Improvement of the predictive value of CD4+ lymphocyte count by β_2-microglobulin, immunoglobulin A and erythrocyte sedimentation rate

Bernhard Schwartländer, Barbara Bek, Horst Skarabis*, Judith Koch, Jörg Burkowitz* and Meinrad A. Koch for the multicentre cohort study group†

Objective: To evaluate whether the use of immunological markers in addition to CD4+ lymphocyte count can improve the prediction of the probability of developing AIDS within a given period.

Design and setting: Prospective multicentre cohort study of homosexual men.

Patients: A total of 447 HIV-positive homosexual men followed prospectively at 6-month intervals (median time of observation, 47 months).

Methods: Estimation of AIDS-free time using lifetable plots by Cutler and Ederer and Weibull parametric models. A stepwise multivariate regression analysis was used to calculate the optimal combination of the parameters studied.

Results: In general CD4+ lymphocyte counts are most important for the prediction of AIDS-free time. The use of serum levels of β_2-microglobulin (β_2M), immunoglobulin A (IgA) and erythrocyte sedimentation rate (ESR) can significantly improve the predictive value of CD4+ lymphocyte counts. However, the usefulness of these parameters depends on the stage of HIV disease. In patients with a CD4+ lymphocyte count $> 500 \times 10^6$/l, only IgA level had a significant predictive value; none of the other parameters significantly improved the model. In patients with a CD4+ lymphocyte count $< 500 \times 10^6$/l, the absolute number of CD4+ cells itself was the most important single predictive parameter, but the prediction of AIDS was significantly improved by the addition of the other parameters investigated. The most powerful combination of parameters in this group was CD4+ count, β_2M and ESR.

Conclusion: Determination of serum IgA, β_2M and ESR in addition to CD4+ lymphocyte count may aid the choice of specific therapeutic regimens or systems of care for HIV-positive individuals.

AIDS 1993, 7:813–821

Keywords: Survival analysis, HIV, AIDS, CD4, β_2-microglobulin, immunoglobulin A, immunological markers.

Introduction

CD4 lymphocyte count is the best prognostic indicator of the course of HIV disease and is routinely used to decide when to initiate antiretroviral treatment with zidovudine or *Pneumocystis carinii* pneumonia (PCP) prophylaxis with pentamidine [1–4]. However, its reliability is limited because of considerable variability due to laboratory [5], biological [6] and individual [7,8] factors. In addition, the progression of HIV disease in individuals who at one point had the same CD4+ lymphocyte count may differ substantially. Because of these limitations many studies have investigated other or additional potential prognostic parameters, including presence of free p24 antigen in serum [9–11], loss of anti-p24 antibodies [5,12], serum neopterin [13–16], serum β_2-microglobulin (β_2M) [10,12,17–20] and serum interferon [21] levels. p24 antigen and anti-p24 antibodies are HIV-specific markers; neopterin and β_2M levels may be elevated because of other infec-

From the AIDS Centre, Federal Health Office and *Free University, Berlin, Germany. †See Appendix.

Requests for reprints to: Dr Bernhard Schwartländer, AIDS-Zentrum im BGA, Reichpietschufer 74–76, D-1000 Berlin 30, Germany.

Date of receipt: 9 December 1992; revised: 26 February 1993; accepted: 5 March 1993.

tions or autoimmune and neoplastic diseases [22,23]. Progression to AIDS is also reported to be associated with increased levels of immunoglobulin (Ig) G [24] and IgA [17,19,24] and an increased erythrocyte sedimentation rate (ESR) [17].

None of these additional parameters has the predictive potential of CD4+ lymphocyte count; their predictive value in combination with CD4+ lymphocyte count is controversial [13,14,17,20,21,25–27].

To evaluate whether the prediction of AIDS can be improved by combining different immunological markers, we analysed the predictive value of CD4+ lymphocyte count, β_2M, immunoglobulins and ESR alone and in combination using data from the German multicentre AIDS cohort study.

Patients and methods

Study population

Data on 447 HIV-positive homosexual men from the prospective multicentre cohort study of the Federal Health Office, Germany (which started in October 1984) were analysed. Individuals with other risk behaviours for HIV infection, such as intravenous drug use, were excluded from the study during recruitment. The mean age at enrolment was 34 years (range, 18–65 years). Cohort members are examined at 6-month intervals. Each visit includes medical history, physical examination, and serological and immunological testing. At enrolment 49% of the subjects were asymptomatic, 31% had minor symptoms such as lymphadenopathy, and 20% had AIDS-related conditions. The median observation period was 47 months (5th and 95th percentiles, 6 and 84 months). During the period of observation 163 (36.5%) individuals developed AIDS. Opportunistic infections were the most frequent AIDS manifestations (71.3%), followed by Kaposi's sarcoma (15.3%) and lymphoma (4.9%).

