucleated cells.[9] Over-production of the HLA complex can cause ß2M to be released into circulation. The lymphocytes are largely responsible for the over-production of this solubilized ß2M, which is normally filtered out of serum through renal glomeruli and ultimately reabsorbed and catabolized by epithelial cells. Serum elevations of ß2M occur, however, in several clinical conditions such as multiple myeloma, nasopharyngeal carcinoma, and a variety of lymphoproliferative disorders.[10,11,12,13] In addition, ß2M has been studied 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.[14,15,16]

ß2-Microglobulin's link to HIV-1

The association between ß2M and HIV-1 infection is well-documented, with ß2M serum levels rising in correlation with disease progression and reaching a peak just before death.[16,17,18,19,20] Some of these early investigations suggested a prognostic role for ß2M, as patients with higher ß2M serum levels tended to develop AIDS sooner than patients with lower levels.[21,22] While recent studies have confirmed the strong correlation among ß2M, CD4 count, and HIV-1 (typical results are shown in Table 1),[23] conflicting results have been reported regarding the prognostic value of ß2M, that is, whether or not the determination of ß2M 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 ß2M and several viral markers, including HIV-1 RNA load (the number of copies of HIV-1 RNA found per ml of plasma; see side bar: HIV-1 RNA Load).[24] 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. At study entry, ß2M 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 RNA 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 ß2M in this study (data not shown) contradict those reported in Table 1 in which ß2M serum levels of all HIV-infected groups were significantly elevated above normal. Further investigation would likely reveal whether or not differences in study populations, analytical methods, or in ß2M assay methodology (i.e., selecting an appropriate upper reference limit) contributed to this discrepancy.

In a similar, but retrospective study, HIV-1 RNA load, ß2M, neopterin, and TNFR-II were evaluated 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 subjects (n = 90) 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), six months later, 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 RNA load and TNFR-II showed a statistically significant difference between each

Table 1. The strong correlation between ß2M serum levels and CD4 counts in HIV-infected individuals.[23]
(n = 200)

CD4 count (cells/µl)	ß2M (µg/L; mean ± SD)
< 50	4.55 ± 1.24
50 - 199	3.82 ± 0.75
200 - 500	3.75 ± 1.10
Healthy controls	1.31 ± 0.22

HIV-1 RNA Load

Although not discussed at length in this article, it should be noted that HIV-1 RNA load, in terms of HIV-1 RNA copies/ml found in the plasma of HIV-infected individuals, is highly associated with progression to AIDS.[1,24] In fact, two models have been proposed in which high HIV-1 RNA load after primary infection is predictive of rapid progression to AIDS, while low HIV-1 RNA load is associated with long-term asymptomatic disease.[27,28] The special handling required of test samples and higher costs render assays for HIV-1 RNA load (PCR and branched DNA signal amplification) less desirable for routine clinical use than the standard immunoassays available for such immune activation markers as B2M.

Univariate vs. Multivariate Studies

A univariate longitudinal study comprises measuring a single variable over a period of time from an individual, e.g., CD4+ T cell lymphocyte count measured at 3-month intervals. In analyzing such data, correlations among the serial measurements are considered.

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, B2M, and neopterin measured at 3-month intervals. In analyzing the relationship among the variables, the correlations among the variables taken at the same time and at different times must be considered.

Table 2. Baseline marker values for three progression groups.[1]
(n = 30 for each group)

	Progression Groups		
Marker	**Slow**	**Moderate**	**Rapid**
HIV-1 RNA* (copies/ml)	1683	6816	17830
TNFR-II* (ng/ml)	3.13	3.64	4.11
CD4 count (cells/µl)	625.5	590.5	534
Neopterin (nmol/L)	10.29	10.35	11.17
ß2M (µg/L)	1.9	2.09	2.52

*Denotes a statistically significant trend across the three progression groups ($p < 0.001$, Friedman's test).

group (Table 2). In addition, univariate and multivariate matched conditional regression models (see side bar: Univariate vs. Multivariate Studies) 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.

It is interesting to note that ß2M correlated significantly with HIV-1 RNA load in this study, although it was not predictive of clinical disease progression. While the authors offered no hypothesis for this, the pre-classification of study subjects by their CD4 count and CD4 count decline may have biased the interpretation of ß2M measurements.

ß2-Microglobulin vs. CD4 count

Two recent studies investigated not only ß2M and CD4 count, but explored the relationship between the two and how this relationship may relate to AIDS onset and death.

In a prospective study involving 539 HIV-infected individuals without AIDS, ß2M and CD4 count were measured approximately every 1.5 months for a period of 21 months.[8] Data were separately analyzed with respect to disease progression to AIDS or death. Contrary to the reports discussed above, univariate analysis indicated that both ß2M and CD4 count were significantly associated with an increased risk of developing AIDS (Table 3).

