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How to cite this article:
A. MOCROFT, M. A. JOHNSON, C. A. SABIN, M. BOFILL, G. JANOSSY and A. N. PHILLIPS (1997). The relationship between beta-2-microglobulin,   CD4 lymphocyte count, AIDS and death in HIV-positive individuals. Epidemiology and Infection,118, pp 259-266
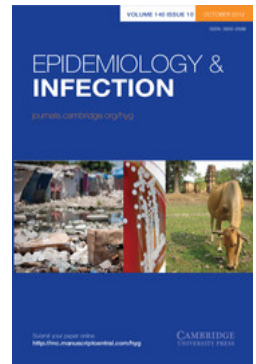
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The relationship between beta-2-microglobulin, CD4 lymphocyte count, AIDS and death in HIV-positive individuals

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SUMMARY

The relationship, in 539 individuals infected with the human immunodeficiency virus (HIV), between two prognostic markers, the CD4 count and beta-2-microglobulin (B2M), and the development of the acquired immunodeficiency syndrome (AIDS) and death was investigated. Cox proportional hazards models were used to determine the risk of AIDS or death. In a multivariate model which adjusted for demographic factors and treatment, the most recent measurements of B2M (relative hazard (RH) 1·37 per g/l higher) and CD4 count (RH 2·17 per log-unit lower) were both significantly associated with the development of AIDS. Similarly, in a multivariate model which additionally adjusted for the development of AIDS as a time dependant covariate, there was a strong relationship with risk of death for the most recent measurements of B2M (RH 1·34 per g/l higher), and CD4 lymphocyte count (RH 1·91 per log-unit lower). A difference in the level of B2M could be used among patients with similar CD4 counts as an indicator of increased risk of progression to AIDS or death. Using the most recent values of these markers provides a better estimate of the risk of AIDS or death, compared to the more common method of analysis, where baseline values of the markers are used.

INTRODUCTION

In patients infected with the human immunodeficiency virus (HIV), both serum beta-2-microglobulin (B2M), a sub-unit of the class I major histocompatibility complex found on the surface of nucleated cells and thought to reflect the degree of immune activation [1], and CD4 lymphocyte counts, have been shown to predict the development of AIDS both individually and in combination [2–8]. The association between CD4 lymphocyte counts and death in patients infected with HIV has also been shown [10–12], but the relationship between B2M and death has been explored less thoroughly [13].

In clinical practice, HIV positive patients without AIDS tend to be seen every few months and markers such as the CD4 count are used to assess whether or not a patient is at risk of progressing to AIDS before their next visit, and to help determine an appropriate time to start therapy (e.g. when the CD4 count falls below 200/mm³). Most studies considering the relationship between markers and disease progression have considered a single value of the prognostic markers at some arbitrary baseline to calculate the subsequent risk of AIDS or death, usually over a follow-up period measured in years [3, 5–9]. To date, few studies have assessed the predictive value of the CD4 count when all measurements taken during

follow-up are accounted for [14–16], and it remains to be demonstrated that B2M and CD4 count independently predict the risk of AIDS or death in this more clinically relevant situation.

We took advantage of a large patient group seen regularly at the Royal Free Hospital in whom B2M and CD4 counts are measured at regular intervals, to determine if both B2M and CD4 counts are independent predictors of AIDS and death using time-updated measurements. We also illustrate and discuss the different results that would be obtained if only baseline values of the prognostic markers were used.

MATERIALS AND METHODS

Patients

All patients seen at the Ian Charleson Day Centre (ICDC), the outpatient facility for patients infected with HIV at the Royal Free Hospital, London, were eligible for inclusion in this study. Of these, all patients with greater than one month of follow-up and for whom at least one CD4 count and B2M measurement were available were included. Diagnosis of AIDS was made according to the Centers for Disease Control 1987 criteria [17]. Date of birth, exposure category, ethnic origin, CD4 lymphocyte counts, B2M levels, and date of death were obtained from the HIV/AIDS unit database. Where possible, dates of death for patients lost to follow-up were obtained from the Communicable Disease Surveillance Centre. The dates of starting antiretrovirals (zidovudine, didanosine or zalcitabine) and prophylaxis for *Pneumocystis carinii* pneumonia (PCP), (nebulized pentamidine, cotrimoxazole, dapsone or clindamycin) were obtained by a retrospective search through all patient notes.

