Immunologic markers of AIDS progression: consistency across five HIV-infected cohorts

Multicohort Analysis Project Workshop. Part I.

Objective: To provide background on five HIV-infected cohorts with documented seroconversion times and serum immunoglobulin (Ig) A and β_2-microglobulin (β_2M), CD4+ cell count and haemoglobin levels. To give a relative risks (RR) regression summary of the prognostic value of serial CD4+ cell count, IgA, β_2M and haemoglobin measurements for clinical AIDS, and to examine whether cofactors such as current age, sex and exposure category affect these RR.

Design: The Multicohort Analysis Project (MAP) workshop was an international collaboration which brought statisticians, immunologists and clinicians from the five cohorts to work together for 10 days. A predefined restricted database was made available by each cohort for the workshop.

Setting: The Medical Research Council (MRC) Biostatistics Unit, Cambridge, UK hosted the MAP workshop from 19 to 30 April 1993.

Subjects: MAP workshop database comprised 1744 patients with documented HIV seroconversion times, with 407 women, over 900 injecting drug users (IDU) and over 500 homosexual men; 363 patients had AIDS and there were 308 deaths.

Main outcome measures: Descriptive statistics on survival and progression to clinical AIDS by cohort and exposure category, CD4+ cell count at AIDS diagnosis and pre-AIDS zidovudine therapy. RR summarizing the joint prognostic significance of serial markers and cofactors such as age, sex and exposure category for progression to clinical AIDS.

Results: Slower progression to AIDS for IDU [95% confidence interval (CI), 0.35–0.71] and heterosexuals (95% CI, 0.19–0.98) compared with homosexual men was confirmed after adjusting for current age-group and serial CD4+ cell counts. CD4+ cell counts at AIDS diagnosis were much higher among homosexual men before than after 1988 (median, 150 and 90×10^6/l, respectively). Little zidovudine use was observed among AIDS cases diagnosed before 1988 (2%) but increased use was recorded after 1988 and 1989 (24%) and even greater use after 1990 (59%). Low serial CD4+ cell count, haemoglobin levels and high serum IgA and β_2M levels were associated with an increased risk of progression to AIDS. CD4+ cell count always provided prognostic information in addition to other markers; IgA and β_2M (95% CI, 1.23–1.50 and 105–1.51, respectively) were jointly prognostic. β_2M did not provide significant extra information (95% CI, 0.91–1.47) to the combination of serial CD4+ cell count and IgA, although haemoglobin did (95% CI: 0.74–0.91 for 10 g/l increase in haemoglobin). Interactions between cofactors, particularly exposure category and serial markers, were used to test for modifications in RR. The association between AIDS risk and serial CD4+ cell count was weaker, and with elevated IgA stronger, for homosexual men; RR associated with high β_2M values were lower for IDU, in whom β_2M may be elevated for reasons other than HIV disease.

Conclusions: IgA and β_2M, which can be measured in small volumes of stored blood, are jointly predictive of progression to AIDS. Results were broadly consistent between cohorts representing different age-groups, seroconversion periods and exposure categories. Some regression effect modifications by exposure category were noted, however, which merit further independent study.

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See Appendix for participants.

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Introduction

AIDS diagnoses have provided most of the available information on past HIV infection rates [1–5]. Because of the long incubation period between HIV infection and AIDS and the limited knowledge about recent infection rates, unlinked anonymous HIV testing programmes [6] have been established to provide information on annual HIV seroprevalence in sentinel groups [2]. However, HIV seroincidence rates are of greater interest and could be calculated, avoiding the bias inherent in self-referral testing, by unlinked anonymous testing programmes if infection markers for estimating the duration of seropositivity could be identified. At best, only stored sera are available from unlinked anonymous testing programmes, usually as single not serial blood samples. Any potential marker must therefore be measurable in stored plasma, ruling out the CD4+ lymphocyte count [7–12], which has received the most attention to date.

Candidate markers for surveillance must be inexpensive, measurable in the typical residual serum volume of <0.5 ml, and exhibit a monotone (increasing or decreasing) trend from HIV infection to AIDS diagnosis that varies little between populations and exposure categories. Likely candidates are serum immunoglobulin (Ig) A [13] and serum β_2-microglobulin (β_2M) [14].

The Multicohort Analysis Project (MAP) workshop, held in Cambridge, UK for 10 days in April 1993 and attended by statisticians, immunologists and clinicians aimed to assess the suitability of these markers in terms of pattern of change during HIV infection, universality of results and applicable methods. A limited database from a number of patient cohorts, in which CD4+ cell count IgA, β_2M, haemoglobin levels and seroconversion times had been recorded, was used by the workshop to assess the usefulness of candidate markers for seroincidence studies. The choice of MAP cohorts was intended to be representative rather than exhaustive. Only consistent findings over a number of cohorts, representing a variety of recruitment strategies and exposure groups, could provide a sound basis for the extension of seroprevalence programmes. Since population monitoring was the goal, the explanatory variables (or cofactors) were limited to those typically available in anonymous HIV seroprevalence studies; i.e., age group, sex, geographical location and exposure category.

Part I of the MAP workshop provides background on the cohorts used, descriptive statistics on the specific data sets, including patterns of zidovudine use over time, a relative risks (RR) regression summary of the prognostic value of serial CD4+ cell count, IgA, β_2M and haemoglobin measurements, and whether cofactors affect these RR.