During the period of investigation 37 subjects received zidovudine before developing AIDS, of whom 31 received it for less than 12 months. Twenty-four subjects received primary prophylaxis for PCP, four for more than 12 months.

Laboratory testing

CD4+ lymphocyte counts (flow cytometry) and ESR determinations (in mm after 1 h) were performed at the study centres. β_2M titres (radioimmunoassay; Pharmacia, Freiburg, Germany) and Ig levels were determined at the central laboratory.

Statistical methods

Survival analyses were performed to determine the predictive value of the various parameters, both alone and in combination. The end-point was the onset of AIDS; the starting-points were the times when a specific threshold of a certain parameter was first observed in an individual. Lifetable plots by Cutler and Ederer were used to show the distribution of AIDS-free time for groups with different starting-points.

Since non-parametric product-limit estimators can be biased for heavily censored data, we used a Weibull parametric model for further analysis. Applicability of Weibull family was checked by plotting $\log(\log(-F(t)))$ as a function of $\log(t)$. We determined whether observed differences were statistically significant by comparing the 95% confidence intervals (CI) of the median AIDS-free time. To determine the relative importance of the different covariates, a stepwise regression model was calculated using the statistical software package LIMDEP 6.0 [28].

Results

We first investigated the extent to which defined starting-points were associated with remaining AIDS-free time (Figs 1a–e). AIDS-free time was strongly correlated with CD4+ lymphocyte count. IgG, IgA, β_2M and ESR also had a predictive value (Fig. 1). However, each of these parameters was less powerful in predicting AIDS-free time than CD4+ count. IgM level had no predictive value and was therefore excluded from further analysis.

To analyse whether the predictive power of the different parameters investigated depends on stage of HIV disease, as reflected by the number of CD4+ lymphocytes, subject data were assigned to groups with different starting-points for CD4+ count (< 200, 200–500 and $> 500 \times 10^6$/l).

For each of these CD4+ lymphocyte count groups, we analysed whether introduction of a second parameter improved the predictive power. For this purpose threshold levels of the other parameters were defined on the basis of the observed results (Figs 1a–e) and clinical or immunological experience (IgG > 16.0 g/l, IgA > 3.5 g/l, β_2M > 3.5 mg/l, and ESR > 9 mm after 1 h). We then calculated AIDS-free time for the three CD4+ count groups after stratification according to the thresholds of the additional parameters.

The results are shown in Tables 1–4 and Figs 2–5. β_2M level had an additional predictive value in all CD4+ count groups (Figs 2a–c). In all groups the median AIDS-free time was at least twice as long in subjects with a β_2M level ≤ 3.5 mg/l compared with subjects with a β_2M level > 3.5 mg/l. However, this difference was only statistically significant for the group with CD4+ counts between 200 and 500×10^6/l.

For IgA, there were substantial differences in AIDS-free time between the groups with CD4+ counts < 200 and $> 500 \times 10^6$/l (Figs 4a–c). ESR had an additional predictive value only in the two groups with CD4+ counts $< 500 \times 10^6$/l (Figs 3a–c). The addi-