Laboratory methods

CD4 analysis

A whole blood lysis method was used and the percentage CD4 lymphocytes was expressed as absolute CD4 counts on the basis of lymphocyte counts. A monoclonal antibody, RFT4, was used, as described previously [17]. More recently, absolute CD4 counts have been obtained directly in an ORTHO Cytoron-Absolute flow-cytometer (ORTHO Diagnostics, High Wycombe, UK). Quality control was routinely monitored in the UK National External Quality Assurance Scheme. A median of 10 CD4 lymphocyte counts per person were available (90 % range 2–39), at a median time interval of 1·3 months (90 % range 0·3–5·3 months).

Beta-2-microglobulin

Beta-2-microglobulin was measured on fresh serum samples, using a commercial radial immunodiffusion (RID) method (NanaRID, Binding Site Ltd, UK). The measurements were routinely monitored in the UK National External Quality Assurance Scheme. A median of 7 measurements (90 % range 1–21) of B2M were made during follow-up, at a median time interval of 1·4 months (90 % range 0·4–5·1 months).

Statistical methods

Disease progression to AIDS or death according to the lowest CD4 count recorded was analysed using Kaplan-Meier curves [19]; this assumes that CD4 lymphocyte count was a monotonic decreasing function of time. The prognostic markers studied, CD4 counts and B2M, were modelled as time-dependant covariates in Cox proportional hazards models. This method models the risk of an event using the latest available value of the marker at the time of each event. Inevitably the marker values fluctuate, which may be due to laboratory techniques or due to changes in the health of the patient. This tends to result in an underestimation of the relative hazard and wider confidence intervals [20]. Treatment with anti-retroviral drugs or PCP prophylaxis was also included in the model as time dependant covariates. Binary variables were created which took the value of zero before a patient began treatment, and switched to one thereafter. When studying the association between B2M, CD4 counts and risk of death, the development of AIDS was additionally fitted as a time dependant covariate. Various transformations (logarithmic, square root etc.) of both CD4 and B2M were performed to determine the best fitting model. Further models which considered only baseline values of the prognostic markers were also fitted using Cox proportional hazards models. There was no evidence that the assumption of proportional hazards did not hold ($P > 0·05$). Interactions between demographic variables and the immunological markers were also included in the model, but their inclusion did not result in a significant improvement in fit. Patients were

followed-up to the date of last follow-up, or 31 August 1994, whichever occurred first. Excluding patients who had died, only 40 patients (7·4%) had not been seen for 12-months at 31 August 1994. All statistical analyses were carried out using Statistical Analysis Software (SAS) [21], and all tests of significance were two-sided.

RESULTS

A total of 539 patients without AIDS at baseline were included in this analysis. The median total follow-up time, from first visit, when the baseline values of CD4 counts and B2M were established, to death or last visit was 21·1 months (90% range 2·8–60·2 months), during which time 54 patients (10·0%) died. The median age at first visit was 30·6 years (90% range 22·0–47·0 years); the male patients were significantly older than the females (median ages 31·1 and 28·8 respectively, $P < 0·0001$ Wilcoxon). Table 1 illustrates the demography of the population, together with the median baseline values of both CD4 lymphocyte counts and B2M, all of which were measured within 3 months of a patient's first visit to the hospital. The majority of the patients were male (448, 83·1%), and homosexual or bisexual (429, 79·6%). As can be seen from Table 1, Caucasian patients had significantly higher median CD4 counts at first visit than patients from other ethnic groups ($P = 0·004$, Wilcoxon). In addition, there were statistically significant differences in median baseline values of CD4 count and B2M between different exposure groups; intravenous drug users (IDU) presented with the highest CD4 counts ($P = 0·03$, Wilcoxon) while homosexuals or bisexuals presented with the lowest values of B2M ($P = 0·01$, Wilcoxon). Patients who were aged more than 35 at baseline had significantly lower CD4 lymphocyte counts and higher levels of B2M ($P = 0·004$ and $P < 0·0001$ respectively, Wilcoxon).

