THE FOLLOW-UP OF A COHORT OF ANTI-HIV SEROPOSITIVE HAEMOPHILIACS FOR UP TO 15 YEARS FROM SEROCONVERSION

THESIS

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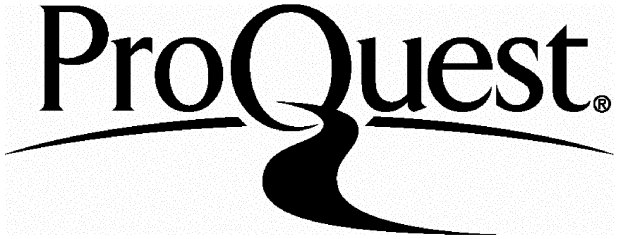

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ABSTRACT

111 men with haemophilia registered at the Royal Free Hospital Haemophilia Centre became infected with HIV between 1979 and 1985 after treatment with infected blood products. These men have been followed for up to 15 years since HIV seroconversion. This thesis presents an epidemiologic follow-up of this cohort of patients.

By the end of 1994, 47 men had developed AIDS and 45 had died, Kaplan-Meier progression rates of 56.5% (95% confidence interval 39.5-73.6) and 46.9% (95% confidence interval 35.6-58.2) by 14 years after seroconversion respectively. Prior to the development of AIDS, 82 of the men had developed at least one more minor condition indicative of their HIV infection. Older individuals and those who seroconverted prior to 1981 and from 1983 onwards appear to have a more rapid progression of disease. The CD4 lymphocyte count, which drops throughout infection, is a strong prognostic marker for disease progression. The rate of CD4 decline, the Immunoglobulin A level and the development of p24 antigenaemia all add some additional prognostic information to that provided by the most recent CD4 count alone. In contrast, the CD8 lymphocyte count simply identifies those individuals with the lowest and most rapidly declining CD4 counts. Whilst the beta-2 microglobulin level appears to provide additional prognostic information to the CD4 count at high CD4 levels, it is of less value at lower counts. The development of a bacterial infection prior to AIDS suggests that a patient's condition is likely to deteriorate, irrespective of their immune status. Despite being the best marker of progression, the CD4 count is, unfortunately, measured imperfectly. This has the effect of reducing the apparent relationship with disease progression and may lead to erroneous conclusions about the value of other covariates in a proportional hazards model.

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1.1 HIV and AIDS

1.1.1 The origins of the AIDS epidemic

From 1981 onwards, increasing numbers of reports of relatively uncommon diseases such as *Pneumocystis carinii* Pneumonia (PCP) and Kaposi's Sarcoma, in young homosexual men prompted concern in the USA[1,2]. These reports coincided with an increase in the number of requests for pentamidine, a drug used in the treatment of PCP, to the Centers for Disease Control (CDC), the agency in the USA responsible for monitoring the use of restricted drugs. In 1982, following an investigation into these cases CDC adopted a definition of what is now called the Acquired Immunodeficiency Syndrome (AIDS)[3]. This original definition included the following:

> *" A person who has had a reliably diagnosed disease that is at least moderately indicative of an underlying cellular immune deficiency (such as an opportunistic infection, or Kaposi's Sarcoma in a person aged less than 60 years), but who, at the same time, has had no known underlying cause of cellular immune deficiency nor any other cause of reduced resistance reported to be associated with that disease."*

AIDS was soon described in other patient groups including blood transfusion recipients[4,5] and haemophilic patients[6]. Subsequently, a number of revisions have been made to the definition, both in terms of the list of diseases used as indicators of underlying immunodeficiency, and to take account of the discovery of the Human Immunodeficiency Virus (HIV) in 1983, thought to be the etiologic causative agent of AIDS[7-9].

More recently a change to the definition was made in 1992[10] when three new AIDS-defining conditions were added to the list along with, for the first time, a diagnosis of severe immunodeficiency in the absence of clinical illness (not adopted in Europe). Details of the conditions which make up the most recent definition, accepted in this country from 1st January 1993, are shown in Table 1.1.