Methods

Participating cohorts

Five cohorts provided data for the workshop: the Edinburgh City Hospital cohort, the Italian Seroconversion cohort, the London Royal Free Hospital Haemophilia cohort, the National Cancer Institute (NCI) cohort, and the Toronto Sexual Contact study cohort.

Edinburgh City Hospital cohort

Recruitment began in late 1984 and by 1992 the cohort consisted of 577 individuals treated at Edinburgh City Hospital, Edinburgh, UK [15]. The earliest HIV infection among Edinburgh's injecting drug users (IDU) was identified in January 1983. Knowledge of drug-using behaviour and sexual contacts, and retrospective testing of sera stored since 1983, provided information on the likely seroconversion intervals. By the start of 1993, a subset of 364 patients had well estimated seroconversion dates, including 129 (35%) women. Median age at seroconversion was 23 years and the modal year of seroconversion was 1983. The midpoints of seroconversion intervals (maximum width, 24 months) were used to date HIV infection.

Overall, 91% of the seroconversion (sub)cohort used in the workshop were IDU. Haematologic and immunologic monitoring of the cohort was extensive; blood was taken at most clinic visits (average intervisit time of 3.5 months), and patient profiles were developed after many visits. In addition, some retrospective infilling of marker data was performed for markers such as IgA and β_2M, which were not initially measured routinely, but for which stored sera could be used subsequently. Ascertainment of AIDS cases and deaths in this cohort is believed to be accurate because cases are localized in certain areas of Edinburgh where they are well known to general practitioners. The main AIDS-care facilities are also concentrated at the City Hospital and flagging of patients lost-to follow-up for at least a year with the Registrar General for Scotland ensures that most AIDS cases and deaths are identified. The data file for the MAP workshop was closed on 10 March 1993.

Italian seroconversion cohort

This cohort consisted of 1004 individuals enrolled at 16 Italian clinical centres with an available HIV-negative test and a subsequent positive test less than 2 years later [16–18]. About 60% of these seroconverters were IDU, 25% were homosexual men, 15% were infected through heterosexual contacts, and 25% were female. Median age at seroconversion was 26 years (range, 14–60 years). Homosexual men were older (median 32 years) than IDU (median, 24 years) and heterosexual contacts (median, 26 years).

Each patient's date of seroconversion was estimated as the midpoint between the last negative and first

positive test. The median lag was 7 months and there were no differences in lag times between different risk, sex and age groups. The median year of seroconversion was 1987 (range, 1980–1992).

Patients were seen approximately every 6 months for clinical and laboratory review. CD4+ cell count and CD8+ cell counts, serum β_2M, serum IgA levels and other biological markers are measured repeatedly, although not at every visit. A cross check with the National AIDS Registry is performed every year in order to rule out AIDS diagnosis in subjects lost to follow-up. Vital status was also ascertained by reviewing patient records from the registry office of the municipality of residence and the National AIDS registry linkage. The last date of Registry review was 31 March 1993, but to allow for reporting delays to the Registry, the cohort was censored at the end of December 1992.

London Royal Free Hospital haemophilia cohort

This cohort consisted of 111 men with haemophilia registered at the Royal Free Hospital Haemophilia Centre, who seroconverted to HIV after receiving infected blood clotting factor concentrate between October 1979 and July 1985 [10,19,20]. Because of their haemophilia, the patients have had blood regularly stored. Consequently, it was possible to test serum samples retrospectively and to estimate dates of seroconversion for all patients.

For 63 patients, the dates of their last negative and first positive HIV tests were available. Seroconversion dates for these patients were estimated as the midpoint between the first positive and last negative test. For the remaining 48 patients, no HIV-negative test was available. For 36 of these patients, their seroconversion date was estimated as the midpoint between October 1979 (the presumed first possible date of infection) and the date of their first HIV-positive test result. For the remaining 12 patients, the date of their first HIV-positive test was taken to be the last possible date of infection, i.e., the time that heat-treated clotting factor concentrate was introduced. Seroconversion dates were thus estimated as September 1982, i.e., mid-way between October 1979 and July 1985. The patients were aged between 2 and 77 years (median 24 years) at seroconversion. For comparison with the other MAP cohorts, patients under 16 years of age were excluded, leaving 86 subjects for analysis. Patients were seen approximately every 3–6 months for clinical and laboratory review, and were followed until 31 December 1992. From 1982, absolute CD4+ cell and CD8+ cell counts were measured at each visit and serum IgA, IgG and IgM levels were recorded. Routine serum β_2M measurement began in December 1990.

NCI cohort

This cohort consisted of 131 homosexual men consecutively enrolled in Spring 1982 by three primary care physicians in Manhattan (New York City) and Washington, DC, [21] and followed for the purposes of this analysis until 31 December 1992. White blood cell counts, lymphocyte counts and CD4+ cell and CD8 cell counts were measured at yearly follow-up visits. Neopterin and β_2M (but not IgA) were measured retrospectively in stored sera [22].