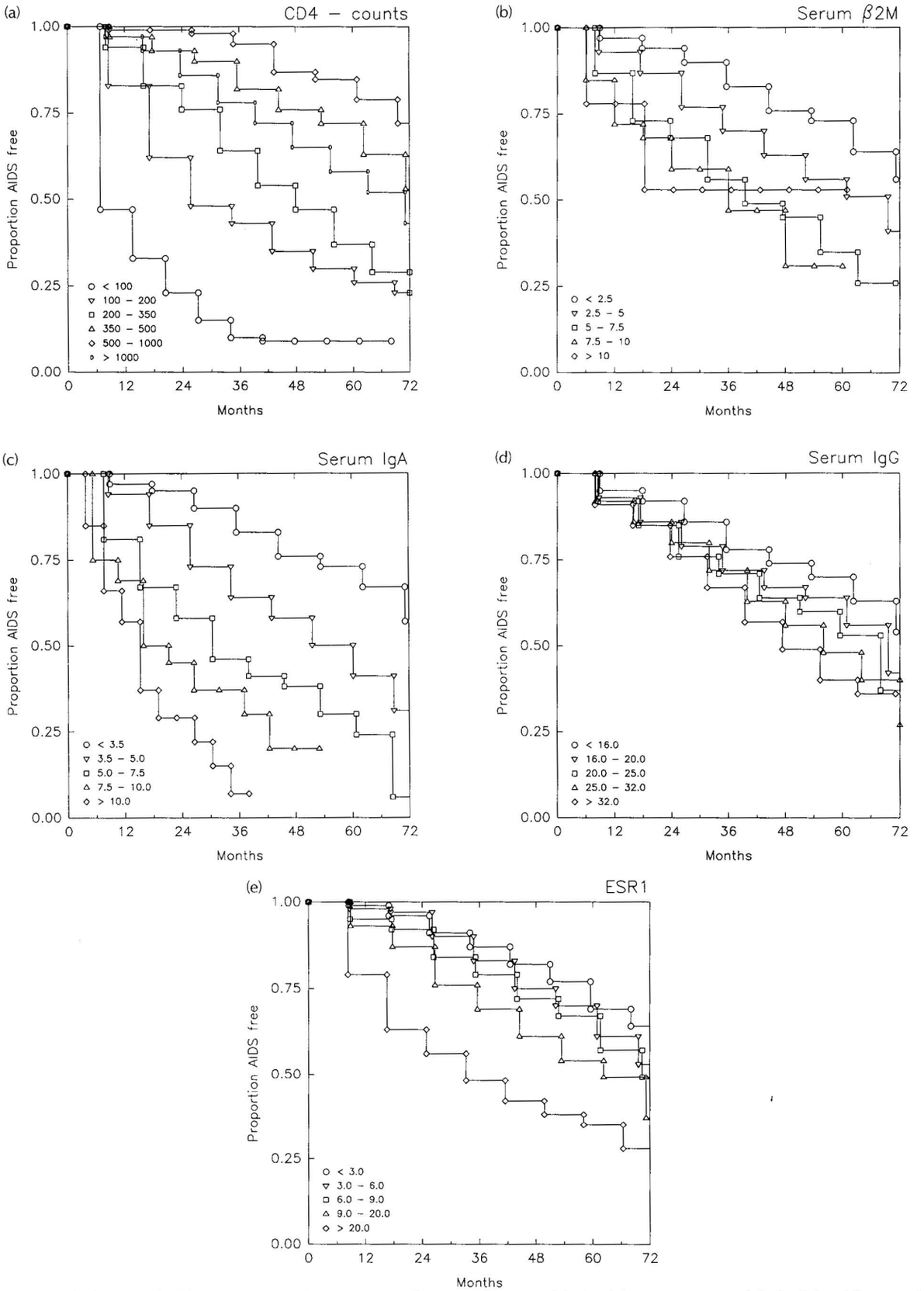

Fig. 1. Survival plots stratified for (a) CD4 lymphocyte count; (b) serum β_2-microglobulin; (c) serum immunoglobulin (Ig) A; (d) serum IgG; (e) erythrocyte sedimentation rate. The end-point for survival analysis was the onset of AIDS; the starting-point was the time when the respective parameter reached a given threshold for the first time in an individual.