The first case of AIDS in the UK was reported in December 1981[11]. Shortly afterwards, the Public Health Laboratory Service's Communicable Disease Surveillance Centre (CDSC) in collaboration with the Communicable Disease (Scotland) Unit set up a voluntary reporting scheme to monitor the incidence of Kaposi's Sarcoma and opportunistic infections in Britain.

Table 1.1 : Clinical conditions included in the current (1993) CDC definition of AIDS[10]

Candidiasis of bronchi, trachea, or lungs
Candidiasis, oesophageal
Cervical cancer, invasive**
Coccidioidomycosis, disseminated or extrapulmonary
Cryptosporidiosis
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen, or nodes)
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV-related*
Herpes simplex: chronic ulcer(s) (>1 month duration); or bronchitis, pneumonitis, or oesophagitis
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month's duration)
Kaposi's Sarcoma
Lymphoma, Burkitt's
Lymphoma, immunoblastic
Lymphoma, primary, of brain
Mycobacterium avium complex or M. *kansasii*, disseminated or extrapulmonary
Mycobacterium tuberculosis, any site (pulmonary** or extrapulmonary)
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis carinii pneumonia
Pneumonia, recurrent**
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain
Wasting syndrome due to HIV*

* Added in 1987

** Added in 1993

The CDC definition of AIDS was adopted in this country in 1982 and reports of 178 cases of AIDS had been received by CDSC by 1st June 1985[12]. In 1986 a second HIV virus (HIV-2) was isolated[13]. The original, and still most common virus is now frequently referred to as HIV-1.

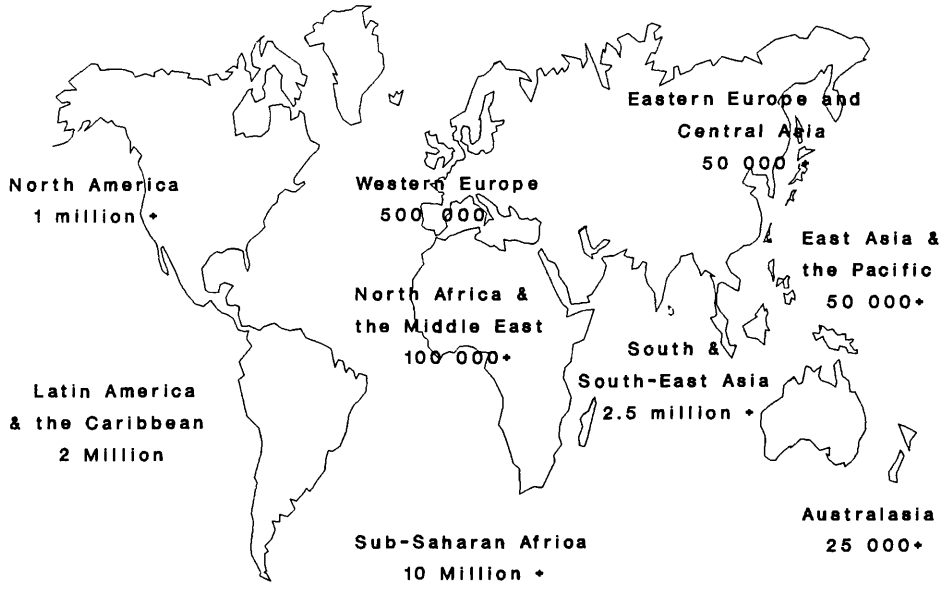
1.1.2 Modes of transmission

HIV is primarily transmitted through body fluids, including blood and semen. Consequently, individuals can be infected through sexual intercourse, sharing of needles, through receipt of contaminated blood or its products, or after needlestick injuries. Because their lifestyle often involves multiple sexual partners and also because of the possibility of a higher risk of transmission through anal rather than vaginal intercourse, homosexual men have been the main group of individuals infected in the UK. However, a large number of intravenous drug users (IVDUs) and blood product recipients are also infected. Amongst children, perinatal transmission is the main cause of infection, with between 9 to 39% of children born to infected mothers becoming infected themselves[14-18].