The men were classified into three groups. Group 1 consisted of 47 men who were HIV-negative at enrolment and seroconverted during the study. For these patients, the date of seroconversion was estimated as the midpoint between the most recent negative test and subsequent positive test. Groups 2 and 3 consisted of 42 HIV-positive men from Washington, DC, and New York City, respectively. For these two groups of prevalent positives, backcalculation probabilities were used to estimate an average seroconversion date of mid-1981 for group 2 and mid-1980 for group 3. The rates of progression to AIDS for individuals in the three different groups were found to be virtually identical [23].

Toronto Sexual Contact Study cohort

This cohort resulted from a single centre longitudinal study of homosexual and bisexual men in Toronto, Ontario, Canada [24,25]. The cohort members were sexual contacts of men diagnosed with AIDS or AIDS-related complex ('primary cases'). Between July 1984 and July 1985, 249 men were enrolled in the study. Of these, 143 were seropositive at enrolment and 16 seroconverted during the study. For men who were seropositive at enrolment, the date of seroconversion was taken as the date of first sexual contact with the primary case. For men who seroconverted during the study, the date of seroconversion was taken as 3 months prior to the visit at which the individual first tested positive. At 3-monthly intervals, the men were interviewed about their sexual behaviours and use of drugs and alcohol, a medical examination was performed and blood was drawn for serologic testing. Complete data are available for these patients to 15 July 1991.

Data collection

The following data were available for each patient from all five cohorts: estimated date of HIV seroconversion, dates of last negative and first positive HIV tests where available, date of enrolment into the study cohort, age at seroconversion, sex, exposure category, dates of AIDS diagnosis, death or last follow-up in the absence of these, and, where applicable, the date of initiation of zidovudine therapy. The date at each patient visit and any available CD4+ and CD8 lymphocyte counts, IgA in μg/l (not performed in NCI cohort) and β_2M measurements in mg/l (only available at enrolment in Toronto cohort) and, where possible, haemoglobin (g/l) levels were recorded.

Statistical analysis

Diagnosis of clinical AIDS according to the Centers for Disease Control and Prevention (CDC) def-

inition [26] and death by any cause were analysed separately using lifetable methods (see Figs 1 and 2). Deaths prior to AIDS were taken as a censoring event in analysis of AIDS progression. For both endpoints, censoring was otherwise based on the date of last follow-up as outlined in the cohort descriptions. Box and whisker plots (see Fig. 3) were used to summarize the most recent CD4+ cell count at or within 1 year prior to AIDS diagnosis. Boxes show the interquartile range and whiskers show 1.5 times the interquartile range, truncated by the range of observation where necessary.

RR regression [27], a generalization of proportional hazards regression allowing for time-dependent covariates, was used to examine the association between various explanatory variables and the risk of AIDS. Explanatory variables could be fixed cofactors such as sex (RR for women versus men), time-dependent cofactors such as calendar period of follow-up (RR during 1988–1989 when uptake of zidovudine was increasing versus pre-1988), or serially measured markers such as CD4+ cell count which changes over time for individual patients.

We report RR estimates and associated P values together with a 95% confidence interval (CI) for the actual RR. For serially measured markers, the tabulated 95% CI are for the RR associated with an increase of 100×10^6/l CD4+ cell cells, 100 µg/l IgA, 1 mg/l β_2M, or 10 g/l haemoglobin. RR regression is well suited to the examination of serially measured immunologic markers and also accommodates left truncation due to delayed entry into a cohort (the delay between seroconversion and enrolment), censoring, and migration in and out of a cohort over time because of temporary loss to follow-up, for example. The moderating effect of cofactors on marker contributions is explored through interaction terms. A stratified version of RR regression can also be used which allows a different baseline hazard for each cohort, or subcohort in the case of Italy, but regression effects are assumed to be constant across strata (except when interaction terms involving stratum are included).

Logarithmic or other transformation of marker values was avoided. However, an important analysis convention was used, whereby the CD4+ cell count at 3 months preceding current time t was taken as the explanatory variable CD4+ cell count, and similarly for other immunologic markers. This avoids using marker values at the time of AIDS diagnosis. It is unlikely that the 3 month shift is materially important early in the course of HIV disease, but was adopted so that any precipitous marker changes around the time of AIDS diagnosis cannot distort risk relationships.

Thus, patients first entered the risk set 3 months after the cohort visit for which marker values were first available. Marker values were sustained for, at most, 1 year's temporary loss-to-follow-up. Thereafter, the patient was removed from the risk set until subsequent marker information became available.

Results

Demographic data

The numbers of individuals from each cohort used in workshop analyses, including the number of women and IDU in each cohort, are displayed in Table 1. Follow-up information for each cohort is reflected in the numbers of AIDS diagnoses, deaths, and individuals considered lost to follow-up since they did not have a regular cohort visit in the last year of cohort follow-up. This is an overestimation of the loss to follow-up for the Edinburgh, Italian and London cohorts which acquired information on major endpoints through other procedures.

The intensity of follow-up in each cohort and the number of patients with information on CD4+ cell count, IgA and β_2M at five or more visits is shown in Table 2. Less data were available for markers other

Table 1. Description of cohorts included in Multicohort Analysis Project workshop.