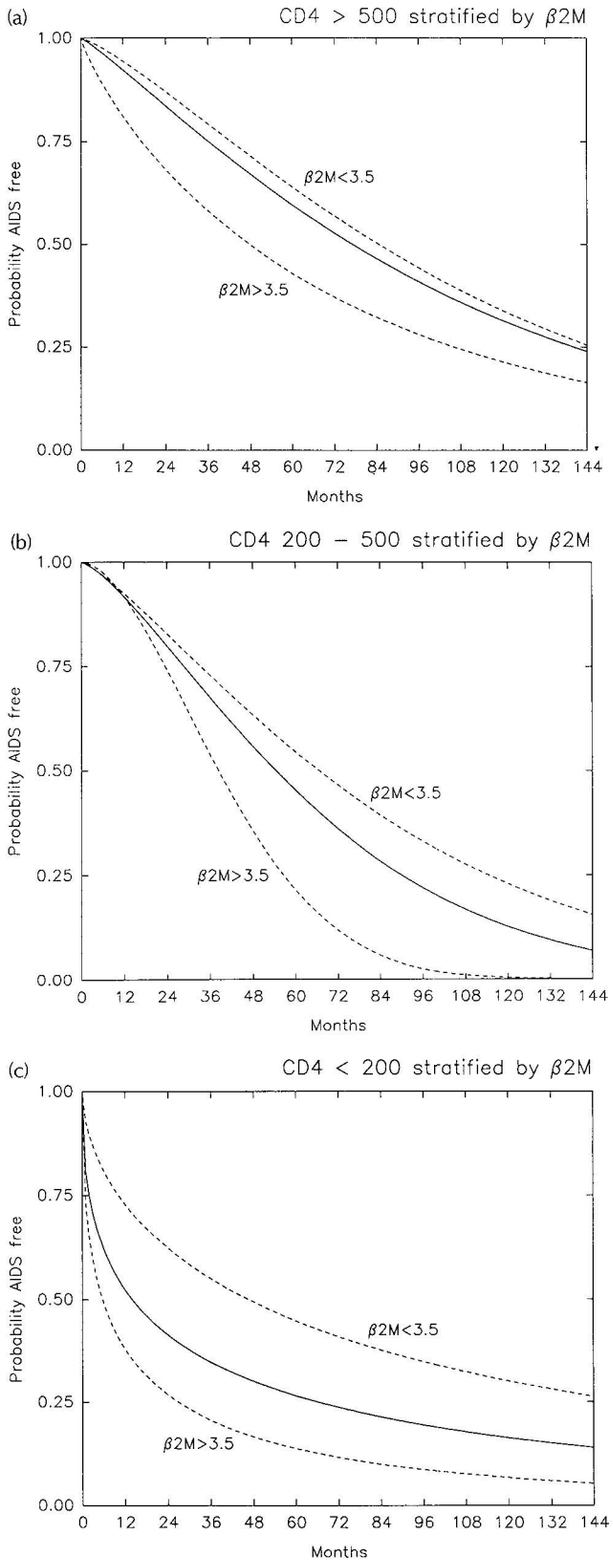

Fig. 2. AIDS-free time (Weibull model) for patients with a CD4+ lymphocyte count > 500 (a), 200–500 (b) and < 200 (c) × 10^6/l stratified for given levels of β_2-microglobulin (β_2M; mg/l) at baseline.

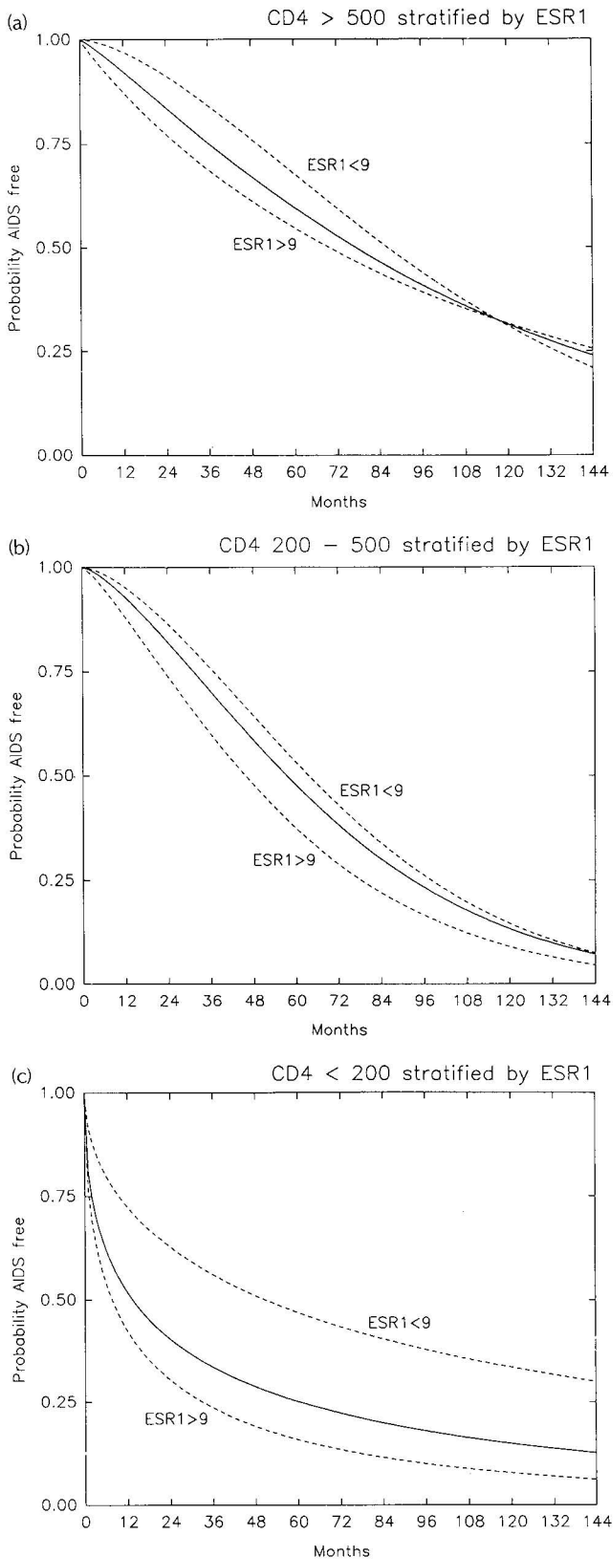

Fig. 3. AIDS-free time (Weibull model) for patients with a CD4+ lymphocyte count > 500 (a), 200–500 (b) and < 200 (c) × 10^6/l stratified for given erythrocyte sedimentation rate (ESR) values (mm after 1 h) at baseline.