1.1.3 The growth of the epidemic

By the 29th December 1994, 1,025,073 AIDS cases had been reported to the World Health Organisation[19]. Reported AIDS cases, however, represent only a minority of cases and it is estimated that there have been around 4 million AIDS cases in the world since the epidemic began[20]. In addition, a further 16 million adults and one million children are estimated to be infected with HIV[20]. Figure 1.1 illustrates the distribution of estimated HIV infections throughout the world. Sub-Saharan Africa is thought to have the majority of infections, with over ten million individuals thought to be infected with HIV. Transmission is mainly by heterosexual contact or from mother to child. In West Africa especially, many individuals are infected with HIV-2 and both sexes appear to be affected equally. Other areas where the epidemic has spread rapidly include: Latin America and the Caribbean (2 million infections); South and South-East Asia (2.5 million infections), in particular Thailand and India; and North America (1 million infections). While many infections in South Asia are amongst heterosexuals of both sexes, the predominant risk groups in the USA and Europe are homosexual men and IVDUs[21].

Figure 1.1 : The estimated distribution of cumulative HIV infections in adults, by continent or region : mid 1994[20]



In the UK a total of 10,304 AIDS cases had been reported to CDSC up to the end of December 1994[22], of whom 3285 are thought to remain alive. The true number of AIDS cases is, however, likely to be a lot higher as a result of both delays in reporting AIDS cases to CDSC and under-reporting. To date, the majority of AIDS cases (7463) are thought to have acquired the virus through sexual intercourse between men, although around 565 AIDS cases have been amongst individuals infected via blood or blood products, including haemophilic patients (460 cases). Geographically, the four Thames regions account for 70% of all AIDS cases reported (7260 cases)[22].

1.2 Inherited coagulation disorders / haemophilia

Inherited coagulation disorders affect 1 in 10,000 of the population. They are a result of mutations of the genes responsible for the synthesis of specific proteins known as clotting factors. The most frequently affected proteins include factor VIII and factor IX, although deficiencies of factors VII, X and XI also exist. In general, the clinical severity of the disorder is related to the degree of dysfunction of the clotting factor concerned[23].

1.2.1 Haemophilia A

The most common coagulation disorder in the UK is haemophilia A which is caused by a defect in factor VIII. Haemophilia A was first documented more than 1700 years ago and the genetics of the condition were first described in the 1800s. The disorder accounts for around 80-85% of all life-long bleeding disorders in man. Haemophilia A is a sex-linked inherited condition in that it is passed through 'carrier' females to their offspring. If male, then the child has a 50% chance of suffering from haemophilia A.

Haemophilia A is characterised by bruising or prolonged bleeding, particularly into the joints and soft tissues. Patients are categorised into mild, moderate or severe on the basis of their level of deficiency (>5, 2-5 and <2% of normal levels of clotting factor respectively). Patients with mild or moderate haemophilia may require very little treatment and may rarely experience bleeding problems. However, individuals with severe haemophilia often suffer from painful bleeding into the joints, muscles or other soft tissues and this may follow even trivial injury or may seem to occur spontaneously. If left untreated these bleeds can lead to permanent disability and crippling.

1.2.2 Other common inherited bleeding disorders

A bleeding disorder due to the indirect consequences of changes in plasma von Willebrand factor was first described in 1926[24]. This is known as von Willebrand's Disease (vWD) and is similar in clinical appearance to Haemophilia A. vWD affects both sexes equally with each child having a 50% chance of inheriting the abnormal gene from their parents. The condition accounts for less than 1% of severe bleeding disorders.

Haemophilia B (or Christmas Disease), a deficiency of factor IX, was first distinguished from haemophilia A in 1952[25,26]. The pattern of inheritance of haemophilia B is the same as that of haemophilia A, although it occurs less frequently in around 1 in 30,000 live male births.

1.2.3 Treatment of haemophilia and vWD

Treatment of both forms of haemophilia and vWD involves infusion with clotting factor. Bleeds can be treated by the infusion of clotting factor after they occur in a therapeutic manner or prophylaxis with lower doses of clotting factor can be given in order to prevent bleeding occurring.