Cohort	Analysis cohort (n)	Seroconversion age [years (mean ±SD)]	No. women	No. IDU	AIDS (n)	Deaths (n)	No. with last visit more than 1 year before closure of analysis file
Edinburgh	364	22.9 (5.1)	129	330	62	79	72
Italy*	1004	27.7 (7.7)	278	599	135	86	484
London	86	30.3 (13.7)	0	0	38	40	10
NCI	131	33.7 (6.8)	0	0	78	73	31
Toronto	159	31.0 (6.7)	0	0	50	30	67
Total	1744		407	929	363	308	
Italy*							
IDU	599	25.2 (4.9)	169	599	80	59	
Homosexual	245	33.7 (9.0)	0	0	41	21	
Heterosexual	160	28.2 (8.9)	109	0	14	6	

*Cohort also tabulated by exposure group. NCI, National Cancer Institute; IDU, injecting drug user.

Table 2. Frequency and patterns of marker visits.

Cohort	Seroconversion year	Mean no. visits	Mean interval between visits (months)	No. marker determinations: CD4 visits (%) 5	CD4 visits (%) 10	CD4 visits (%) 20	IgA visits (%) 5	IgA visits (%) 10	β_2M (%) 2	β_2M (%) 5	Total β_2M visits
Edinburgh	1983	20.3	3.5	285 (78)	234 (64)	96 (26)	160 (44)	68 (19)	255	18 (5%)	960
Italy	1986	5.5	8.8	383 (38)	110 (11)	1	116 (12)	21 (2)	349	33 (3%)	1308
London	1982	24.5	4.6	75 (87)	63 (73)	41 (48)	66 (77)	37 (43)	44	27 (31%)	303
NCI	1980–1981	7.4	14.5	50 (38)	2 (2)	0	ND	ND	99	1 (1%)	393
Toronto	1984	14.4	3.7	131 (82)	95 (60)	41 (26)	132 (83)	96 (60)	0	†	135
Italy*											
IDU	1986	4.7	10.3	178 (30)	49 (8)	1	58 (10)	10 (2)	152	21 (4%)	635
Homosexual	1986	7.2	6.9	136 (56)	46 (19)	0	33 (13)	7 (3)	132	8 (3%)	446
Heterosexual	1988	5.7	6.9	69 (43)	17 (11)	0	25 (16)	4 (2)	39	4 (2%)	227

*Cohort also tabulated by exposure group; †at enrolment only. NCI, National Cancer Institute; IDU, injecting drug user; ND, not done.

than CD4+ cell count. Because the number of visits was related to patient survival, patients with longer survival time were expected to have more measurements than those with shorter survival time. In addition, the interval between visits also determined the number of marker visits (Table 2).

Progression to AIDS and survival

Figure 1a shows Kaplan–Meier survival curves by cohort. While survival was longer in the Edinburgh and Italian cohorts, both consisted predominantly of IDU who tend to be younger at seroconversion (Fig. 2a). The cohorts of homosexual men, in particular the NCI cohort, had significantly poorer survival. The general pattern for progression to AIDS (Fig. 1b by cohort and 2b by exposure groups) was similar to that for mortality; the IDU cohorts appeared to be slower progressors, while the cohorts of homosexual men progressed more quickly. The confounding effect of age is addressed in later RR regression. There were few events (AIDS diagnoses or deaths) among heterosexuals in any of the cohorts, making it difficult to draw conclusions about this exposure group. Analysis for endpoints involving CD4+ cell counts resulted in similar patterns by exposure group [28].

CD4+ cell counts at AIDS

Figure 3 illustrates the most recent CD4+ cell count at or within 1 year prior to AIDS diagnoses, by cohort, and for homosexual men, by year of AIDS diagnosis. Higher CD4+ cell counts at AIDS were observed in patients from the NCI and Toronto cohorts, approximately 30% of whom had developed Kaposi's sarcoma as their first AIDS manifestation. A particularly strong trend in CD4+ cell counts at AIDS diagnosis by year was observed among homosexual men. Prior to 1988, CD4+ cell counts at AIDS diagnosis were much higher in this group (median, 150×10^6/l) than from 1988 onwards (median, 90×10^6/l).