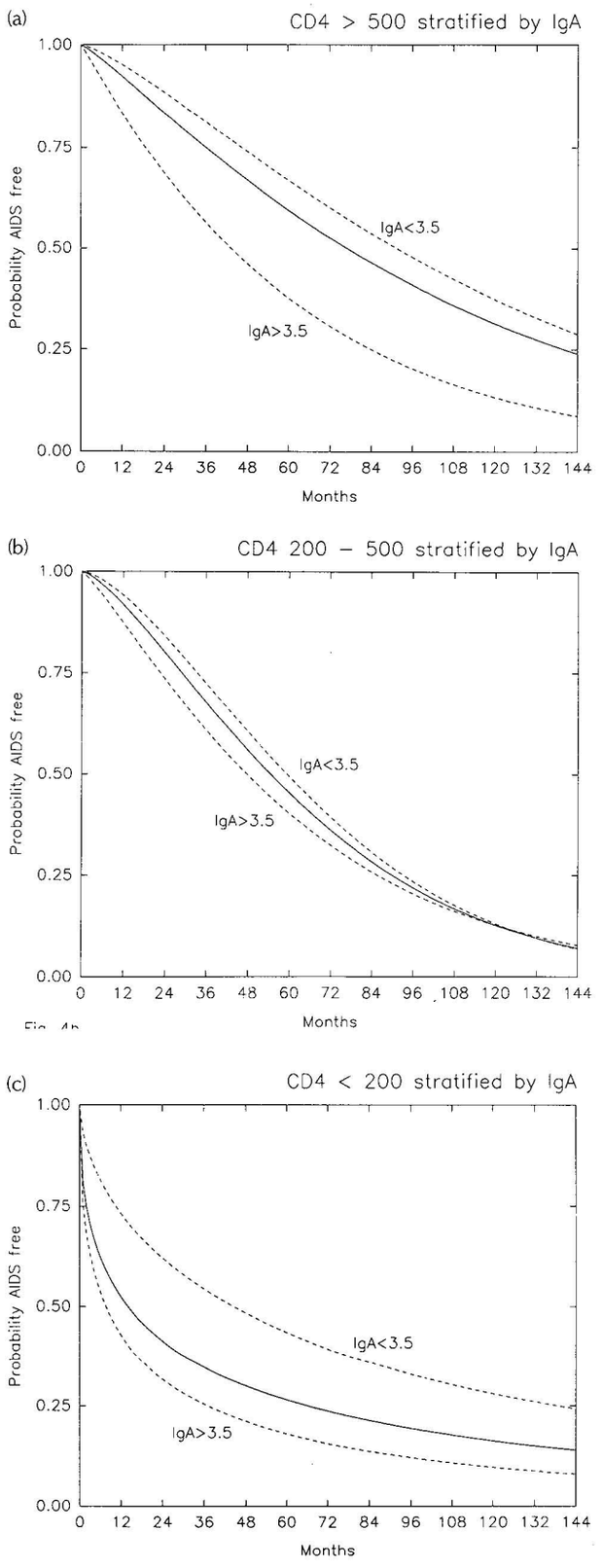

Fig. 4. AIDS-free time (Weibull model) for patients with a CD4+ lymphocyte count > 500 (a), 200–500 (b) and < 200 (c) × 10^6/l stratified for given levels of serum immunoglobulin A (IgA; g/l) at baseline.

tional predictive value of IgG was low in all CD4+ count groups (Figs 5a–c); it was therefore excluded from further analysis.

This first analysis showed that the predictive value of additional parameters varied depending on CD4+ count group. To assess the optimal combination of parameters for each of the three CD4+ count groups, we calculated the probability of developing AIDS using a stepwise regression model with CD4+ count, IgA, β_2M and ESR as independent variables. We evaluated all combinations in order to control for interactive effects between the different parameters.

The results of this analysis are shown in Figs 6a–c. The most powerful predictor for each of the CD4+ count groups was introduced as the first parameter and is shown at the top of the tree. Significant improvement ($P \leq 0.05$) of the model (by addition of a given covariate) is illustrated by solid lines. Dotted lines illustrate combinations that did not significantly improve ($P > 0.05$) the model.

The combination of parameters giving the best prediction of developing AIDS differed in all three CD4+ count groups. Only IgA level had a significant predictive value in the group with CD4+ counts > 500 × 10^6/l. None of the other parameters significantly improved the model (Fig. 6a). In the two groups with CD4+ counts < 500 × 10^6/l, the absolute number of CD4+ cells itself was the most important single predictive parameter (i.e., to predict the probability that a patient with a CD4 lymphocyte count < 200 × 10^6/l will develop AIDS within a given period, it is of greatest significance to know whether that patient's CD4+ count is, for example, 20 or 190 × 10^6/l).