Before the discovery that a haemophilia-associated bleeding episode could be successfully treated by transfusion of whole blood in 1840[27], patients with haemophilia were untreated and often died at a young age of haemorrhage. Until the identification of the main blood types in 1900 complications with incompatible blood types meant that blood transfusions were rarely used. In the early 1900s, however, blood transfusions began to be used more often, their use becoming widespread after the Second World War. In 1923 transfusions of plasma were first used as therapy for haemophilia patients[28], and these remained in common use until the 1960's. However, because of the small amount of factor VIII in plasma, its short half-life and the problems of circulatory volume overload, the majority of haemophilia patients still died early in life. Prior to the AIDS epidemic, intracranial bleeding was the cause of death in 25% of haemophilic patients, and the median life expectancy before 1960 was only 10.2 years[29].

In 1964, Pool analysed the content of the precipitate that formed during the thawing of frozen plasma and found that it contained a high amount of factor VIII[30]. This *cryoprecipitate* allowed patients to treat themselves at home for the first time and resulted in fewer problems with joint bleeds and arthritis in later years. Life expectancy for men with haemophilia improved to 56.8 years between 1961 and 1980[31]. From the late 1960's the production of highly purified preparations of factor VIII concentrate were also used.

1.2.4 The introduction of HIV into the haemophilia community

Initially cryoprecipitate was produced from single units of plasma which were transfused directly. However, in order to produce the large quantities of concentrate required, plasma donations were subsequently pooled to form *intermediate purity clotting factor concentrates*[32]. As a result of intermediate purity concentrate usage, almost all haemophilic individuals became infected with hepatitis C virus (HCV) and many patients were also infected with hepatitis B virus (HBV), both being associated with chronic liver-related problems. In the early 1970s a lack of British products led to a decision to import factor VIII concentrates prepared from the plasma of paid donors in the USA. From 1979, HIV began to be transmitted in the UK, firstly through these imported batches of concentrate and subsequently through UK produced National Health Service concentrates, although the latter resulted in a much lower rate of transmission of HIV. As the number of infected donors in the USA increased, the number of contaminated batches grew at a rapid rate. As a consequence, in the UK over 1200 haemophilia patients were infected with the virus[33], predominantly those who had severe

haemophilia. Due to their smaller need for clotting factor concentrates, far fewer individuals with milder forms of haemophilia were infected

Attempts to inactivate viruses in clotting factor concentrates were started in response to the recognition of non-A non-B hepatitis. These efforts were intensified with the advent of AIDS and since 1985 concentrates have either been heat treated or subjected to other sterilisation procedures. In addition, both blood donors and samples of the donated blood are now screened for HIV and HCV. Worldwide surveillance of transfusion-transmitted disease is carried out and since 1986 there has been no transmission of HIV from sterilised products. However, the transmission of HIV may still occur in parts of the world where unsterilised cryoprecipitate is the mainstay of treatment, e.g. in India[34]. Although generally safe, the transmission of some other viruses is still, however, possible[35,36]. Recently recombinant DNA technology has made it possible to clone the factor VIII gene[37,38] and recombinant factor VIII is now a licensed blood product in the UK. However, whilst these products pose no threat of viral transmission, their cost remains prohibitively expensive[24].

1.3 Project aims and objective

The main aim of this thesis is to provide a report of an epidemiologic follow-up of 111 patients registered at the Royal Free Hospital Haemophilia Centre infected with HIV. More specific aims are:

i) To describe the cohort in terms of demographics and clinical events which have occurred (including the development of pre-AIDS HIV-related conditions, the development of AIDS and death).

ii) To study the effect of these demographic factors and other potential 'co-factors' on the progression of HIV disease, as determined by progression to AIDS, death or to the first HIV-related clinical event.

iii) To study the pattern of immune decline, as described by the CD4 lymphocyte count, throughout HIV infection, and to evaluate the use of the CD4 count as a prognostic marker.

iv) To study the pattern of other potential markers throughout infection, including the CD8 lymphocyte count, beta-2 microglobulin (B2M) level, Immunoglobulin A (IgA) level, p24 antibody and p24 antigen status.

v) To investigate the relationships between these potential markers and the CD4 count and to assess whether they add any extra prognostic information in addition to that provided by the CD4 count.

vi) To investigate the effect of measurement variability and missing values on the estimate of the relative hazard associated with a drop in the CD4 count.