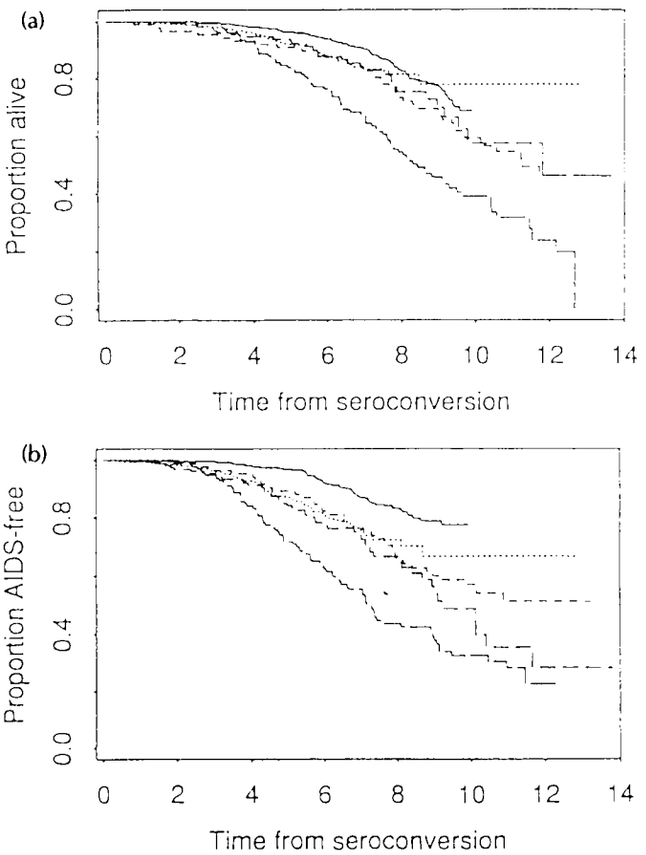

Fig. 1. (a) Survival, time from seroconversion; (b) progression to AIDS, time from seroconversion. (——), Edinburgh; (.....), Italy; (- - - -), London; (– – –), Toronto; (— —), National Cancer Institute).

Patterns of treatment use over time

Treatment was predominantly non-randomized; however, its use may be of importance in interpreting marker values and prognosis, and should therefore be considered. Table 3 shows the number of AIDS cases diagnosed in various calendar periods and

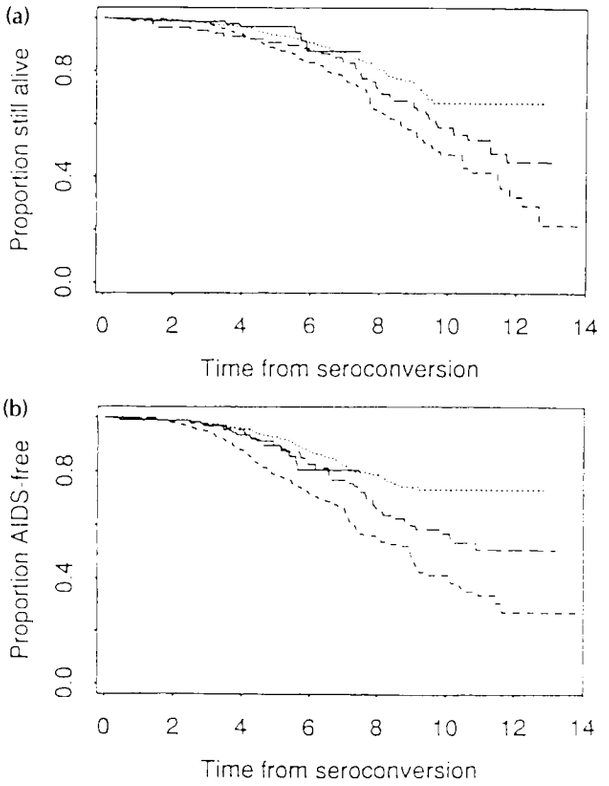

Fig. 2. (a) Survival, time from seroconversion; (b) progression to AIDS, time from seroconversion. (- - - - .), homosexuals; (.....), intravenous drug users; (——), heterosexuals; (– – –), haemophiliacs.

how many had received zidovudine treatment prior to diagnosis for each cohort. In 1987 antiviral treatment became available for HIV-infected individuals with CD4+ cell counts $< 200 \times 10^6$/l. Very little zidovudine use was observed in AIDS cases diagnosed before 1988, but increasing use was seen in those diagnosed after 1988 and 1989. Information on other treatments or prophylaxis for opportunistic infections was not available at the MAP workshop, but during the periods studied most treatment regimens would have included zidovudine.

For patients with CD4+ cell counts $> 500 \times 10^6$/l, zidovudine use was limited. For patients with lower CD4+ cell counts and AIDS, use increased with calendar time. From 1988 to 1989 about 50% of individuals with counts $< 200 \times 10^6$/l received zidovudine. Since 1990, it has become even more common for individuals with low CD4+ cell counts to receive treatment; the use of zidovudine in patients with CD4+ cell counts $200–499 \times 10^6$/l also increased, particularly among the Edinburgh cohort. In summary, the grouping of calendar time into 1987 or before, 1988–1989 and 1990 or later may provide a useful indicator of changes in patient management for RR regression.