For patients with CD4+ counts between 200 and 500 × 10^6/l, the combination of CD4+ count, β_2M and ESR best predicted progression to AIDS (Fig. 6b). The addition of β_2M or ESR to any combination of the other covariates improved the model significantly.

In the group with CD4+ counts < 200 × 10^6/l, the addition of any of the other covariates to CD4+ count improved the prediction of AIDS significantly (Fig. 6c). As for subjects with CD4+ counts between 200 and 500 × 10^6/l, the most powerful combination was CD4+ count, β_2M and ESR. This combination was not improved by the addition of IgA.

Discussion

Our objective was to determine whether the predictive value of CD4+ lymphocyte count can be improved by the use of additional prognostic markers. Our data show that β_2M, IgA and ESR supply such additional information.

An important result of this study is that the usefulness of the parameters analysed depends on the stage of HIV disease as indicated by CD4+ lymphocyte count.

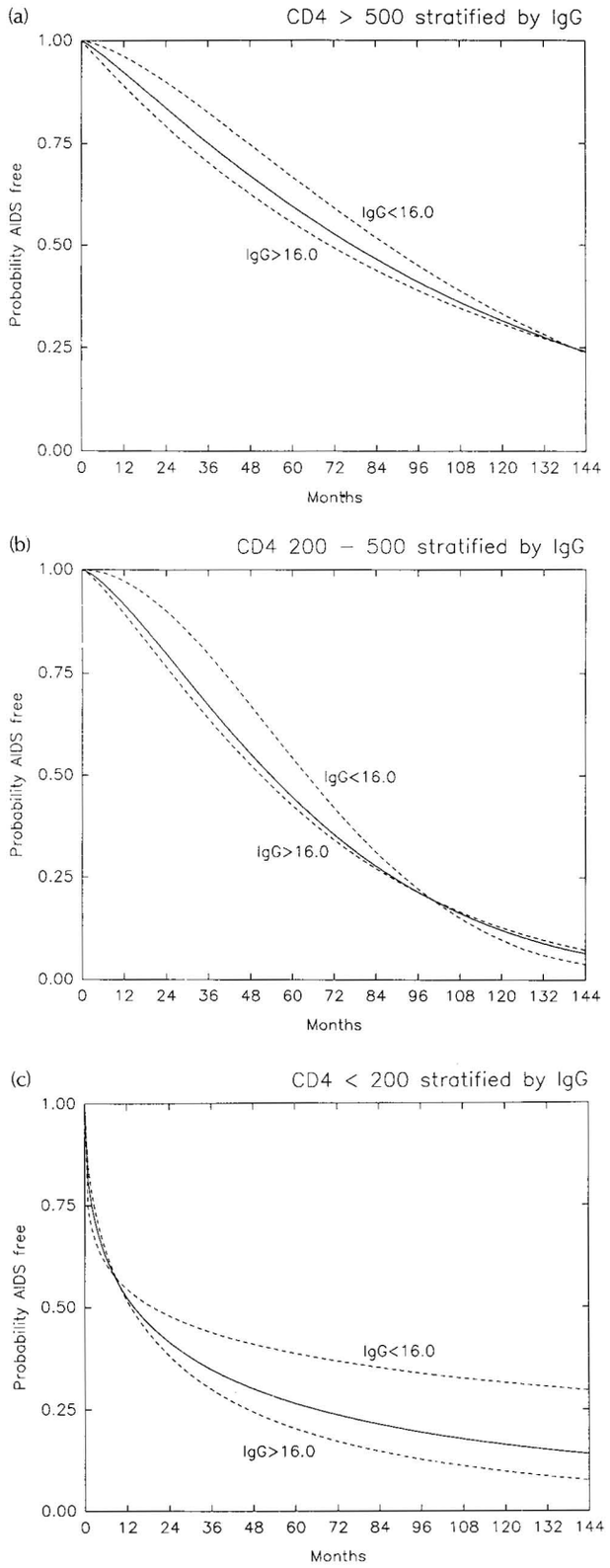

Fig. 5. AIDS-free time (Weibull model) for patients with a CD4+ lymphocyte count > 500 (a), 200–500 (b) and < 200 (c) × 10^6/l stratified for given levels of serum immunoglobulin G (IgG; g/l) at baseline.