One hundred and thirty-one patients (24·3%) developed AIDS over a median follow-up time of 18·4 months (90% range 1·8–56·5 months). The initial AIDS defining diagnoses made include 31 of oesophageal candidiasis (23·7%), 22 of PCP (16·8%), 20 of Kaposi's sarcoma (15·3%) and 10 of HIV wasting syndrome (7·6%). At the start of this study, 18 patients (3·3%) had already commenced treatment with zidovudine and 17 patients (3·2%) were receiving primary PCP prophylaxis. During subsequent follow-up, 155 patients (28·8%) commenced treatment with antiretrovirals, the majority of whom were treated with zidovudine, a similar number of patients (172, 31·9%) started PCP prophylaxis.

Figure 1 illustrates the Kaplan–Meier progression rates to AIDS and death according to the lowest CD4 count measured. This clearly shows the relationship between decreasing CD4 counts and the risk of AIDS and death. The risk of AIDS remained small until a CD4 count of 200/mm³ was reached. Kaplan–Meier estimates suggest that 9% of patients will have a diagnosis of AIDS when the CD4 lymphocyte count has declined to 200/mm³. After this time the risk of AIDS increased exponentially. Similarly, the risk of death was negligible until the CD4 count fell to 50/mm³, but again, increased dramatically after this time.

Table 2 shows the univariate relative risks for the development of AIDS associated with age, sex, ethnic origin, exposure category, treatment, B2M and CD4 lymphocyte count. In the univariate analysis, both a higher B2M measurement and lower CD4 counts during follow-up were associated with a significantly raised risk of AIDS (relative hazard (RH) per g/l higher and 2·50 per log-unit lower respectively; $P < 0·0001$ for both). Treatment and age were also significant predictors for the development of AIDS; there was a 36% increase in risk of AIDS for every 10 year higher age (RH 1·36; 95% confidence interval (CI) 1·12–1·63, $P = 0·002$) and a 110% increased risk of AIDS in those patients who started antiretroviral treatment (RH 2·10, 95% CI 1·46–3·02, $P < 0·0001$). B2M and CD4 count were correlated at baseline (correlation coefficient −0·385, $P < 0·0001$), thus patients with high B2M levels had lower CD4 counts and it is essential to adjust for these at the same time. Age, ethnic group and exposure category were also related to each other, thus a multivariate analysis was needed to clarify the strongest independant effects. In addition to mutually adjusting for B2M and CD4 lymphocyte counts, the multivariate analysis also adjusted for age, treatment, and demographic factors such as gender and ethnic origin. These results showed that B2M and CD4 counts were independant markers for the development of AIDS, and patients with similar CD4 counts but in whom the B2M level was higher were at an increased risk of developing AIDS. Patients who are treated with antiretrovirals were at a reduced risk of AIDS in the multivariate analysis (RH 0·56; 95% Cl 0·29–1·05, $P = 0·07$), which indicates that despite the fact that patients who started treatment were more ill (as measured by low CD4 counts and high B2M levels) there was some reduction

Table 1. *A description of HIV positive patients at the Royal Free Hospital*

	Total		AIDS No.	Death No.	CD4 count at baseline			B2M at baseline		
	No.	%			Mean (s.d.)	Median (90% range)	P*	Mean (s.d.)	Median (90% range)	P*
All patients	539	100	131	54	434 (287)	400 (40–910)	n.a.†	3·1 (1·7)	2·9 (1·8–5·1)	n.a.†
Gender										
Male	448	83·1	115	47	440 (289)	412 (40–900)	0·2	3·1 (1·1)	2·9 (1·9–5·1)	0·2
Female	91	16·9	16	7	405 (276)	380 (30–1010)	—	3·0 (0·9)	2·7 (1·7–4·7)	
Age group										
≤ 25	85	15·8	16	6	489 (380)	410 (90–1000)	0·004	2·7 (0·8)	2·7 (1·6–3·8)	0·0001
26–35	306	57·1	68	24	450 (274)	420 (40–920)	—	3·1 (1·1)	2·8 (1·9–5·1)	—
> 35	148	27·5	47	24	369 (237)	356 (40–750)	—	3·4 (1·1)	3·2 (2·1–5·2)	—
Ethnic origin										
Caucasian	429	79·6	110	47	452 (297)	420 (50–940)	0·0001	3·2 (1·1)	2·9 (1·9–5·1)	0·07
Other	110	20·4	21	7	363 (228)	350 (30–740)	—	2·9 (0·9)	2·8 (1·7–4·6)	—
Exposure category										
Homo/bisexual	345	64·0	98	41	440 (266)	420 (50–910)	0·03	3·1 (1·0)	2·8 (1·8–5·0)	0·01
IDU	48	8·9	11	3	486 (271)	495 (20–880)	—	3·3 (1·1)	3·1 (1·9–5·1)	—
Heterosexual	97	18·0	19	7	371 (246)	350 (30–900)	—	3·0 (1·0)	2·8 (1·7–4·7)	—
Other	49	9·1	3	3	461 (456)	350 (120–1110)	—	3·5 (1·3)	3·4 (2·1–5·3)	