1.4 Layout of thesis

Chapter 2 contains a description of the data available for analysis and the methods of statistical analysis used. Chapter 2 also includes a brief description of the Haemophilia Centre itself and details of the regular clinical follow-up of patients in the cohort. Chapter 3 summarises the cohort in terms of their demographics and clinical disease progression. Chapter 4 describes the effect of these demographic factors on progression of HIV disease. The role of the CD4 count as a prognostic marker in HIV infection, and its pattern of change throughout infection is described in Chapter 5, and the potential roles of other laboratory markers are discussed in Chapter 6. Finally, Chapter 7 contains the results of an investigation into the effect of variability on the value given to the CD4 count as a prognostic marker. Rather than devote separate chapters to a review of the literature and to conclusions, each chapter will start with a review of the literature related to the results contained within that chapter and will finish with any concluding remarks.

CHAPTER 2 : METHODS: THE ROYAL FREE HOSPITAL HAEMOPHILIA COHORT, DATA COLLECTION AND STATISTICAL METHODS

2.1 The Royal Free Hospital Haemophilia Cohort

2.1.1 The Haemophilia Centre

The Royal Free Hospital Haemophilia Centre was established in 1964 under the direction of Dr Katharine Dormandy. Initially treatment was predominantly on an outpatient basis with five outpatients. Since the opening of the present Katharine Dormandy Haemophilia Centre at The Royal Free Hospital in 1978, however, the centre has seen enormous expansion with over 1500 patients with congenital clotting factor disorders now registered at the centre. Patients attend from a variety of geographic areas, including some from outside the UK. Care is provided for patients of all ages, including children.

2.1.2 The origins of the cohort

In 1982 the first reports of AIDS began to reach the UK from the USA, identifying that T4/T8 ratios were inverted in patients developing AIDS. Staff in the Department of Immunology at the Royal Free Hospital began to perform lymphocyte subsets under the guidance of Dr Peter Kernoff (Director of Centre 1978 to 1991) in order to identify patients with inverted T4/T8 ratios who were possibly infected. These early procedures were summarised in a paper, published in 1985[39], describing the finding that the degree of immunodeficiency found was related to the amount and type of blood product received. After the introduction of a commercial test for HIV seropositivity in 1984, stored serum samples from all patients were tested for HIV. In 1986 those patients who had tested HIV positive and were in regular care at the Centre were recruited into the haemophilia cohort. All HIV negative patients receiving clotting factor concentrates continue to undergo surveillance with an annual HIV test.

2.1.3 Follow-up procedures

It is the intention that severe haemophilic patients attend the Centre for clinical review every six months and those with mild haemophilia once a year. Patients infected with HIV, however, are generally seen more often for routine review according to their clinical condition. Patients are reviewed by a doctor together with the social worker and a nurse. Regular clinical reviews are shared by a Haematology doctor-in-training

(registrar/senior registrar) and one of the consultant haematologists. Clinical details and information on treatments prescribed are entered into patient notes which are kept in the Haemophilia Centre. At each visit blood samples are taken and stored at -20°C. Each week all centre staff responsible for delivering the comprehensive care (i.e. doctors, nurses, pharmacist, physiotherapist, social worker, welfare rights officer) and myself, attend a meeting in the Centre with the aim of exchanging information about patients who have been seen in the Centre over the previous week.

2.1.4 Haemophilia treatment

Until 1990 all patients received intermediate-purity products from a number of manufacturers. From 1990 onwards, on the recommendations of the UK Haemophilia Centre Directors[40], HIV positive patients at the centre were switched to monoclonal-antibody purified clotting factor concentrates.