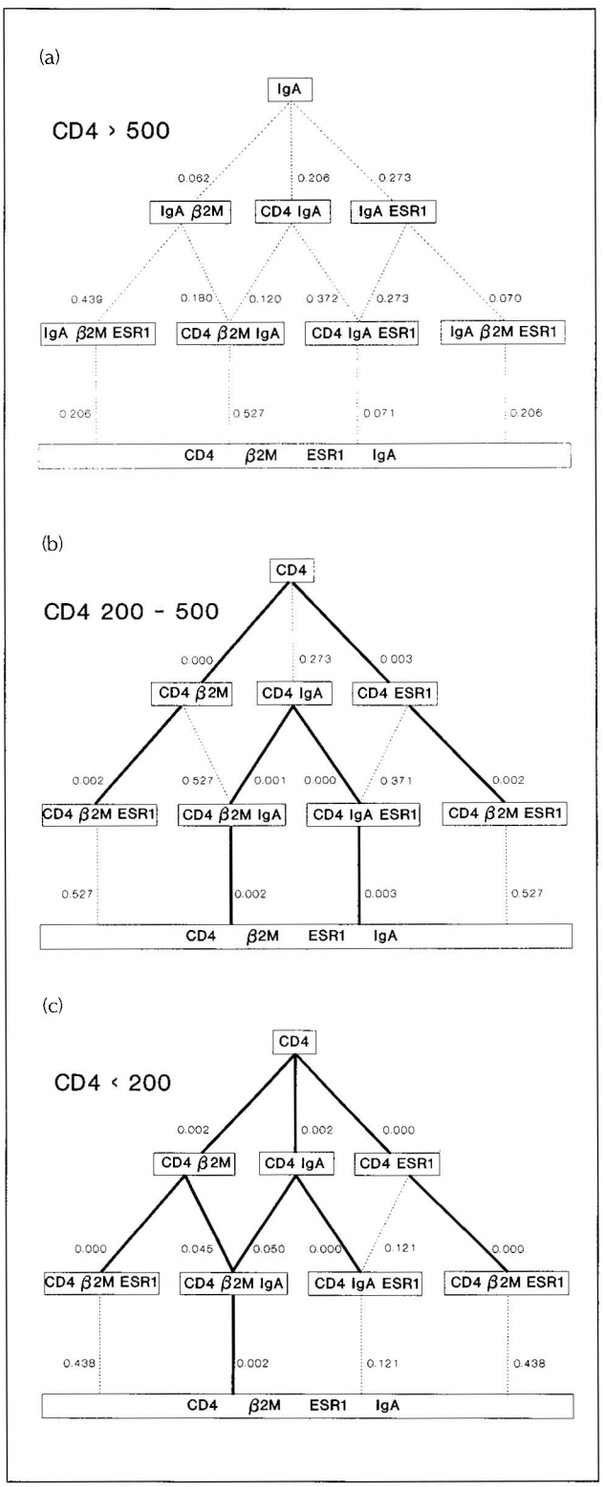

Fig. 6. Evaluation of the predictive value of different combinations of CD4+ lymphocyte count, β_2-microglobulin (β_2M), immunoglobulin A (IgA) and erythrocyte sedimentation rate (ESR) using a multivariate stepwise analysis. The most powerful predictor for each of the CD4+ count groups is shown at the top of the tree. A significant improvement ($P \leq 0.05$) of the model by adding a given covariate is illustrated by solid lines. *P* values are shown next to the lines.

Table 1. Distribution of AIDS-free time (Weibull distribution) stratified by CD4+ lymphocyte count and β_2-microglobulin (β_2M).

CD4+ count (× 10^6/l)	β_2M (mg/l)	No.	AIDS-free time (months) 25% percentile	Median (95% CI)	75% percentile
>500		269	146	81 (64–98)	38
	≤3.5	235	146	85 (67–103)	43
	>3.5	34	106	48 (15–81)	17
200–500		258	93	56 (47–65)	30
	≤3.5	193	114	67 (51–82)	34
	>3.5	65	57	38 (31–45)	24
<200		109	66	14 (6–22)	2
	≤3.5	45	154	47 (6–87)	10
	>3.5	64	27	6 (2–10)	1

CI, confidence interval.

Table 2. Distribution of AIDS-free time (Weibull distribution) stratified by CD4+ lymphocyte count and serum immunoglobulin A (IgA).

CD4+ count (× 10^6/l)	IgA (g/l)	No.	AIDS-free time (months) 25% percentile	Median (95% CI)	75% percentile
>500		269	146	81 (64–98)	38
	≤3.5	212	156	92 (71–113)	47
	>3.5	57	84	43 (26–61)	19
200–500		258	93	56 (47–65)	30
	≤3.5	183	93	59 (49–70)	33
	>3.5	75	86	48 (31–64)	23
<200		109	66	14 (6–22)	2
	≤3.5	34	139	45 (3–86)	11
	>3.5	75	37	8 (2–13)	1

CI, confidence interval.

Table 3. Distribution of AIDS-free time (Weibull distribution) stratified by CD4+ lymphocyte count and serum immunoglobulin G (IgG).