* *P*, P-value for testing for differences between groups using Wilcoxon test.
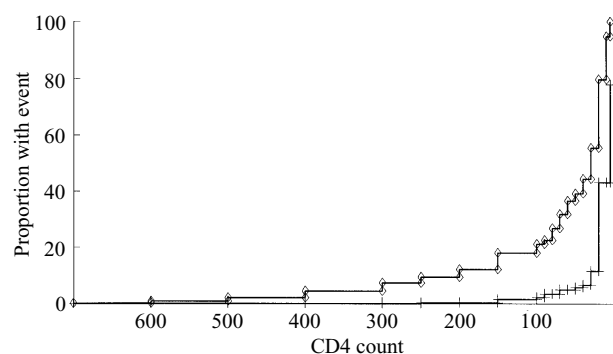
† n.a., not applicable.

Fig. 1. Kaplan-Meier progression rates to AIDS and death according to the lowest CD4 count measured. (◇) progression to AIDS, (+) progression to death.

in the risk of AIDS in patients who start treatment with antiretrovirals.

Table 3 presents similar statistics to Table 2, with death, rather than AIDS, as an endpoint. The multivariate analysis has adjusted for the same variables as above, and in addition this model has been adjusted for the development of AIDS as a time dependant variable. The results show that both CD4 count and B2M were strong prognostic markers (RH 1·91 per log-unit lower and 1·34 per g/l higher; $P < 0·0001$ and $P = 0·004$ respectively). In addition, the

development of AIDS was a strong predictor of death; patients who develop AIDS were nearly 27 times more likely to die than those patients who had not developed AIDS, even after adjustment for CD4 lymphocyte counts and B2M (RH 26·59; 95% Cl 5·06–139·68, $P < 0·0001$). In contrast to the data shown in Table 2, increasing age significantly increased the risk of death, even after adjustment for all other variables (RH 1·56; 95% Cl 1·09–2·24, $P = 0·02$).

All the analyses presented above concentrate on the role of the most recent CD4 and B2M measurement for predicting the development of AIDS or death. Of interest is how this predictive value changed if only the baseline measurements were used to predict the outcome. Table 4 shows the effect of incorporating only baseline values of the laboratory markers on the estimates of the relative hazard. Once again, the multivariate model was adjusted for the same variables as discussed above for Table 2. Model 1 shows the relative hazards from Table 2 for comparison. Model 2 shows the results which would have been obtained if only one value of each marker were available at the patient's baseline visit. In the univariate model, the relative hazard of AIDS or death associated with either B2M or CD4 lymphocyte

Table 2. *Univariate and multivariate relative hazards of AIDS*

Variables modelled using Cox proportional hazards model	Univariate		Multivariate	
	RH (95% Cl)	*P*-value	RH (95% Cl)	*P*-value
B2M (per g/l increase)*	1·79 (1·56–2·05)	0·0001	1·37 (1·13–1·65)	0·0012
CD4 (per log decrease)*	2·50 (2·21–2·83)	0·0001	2·17 (1·77–2·66)	0·0001
age (per 10 yr increase)	1·36 (1·12–1·63)	0·002	1·12 (0·82–1·51)	0·5
PCP prophylaxis*	3·44 (2·12–5·33)	0·0001	0·72 (0·51–1·78)	0·6
ARV treatment*	2·10 (1·46–3·02)	0·001	0·56 (0·29–1·05)	0·07
Gender†				
Female	0·64 (0·38–1·06)	0·08	0·64 (0·27–1·52)	0·3
Exposure category‡				
IDU	0·95 (0·49–1·83)	0·9	1·11 (0·45–2·70)	0·8
Heterosexual	0·80 (0·49–1·31)	0·4	1·11 (0·46–2·39)	0·8
Other	1·00 (0·57–1·76)	1·0	1·04 (0·46–2·39)	0·9
Ethnic origin§				
Caucasian	1·54 (0·97–1·03)	0·07	0·60 (0·30–1·22)	0·2

* Variables were modelled as time dependant covariates.
† Relative risk compared to males.
‡ Relative risk compared to homosexual/bisexuals.
§ Relative risk compared to all other ethnic groups combined.