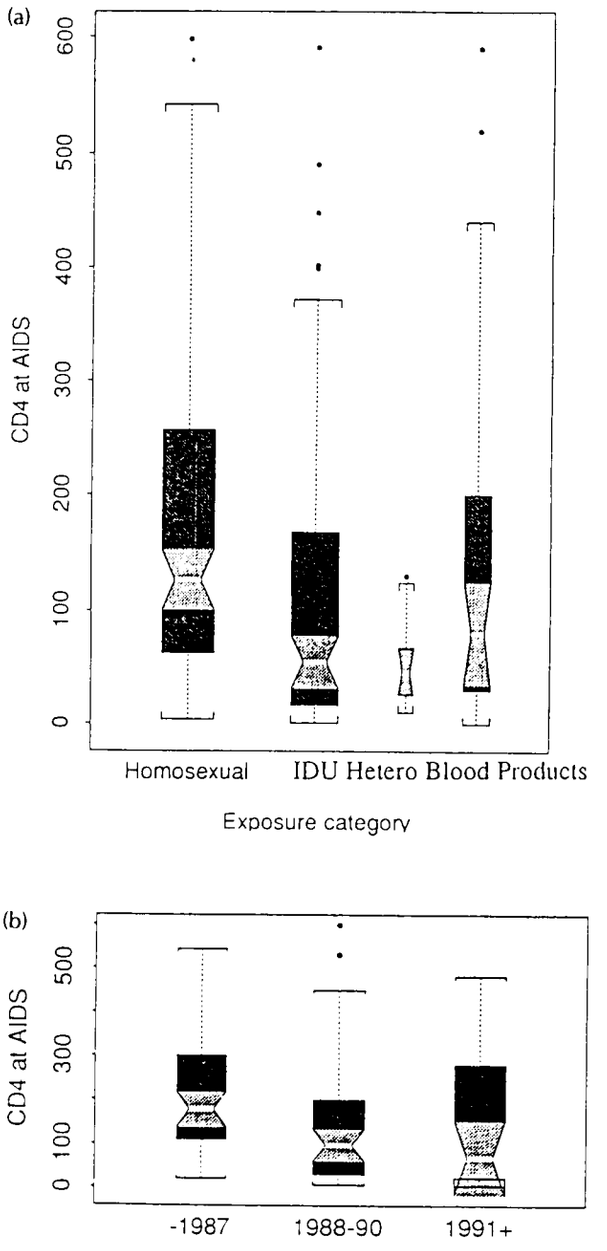

Fig. 3. CD4 counts at AIDS diagnosis by risk by (a) exposure category. (b) Homosexuals only. IDU, injecting drug user. [Misalignment of the notches on different boxes indicates significant differences between medians (MINITAB Reference Manual, Release 8); box width is proportional to number of patients.]

RR regression

We used RR regression to determine whether IgA and β_2M levels, alone or in combination, influence AIDS progression. Their prognostic value, distinct from the well established marker of CD4+ cell count, is of particular interest. The role of haemoglobin was also briefly investigated since there was some

Table 3. Number of AIDS cases who had previously received zidovudine by calendar year of AIDS diagnosis.

	No. AIDS cases (% with zidovudine)			
	1987 or before	1988	1989	1990 or later
Edinburgh	4 (0)	5 (20)	16 (44)	37 (78)
Italy	7 (0)	9 (11)	16 (13)	104 (46)
London	16 (6)	9 (6)	4 (25)	9 (89)
NCI	47 (2)	7 (43)	8 (25)	16 (69)
Toronto	26 (0)	10 (0)	5 (40)	9 (78)
Total	100 (2)	40 (18)	48 (29)	175 (59)

NCI, National Cancer Institute.

evidence from the Edinburgh cohort to suggest that this marker may influence progression to AIDS [29]. The effects of sex, current age group and calendar year on patients' risk scores were also examined because this information is usually available from anonymous serosurveillance programmes.

Cofactors

We examined the four cofactors (age, sex, calendar year and exposure group) singly, and then jointly with and without consideration of serial CD4+ cell counts. Stratification by cohort was not incorporated since exposure category was effectively an alias for our stratum definition. In single cofactor analyses, an increasing rate of progression to AIDS was seen with older current age (Table 4). Calendar year and sex had no major effects, although there was some evidence for slower progression among women. The Kaplan–Meier plots, showing slower progression rates for IDU and heterosexual populations than homosexual men, were confirmed. Moreover, these differences in progression by exposure group were also present in multifactorial models after adjustment for age, and after adjustment for age and CD4+ cell count. Age, although significantly related to AIDS progression after adjustment for exposure category, had no demonstrable effect after adjustment for both serial CD4+ cell count and exposure group.

Serial markers

Three-month lagged marker values (CD4+ cell count, IgA, β_2M) were each studied as predictors of AIDS progression for each cohort (Table 5). There were consistently significant relationships across the cohorts for all markers. Low CD4+ cell count and haemoglobin levels and high IgA and β_2M levels were associated with an increased risk of progression to AIDS. From the NCI cohort, the coefficient for CD4+ cell count was somewhat smaller and that for β_2M somewhat larger than those for the other cohorts. This cohort of homosexual men seroconverted earlier than the others, which may be an important factor in interpreting its CD4+ cell results. It was the only non-IDU cohort with substantial information on β_2M.

The β_2M results warrant additional comment. Significant relationships were observed in the three cohorts (Edinburgh, Italy and NCI) with most information on serial β_2M values. The London cohort, which had few β_2M measurements until very late in follow-up, generated a wide CI and was not particularly informative. The Italian cohort, which consisted predominantly of IDU, generated a slightly higher coefficient than the Edinburgh, mainly IDU, cohort but contained three exposure groups. The coefficient for the IDU subcohort of the Italian data was more consistent with the Edinburgh cohort (Table 5). Because β_2M values are known to be higher in IDU [30] than

Table 4. Cofactor analyses.