Furthermore, multivariate analysis, mutually adjusting for ß2M and CD4 count, and also adjusting for treatment and demographic factors, demonstrated that both ß2M and CD4 count were independent markers of progression to AIDS. With death, rather than AIDS, as the endpoint, multivariate analysis, adjusted for the same parameters mentioned above, indicated that again both ß2M and CD4 count were strong prognostic markers (Table 3).

When the data were analyzed using only the baseline marker 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 ß2M and CD4 count for both progression to AIDS and death. Similarly, multivariate analysis using only baseline values resulted in altered predictive ability of both ß2M and CD4 count.

Analyzing the data further, the authors concluded that it is the most recent determination of ß2M 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 ß2M serum levels are at increased risk of disease progression. Of note, ß2M supplied information supplemental to and independent of CD4 count. The authors also demonstrated the value of serial measurements of ß2M over just a single measurement.

Another in-depth study evaluated CD4 count, ß2M, neopterin, and the relationship among these markers in 198 HIV-infected individuals for a period of approximately ten years.[25] This study investigated the relationship, if any, between the onset of AIDS and the levels of these markers at various time periods:

(i) pre-seroconversion,
(ii) 0 - 12 months post-seroconversion,
(iii) 1 - 2.5 years post-seroconversion.

[Note: For time period (iii), changes in marker levels were used 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.

Time period (i): The results showed that the general trends of the pre-seroconversion levels of these markers was consistent throughout the follow-up period: If pre-seroconversion CD4 count was high, CD4 count generally remained high. Likewise, if pre-seroconversion ß2M or neopterin levels were low, they remained low throughout. As in the other studies discussed above, the markers were independent of one another.

Time period (ii): During the first year post-seroconversion, CD4 count inversely correlated with both ß2M and neopterin levels: If first-year ß2M or neopterin was low, future CD4 count was high. Similarly, if first-year ß2M 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.

Time period (iii): Regarding changes in marker levels that occur 1 - 2.5 years post-seroconversion, those patients with the greatest ß2M or neopterin increases had the lowest future CD4 count, and those with the smallest ß2M or neopterin increases had the highest future CD4 count.

Throughout the study, ß2M and neopterin levels were highly correlated with each other, and while CD4 count was inversely correlated with ß2M and neopterin, the association was much weaker. Although CD4 count had little affect on future ß2M and neopterin levels, the reverse associations were quite strong: Changes in ß2M and neopterin levels strongly correlated with future CD4 count. In particular, ß2M and neopterin levels in the 0 - 12 month post-seroconversion period were the best predictors of future CD4 count. This suggested to the authors that immune system activation, marked by significant increases in ß2M 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 (Table 4). Individuals with the lowest CD4 counts and highest ß2M and neopterin levels in the 0 - 12 month post-seroconversion period were at the greatest risk of developing AIDS, while those with the highest CD4 counts and lowest ß2M 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.

The authors noted that although individuals with higher pre-seroconversion CD4 counts maintained higher CD4 counts throughout the course of infection, these 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 authors 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 post-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 system, as determined by increased levels of ß2M and neopterin.

Closing remarks

It is clear from the few studies discussed here that an undeniable link exists between ß2M and HIV-1 disease progression. During the course of HIV-1 infection, ß2M serum levels correlate with CD4 count, AIDS onset, and death. In addition, the link observed between ß2M and HIV-1 RNA plasma load, as well as the apparent effect of immune system activation during the first year post-infection on 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 of ß2M is limited, serial measurements of immune activation markers such as ß2M were very useful in monitoring the course of disease in HIV-infected individuals. Furthermore, this was demonstrated in two studies, one prospective and one retrospective, involving very large subject populations. The discrepancies reported may be due to smaller study populations, differences in assay methodologies, or to the pre-classification of patients based on CD4 count, as ß2M was repeatedly shown to be independent of CD4 count.

Future studies should not only include more in-depth evaluations of serial measurements of ß2M, but perhaps should also include similar time-course studies of neopterin and TNFR-II, although immunoassays for these markers are currently not as readily available as those for ß2M.

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Table 3. Univariate and Multivariate Relative Hazards of AIDS and Death.[8] Cox proportional hazards model.

	Relative Hazard of AIDS	
Marker	**Univariate**	**Multivariate**
ß2M (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	
Marker	**Univariate**	**Multivariate**
ß2M (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 4. Relative Hazard of AIDS.[25] Cox proportional hazards model.

	Relative Hazard of AIDS		
		0 - 12 Months	**1 - 2.5 Years**
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*
ß2M - Low	1.0	1.0	1.0
ß2M - Medium	1.014	1.628	1.794
ß2M - 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 ($p < 0.05$).