Table 3. *Univariate and multivariate relative hazards of death*

Variables modelled using Cox proportional hazards model	Univariate		Multivariate	
	RH (95% Cl)	*P*-value	RH (95% Cl)	*P*-value
B2M (per g/l increase)*	2·06 (1·76–2·41)	0·0001	1·34 (1·10–1·64)	0·0004
CD4 (per log decrease)*	2·99 (2·36–3·79)	0·0001	1·91 (1·40–2·62)	0·0001
age (per 10 yr increase)	1·76 (1·34–2·31)	0·0001	1·56 (1·09–2·24)	0·02
PCP prophylaxis*	5·37 (2·72–10·63)	0·0001	0·60 (0·24–1·50)	0·3
ARV treatment*	2·99 (1·52–5·86)	0·0015	0·59 (0·23–1·49)	0·3
Gender†				
Female	0·71 (0·62–1·57)	0·4	1·65 (0·52–5·28)	0·4
Exposure category‡				
IDU	0·91 (0·28–2·99)	0·9	0·76 (0·18–3·27)	0·7
Heterosexual	0·76 (0·34–1·72)	0·5	0·86 (0·57–6·02)	0·3
Other	1·56 (0·73–3·44)	0·2	2·02 (0·80–5·12)	0·1
Ethnic origin§				
Caucasian	1·54 (0·97–5·26)	0·06	2·50 (0·75–8·33)	0·1
Diagnosis of AIDS*	50·49 (17·38–146·73)	0·0001	26·59 (5·06–139·68)	0·0001

* Variables were modelled as time dependant covariates.
† Relative risk compared to males.
‡ Relative risk compared to homosexual/bisexuals.
§ Relative risk compared to all other ethnic groups combined.

count dropped towards one, indicating that some important information has been lost. In the multivariate models of the risk of AIDS or death the prognostic value of the B2M appears similar to that obtained when values were updated over time, whilst that of the CD4 count is much reduced. This does not necessarily imply that measuring B2M throughout follow-up does not provide any additional information as Model 2 is under adjusted for the risk of AIDS or death associated with the CD4 count. A

Table 4. *Univariate and multivariate relative hazards of AIDS and death using baseline values of the prognostic markers*

	Univariate		Multivariate	
	RH (95% Cl)	*P*-value	RH (95% Cl)	*P*-value
Progression to AIDS				
Model 1. CD4 and B2M time-updated covariates				
B2M (per g/l increase)	1·79 (1·56–2·05)	0·0001	1·37 (1·13–1·65)	0·0012
CD4 (per log decrease)	2·50 (2·21–2·83)	0·0001	2·17 (1·77–2·66)	0·0001
Model 2. CD4 and B2M fixed at baseline				
B2M (per g/l increase)	1·51 (1·38–1·66)	0·0001	1·42 (1·25–1·60)	0·0001
CD4 (per log decrease)	1·96 (1·74–2·21)	0·0001	1·62 (1·37–1·90)	0·0001
Progression to death				
Model 1. CD4 and B2M time-updated covariates				
B2M (per g/l increase)	2·06 (1·76–2·41)	0·0001	1·34 (1·10–1·64)	0·0004
CD4 (per log decrease)	2·99 (2·36–3·79)	0·0001	1·91 (1·40–2·62)	0·0001
Model 2. CD4 and B2M fixed at baseline				
B2M (per g/l increase)	1·71 (1·50–1·95)	0·0001	1·55 (1·23–1·94)	0·0001
CD4 (per log decrease)	1·85 (1·52–2·25)	0·0001	1·48 (1·16–1·76)	0·0001

multivariate model which accounts for the baseline value of B2M and updated CD4 counts revealed that the risk of AIDS increased by 24% (RH 1·24; 95% Cl 1·13–1·36, $P < 0.0001$) for a 1 g/l higher B2M value and the risk of death increased by 18% (RH 1·18; 95% Cl 1·04–1·34, $P = 0.014$). Clearly, using updated values of both prognostic markers provides valuable information about the risk of AIDS or death.