	RR [significance level] (95% CI)		
Cofactors	Singly (257 AIDS diagnoses)	Jointly (257 AIDS diagnoses)	Jointly with CD4 count (251 AIDS diagnoses)
Exposure group (baseline: homosexual men)			
IDU	0.46 [0.000] (0.35–0.61)	0.69 [0.038] (0.49–0.98)	0.50 [0.000] (0.35–0.71)
Heterosexual	0.34 [0.006] (0.16–0.73)	0.53 [0.134] (0.24–1.21)	0.43 [0.045] (0.19–0.98)
Blood	0.77 [0.183] (0.53–1.13)	0.94 [0.756] (0.63–1.40)	0.84 [0.392] (0.56–1.26)
Age (baseline: under 25)			
25–29	1.58 [0.092] (0.93–2.68)	1.59 [0.084] (0.94–2.68)	1.12 [0.683] (0.66–1.90)
30–34	2.08 [0.007] (1.22–3.56)	2.09 [0.006] (1.24–3.55)	1.27 [0.384] (0.74–2.17)
35–39	2.16 [0.009] (1.22–3.82)	2.13 [0.009] (1.20–3.76)	1.35 [0.307] (0.76–2.38)
≥40	2.60 [0.001] (1.47–4.60)	2.51 [0.001] (1.43–4.41)	1.38 [0.264] (0.79–2.42)
Sex (baseline: male)			
Female	0.68 [0.100] (0.43–1.08)	0.69 [0.103] (0.44–1.08)	0.71 [0.137] (0.46–1.12)
Calendar year (baseline: before 1988)			
1988–1989	0.79 [0.273] (0.52–1.20)		
≥1990	1.01 [0.960] (0.64–1.59)		
CD4 count (per 100×10^6/l)			0.50 [0.000] (0.46–0.55)

RR, relative risk; CI, confidence interval; IDU, injecting drug user.

Table 5. Marker analyses per cohort.

	Cohort	RR [significance level]	95% CI	No. AIDS diagnoses
CD4 count	Edinburgh	0.37 [0.000]	(0.28–0.50)	46
100×10⁶/l	Italy	0.49 [0.000]	(0.42–0.58)	76
	London	0.37 [0.000]	(0.27–0.50)	34
	NCI	0.61 [0.000]	(0.51–0.73)	45
	Toronto	0.42 [0.000]	(0.33–0.53)	48
IgA	Edinburgh	1.35 [0.000]	(1.24–1.48)	39
100 µg/l	Italy	1.30 [0.000]	(1.17–1.43)	25
	London	1.22 [0.000]	(1.13–1.33)	30
	NCI	–	–	–
	Toronto	1.48 [0.000]	(1.29–1.70)	48
β_2M	Edinburgh	1.29 [0.016]	(1.05–1.59)	21
mg/l	Italy	1.54 [0.000]	(1.24–1.91)	21
	London	1.31 [0.700]	(0.33–5.20)	3
	NCI	2.23 [0.000]	(1.59–3.14)	30
	Toronto	–	–	–
	Italy IDU	1.32 [0.046]	(1.01–1.73)	13
Hb	Edinburgh	0.75 [0.001]	(0.63–0.88)	46
10 g/l	Italy	0.57 [0.000]	(0.46–0.71)	28
	London	–	–	–
	NCI	–	–	–
	Toronto	0.73 [0.000]	(0.65–0.82)	48

RR, relative risk; CI, confidence interval; Ig, immunoglobulin; β_2M, β_2-microglobulin; Hb, haemoglobulin; NCI, National Cancer Institute; IDU, injecting drug user.

in other exposure groups, separate analyses for this marker would be more appropriate.

Because of the consistency of the results shown in Table 5, the data were then pooled and RR regression was fitted with stratification by cohort (and by exposure group for the Italian data). However, because the NCI cohort results differed from other cohorts (Table 5), this cohort was sometimes dropped for comparison purposes. We examined CD4+ cell count, IgA and β_2M alone and in combination (Table 6). The latter analyses were restricted to the three cohorts with information on all markers (Edinburgh, Italy and London).

Table 6. Marker analyses with stratification by cohort*.

Markers	RR [significance level]	95% CI	No. AIDS diagnoses
Singly			
CD4	0.44 [0.000]	(0.39–0.50)	158
IgA	1.29 [0.000]	(1.22–1.37)	98
β_2M	1.36 [0.000]	(1.18–1.57)	47
Jointly: pairs and triple			
(a) CD4	0.47 [0.000]	(0.39–0.55)	96
IgA	1.14 [0.000]	(1.07–1.21)	
(b) CD4	0.38 [0.000]	(0.28–0.50)	47
β_2M	1.28 [0.013]	(1.05–1.56)	
(c) IgA	1.36 [0.000]	(1.23–1.50)	39
β_2M	1.26 [0.012]	(1.05–1.51)	
(d) CD4	0.45 [0.000]	(0.33–0.61)	39
IgA	1.20 [0.002]	(1.07–1.34)	
β_2M	1.16 [0.234]	(0.91–1.47)	

*Combined data from Edinburgh, Italy, London cohorts. RR, relative risk; CI, confidence interval; Ig, immunoglobulin; β_2M, β_2-microglobulin.

CD4+ cell count always provided significant prognostic information in addition to the other markers; moreover, as reported previously for the Edinburgh cohort alone [29], IgA and β_2M were significant prognostic markers in a bivariate model. While β_2M is still moderately predictive with CD4+ cell count, it did not contribute extra prognostic information to the combination of CD4+ cell count and IgA. There was no variation in marker coefficients when other cofactors (age, sex and calendar year) were included [31].

The potential value of haemoglobin was briefly examined using the Edinburgh, Italian and Toronto cohorts. It added significant explanatory information to serial CD4+ cell counts and IgA. The estimated RR associated with a 10 g/l increase in haemoglobin was 0.82 (95% CI, 0.74–0.91), but because haemoglobin cannot be measured in stored sera, it was not considered further.