CD4+ count (× 10^6/l)	IgG (g/l)	No.	AIDS-free time (months) 25% percentile	Median (95% CI)	75% percentile
>500		269	146	81 (64–98)	38
	≤16.0	132	140	87 (66–109)	48
	>16.0	137	140	71 (48–94)	30
200–500		258	93	56 (47–65)	30
	≤16.0	88	92	64 (48–81)	41
	>16.0	170	88	51 (40–62)	26
<200		109	66	14 (6–22)	2
	≤16.0	25	233	19 (0–58)	1
	>16.0	84	47	13 (6–20)	3

CI, confidence interval.

This finding is in agreement with the results of a recent study of a cohort of homosexual men with known date of seroconversion [21], which analysed the predictive power of different immune markers at specified times after HIV infection. Because the date of seroconversion for HIV-positive individuals is rarely known in a clinical setting, it is difficult to apply these results to most of the patients in clinical care.

Table 4. Distribution of AIDS-free time (Weibull distribution) stratified by CD4+ lymphocyte count and erythrocyte sedimentation rate (ESR).

CD4+ count (× 10^6/l)	ESR (mm after 1 h)	No.	AIDS-free time (months) 25% percentile	Median (95% CI)	75% percentile
>500		420	145	85 (72–98)	43
	≤9	303	122	82 (71–94)	50
	>9	117	183	80 (47–114)	28
200–500		402	93	57 (50–65)	31
	≤9	249	94	63 (54–73)	39
	>9	153	83	47 (37–57)	23
<200		170	60	13 (7–19)	2
	≤9	50	183	49 (3–95)	9
	>9	120	34	8 (4–12)	1

CI, confidence interval.

We stratified subjects according to the time when their CD4+ lymphocyte count first fell below a specified threshold. Since measurement of CD4+ count of HIV-positive individuals in clinical care is routine, the results of our study are applicable to the majority of patients. Controversial results from previous studies on the predictive value of the parameters analysed may in part be explained by lack of stratification according to the stage of HIV disease.

The most important parameter for the prediction of AIDS was absolute number of CD4+ cells for subjects with a CD4+ lymphocyte count $< 500 \times 10^6$/l, whereas only serum IgA level correlated with AIDS-free time in subjects with a CD4+ count $> 500 \times 10^6$/l.

In patients with CD4+ counts $< 500 \times 10^6$/l the additional parameters improved the predictive power of the absolute number of CD4+ cells significantly. The best combination was CD4+ count, β_2M and ESR.

There is no clear explanation why serum IgA level had a predictive value in patients with higher CD4+ counts. It may reflect non-specific stimulation of the humoral immune system, rather than being a consequence of early mucosal infections. Increased levels of β_2M are associated with cell destruction, signalling the multiplication of the virus or other cytocidal events, such as autoimmune phenomena or inflammatory processes. Increased ESR may simply indicate advanced immune deficiency and the ensuing increasingly severe opportunistic infections. Infections such as oral candidiasis have been shown to predict progression to AIDS [29]. In our study analyses using such 'minor' opportunistic infections as indicators of AIDS-free time did not show significant results. A possible reason for this could be the difficulty in quantifying the severity of these infections and/or confounders such as self-medication (not uncommon in the homosexual community in Germany).

Antiretroviral therapy and prophylaxis should be taken into account when estimating AIDS-free time. Our results were not altered by exclusion of the small number of subjects who had received zidovudine and/or pentamidine prophylaxis. Because of their small number, comparison of patients who had received antiretroviral therapy and/or prophylaxis with patients who had not was not meaningful. However, several studies have shown that zidovudine and pentamidine prolong AIDS-free time [30–32]; groups of subjects where the majority have received zidovudine or primary prophylaxis of opportunistic infections may take a significantly longer time to develop AIDS. Median AIDS-free times and the prognostic values of the immunological markers calculated in our study should be considered baseline values for untreated patients.

Recent studies have shown that elevated β_2M levels decline on zidovudine treatment [33–36]. Further investigations are necessary to determine whether such parameters are useful predictors of AIDS-free time in patients receiving treatment.

The determination of serum IgA, β_2M and ESR in addition to CD4+ lymphocyte count may aid the choice of specific therapeutic regimens or systems of care — for example, when (if at all) a subject should begin to take zidovudine or other antiretroviral compounds.

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Appendix

The multicentre cohort study group of the Federal Health Office, Germany comprises: B. Friedrich-Jänicke, Landesinstitut für Tropenmedizin, Berlin; E.B. Helm, Zentrum Innere Medizin der Universität, Frankfurt/Main; S. Schwander, Bernhard Nocht Institut, Hamburg; R. Gerdelmann, Staatl. Medizinaluntersuchungsamt, Hannover; H. Rasokat, Universität Hautklinik, Köln; E. Thoma, Dermatologische Klinik der Universität, München; J. Bogner, Med. Poliklinik der Universität, München; F.E. Krapf, Immunologisches Institut der Universität, Erlangen, Germany.