DISCUSSION

We have demonstrated that in HIV positive individuals the most recently measured CD4 lymphocyte count and B2M level independently predicted AIDS and death. Previous studies have illustrated that the CD4 count is an important prognostic marker when modelled as a time-updated variable [4, 14–16], but to our knowledge ours is the first which illustrates that the latest value of B2M provides extra information in addition to that provided by the CD4 count in this context. In common with Kerlikowske and colleagues [13], we have also demonstrated in a much larger and more heterogeneous patient group that B2M is also a prognostic marker for death.

It is well documented, mainly in groups of patients with haemophilia, that there is an association between increasing age and progression to AIDS, with older individuals progressing to AIDS more rapidly [22–24]. After adjustment for various demographic variables and the markers studied, we did not find a significant

relationship between age and progression to AIDS. This may be due, in part, to the exposure categories of our patient group. However, even after adjustment for all other factors, the relationship between age and death was statistically significant, in common with some studies that have looked at the relationship between age and death in patients with AIDS [25–26].

In the univariate analyses, we found an increased risk of both AIDS and death for those patients treated with antiretrovirals or who had commenced PCP prophylaxis, which reflects that treatment is given to patients with deteriorating health. However, the results of the multivariate analysis showed that both antiretroviral treatment and prophylaxis for PCP were associated with a decreased risk of both AIDS and death, although these were not statistically significant due to the smaller power of our study, consistent with the findings that both treatments can reduce the risk of progression to AIDS and prolong survival [27–28].

Several other 'activation markers', such as neopterin and IgA have also been proposed as markers of disease progression [6, 14, 29–30]. Beta-2-microglobulin and neopterin have been shown to be highly correlated; both appear to predict disease progression equally well, but one does not provide any additional information on top of that already provided by the other [27, 29]. The ease of measurement of neopterin in urine often makes it more acceptable for use in routine clinical monitoring, particularly in Africa

where limited resources and facilities are available [31]. However, all these activation markers have been shown to be highly correlated with plasma viral load [32], and it remains to be seen whether they will predict disease progression in addition to viral load and CD4 lymphocyte count.

We have shown that the apparent prognostic value of a laboratory marker differs when only a single measurement of the marker is available at some arbitrary baseline. This is because both the CD4 count and B2M levels change during infection and to a different extent in different patients, such that values at the time of the baseline measurement become a relatively imprecise measure of the values at the time of AIDS or death. The two methods of analysis have different interpretations and clinical significance. To assess patient prognosis over the next 3 months or so, more information is provided by the time-updated analysis, in contrast to using baseline measurements, where the question of longer term prognosis is addressed. The difference between these models is not often highlighted; Spijkerman and colleagues [33] recently showed that the relationship between syncytium inducing phenotype of HIV and AIDS was no longer significant after adjustment for the CD4 count as a continuous time dependant covariate, and attributed this to differences in rate of CD4 decline between patients infected with syncytium inducing and non-syncytium inducing phenotypes.

There are several issues to consider in interpreting the results of this analysis. Bias may arise if patients lost to follow-up are not lost randomly; there was no evidence that this was the case. CD4 lymphocyte counts have been measured more regularly than B2M in our patient group, and although the time between successive measurements was not dissimilar for both markers this may have biased our results to show a weaker relationship between B2M and CD4 count than actually exists. However, both B2M and CD4 count were shown to be strong independent prognostic markers for AIDS and death.

To conclude, we have shown that the latest measurement of B2M provides significant prognostic information in addition to that provided by the latest CD4 count. Thus among patients with similar CD4 counts, those with higher levels of B2M are at an increased risk of disease progression over the following few months. We have also illustrated that B2M is an independent prognostic marker for death in patients infected with HIV, and thus that the continued measurement of these markers can help

with patient monitoring. Further, we have discussed the results of modelling baseline values of prognostic markers; future markers which are proposed to predict the development of AIDS independently of the CD4 count should additionally be modelled as time-updated covariates to determine if the most recent measurement adds to the information provided by the latest value of the CD4 lymphocyte count.

ACKNOWLEDGEMENTS

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