Marker effects modified by cofactors

Interactions between cofactors and markers were used to test for regression effect modification. Neither sex nor calendar year was found to modify RR associated with any of the markers. There was a significant interaction between age and β_2M, which indicated that the RR associated with changes in β_2M increased with age, but this was resolved by including an interaction term between the NCI cohort and β_2M [31].

Exposure group was found to modify the effects of all three markers [31]. There was a weaker association between CD4+ cell count and AIDS risk (which included Kaposi's sarcoma) in homosexuals that in or IDU and blood-product recipients. In addition, the risks associated with high β_2M levels were less for IDU than homosexuals; β_2M levels are known to be elevated in IDU compared with other HIV-infected individuals and therefore values may be influenced by other factors. A possible interaction between IgA and HIV infection acquired through sexual intercourse between men was evident; relative AIDS risk associated with increased IgA was higher for homosexual men than for other exposure groups.

Discussion

In interpreting the MAP workshop, the different methods of recruitment and selection of study participants, intensities of follow-up, procedures regarding data collection and management, laboratory procedures for immunologic marker measurements and techniques for estimation of HIV seroconversion dates should be noted. However, intense efforts were made to achieve functional similarities between the data sets used for the various analyses.

Data were only pooled across cohorts for analysis after careful examination of individual cohort analyses. The MAP database comprised 1744 patients with documented seroconversion times (including over 400 women and over 900 HIV-infected IDU); all major exposure categories and age-groups were represented and patients had been immunologically and clinically monitored.

A particularly strong trend in CD4+ cell count at AIDS diagnosis by year of diagnosis was observed in homosexual men. Prior to 1988, CD4+ cell counts at AIDS diagnosis in this group were much higher (median, 150×10^6/l) than from 1988 onwards (median, 90×10^6/l). The homosexual MAP cohorts were the first to be infected, at a time when experience and expertise in the clinical management of HIV infection was limited, and treatment regimens, such as zidovudine, were not available. Moreover, the benefits of pentamidine and cotrimoxazole for *Pneumocystis carinii* pneumonia prophylaxis had not been realised. Our documentation of increased pre-AIDS uptake of zidovudine therapy by calendar year and CD4+ cell count [32] among cohorts provides data for future financial planning. The information is also useful for short-term AIDS predictions using back-calculation [2,33].

Important data from the RR of progression to AIDS emerged from the MAP workshop. Current age and exposure group had separate explanatory value for progression to AIDS when immunologic markers were not fitted; however, after adjustment for CD4+ cell count, only exposure group remained significant with evidence of faster progression to AIDS for homosexual men, perhaps attributable to Kaposi's sarcoma [34]. This is in contrast to the results of Phillips *et al.* [10] who found, in a single cohort including children, that age remained significant after adjusting for CD4+ cell count; and of Pezzotti *et al.* [34] who found, in a single cohort without adjusting for CD4+ cell count, that exposure group effects disappeared after adjusting for age. Further studies are required to determine whether exposure group has a specific effect on AIDS progression which is not caused by differences in the time of seroconversion [34].

In our analyses, RR estimates associated with changed marker values were quite consistent across cohorts apart from some discrepancy noted for estimates corresponding to the NCI cohort. CD4+ cell count was the most informative of the three markers, as has been shown previously [9,25,35] followed by IgA; β_2M was the least informative when all three markers were included in a single model. There may be a lack of power, however, in analyses of β_2M because β_2M levels were measured less frequently than the other markers and less data were available. The larger coefficient associated with β_2M in the NCI cohort may be related to the frequency of Kaposi's sarcoma as the first AIDS manifestation among homosexual men, since β_2M has been shown to be predictive of the development of this cancer [22]. The decline in β_2M, identified in the MAP workshop (see Part II) more than 6 years after seroconversion, might also explain its prognostic value for Kaposi's sarcoma and its overall third ranking as a marker when no allowance (as here) is made for non-linear RR.

Effect modifications due to age were found to disappear when the NCI cohort was eliminated from the analyses and those due to exposure group were less significant but could not be ruled out [31,32].

Treatment, in particular zidovudine, was not incorporated into regression models because treatment was not assigned randomly and the criteria for use may have differed between cohorts [36]. Current observational evidence also suggests that treatment effects are short term for the markers studied.

Differences in the methods used to estimate seroconversion times in the cohorts studied are problematic, but should not affect the qualitative conclusions reached. We have assumed random censoring because of non-AIDS deaths. This may introduce some underestimation of the AIDS risk, particularly in the IDU cohorts where the rate of such events is highest and may also be related to immunosuppression.

After completing Part I, other workshop investigations [32] identified a problem concerning the change in the methods for measuring IgA and β_2M in the Edinburgh cohort during 1990. Although this introduced a shift in the values reported, it should not influence the RR regressions presented here because the Edinburgh cohort had a narrow range of seroconversion times and therefore values compared at any time post-seroconversion were probably measured in the same manner.

Finally, although markers are predictive of progression to AIDS, they are not necessarily useful for estimating time since seroconversion, as required for HIV serosurveillance. This use of the markers is examined in Part II.

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