2.1.5 HIV treatment

Routine HIV-related treatment used in the centre includes the use of zidovudine and prophylaxis for PCP and candidiasis. Zidovudine has been available since 1987, firstly for patients with AIDS and symptomatic disease, and from October 1988 for asymptomatic individuals as part of the MRC/ANRS Concorde trial of immediate versus deferred zidovudine. Secondary prophylaxis against PCP with pentamidine has been used since March 1988 (300mg 2-weekly) and primary prophylaxis since February 1989 (300mg monthly). From 1992 it became apparent that co-trimoxazole was more effective as prophylaxis than pentamidine and also provided protection against toxoplasmosis. Where possible patients were changed to co-trimoxazole (960mg three times a week), although the development of skin sensitivity to co-trimoxazole occasionally necessitates a switch back to pentamidine. Secondary prophylaxis for candidiasis with fluconazole has been used since March 1988 and primary prophylaxis since April 1990 (both 150mg weekly).

Zidovudine and primary prophylaxis for PCP and candidiasis are instigated once a patient's CD4 count falls below 200 cells/mm^3. Patients developing either PCP or candidiasis are offered secondary prophylaxis regardless of their CD4 count. Prophylaxis for *mycobacterium avium intracellulare* (MAI) infection with either rifabutin 300mg daily or clarithromycin 1g daily has been used since May 1994.

2.2 Data collected on the cohort

A large amount of data is routinely collected on patients in the cohort (Table 2.1) and is entered onto a computer data set (held as a Statistical Analysis System (SAS)[41] data set). The computer data set is updated once a year in the first two weeks of January where possible. The development of new clinical conditions and treatments are abstracted from patient notes by Dr Christine Lee (Director of Centre and co-PhD Supervisor). Non-AIDS clinical conditions recorded are bacterial infections, skin complaints, herpes zoster, thrombocytopenia (a platelet count $< 50 \times 10^9$/l) and oral candida. Information is only collected on the first occurrence of each of these conditions. In contrast, all occurrences and recurrences of AIDS-defining conditions are noted. Conditions are classified as AIDS-defining according to the definition of AIDS in use at the time of diagnosis. Patients are not retrospectively diagnosed as having AIDS once a new definition is adopted. Laboratory results are stored in the patient notes and data from these are extracted by Dr Lee (lymphocyte subsets, p24 antigenaemia and beta-2 microglobulin measurements) and myself (Immunoglobulins A, G and M, platelet counts) at the end of each year. All of the data is transferred onto data sheets and entered onto the computer by myself. The data is then checked for inconsistencies and any unusual values for a particular patient are checked back in the patient notes. If necessary, values can be further checked on the hospital Pathology Computer System which contains all test results carried out on patients at the hospital.

Non-routine data is collected on an *ad hoc* basis. The research team for the cohort, which consists of representatives of the Departments of Virology, Immunology and Public Health along with Dr Lee, meets approximately twice a year at which time members may suggest non-routine measurements which they are interested in studying. These may be measured either in fresh blood or in the stored serum samples. Where stored samples are to be used, a decision is made as to which samples should be extracted for study and these samples are then taken to the laboratory where the tests are to be performed. The results are sent back to myself and are entered directly onto the computer for analysis. The data is then verified for errors in data entry.

2.3 Analyses included in thesis

Because of space restrictions for this thesis, the work which is included represents only a small proportion of the analyses I have carried out on the cohort. Firstly, only a selected set of laboratory markers have been included.

Table 2.1 : Data collected and analysed in thesis

Demographic and baseline data	Date of last negative HIV test Date of first positive HIV test Date of birth Social class (I / II / III-N / III-M / IV / V) Haemophilia type and severity (Severe A / Mild A / B / vWD)
Clinical details	CMV serostatus (positive / negative) Date of first bacterial infection Date of first skin complaint Date of first episode of herpes zoster Date of first episode of thrombocytopenia ($< 50 \times 10^9/l$) Date of first episode of oral candida Date of first and all subsequent AIDS-defining conditions
Laboratory markers	Dates and measurements of the following laboratory markers : CD4 counts, CD8 counts, beta-2 microglobulin*, IgA levels, IgG levels*, IgM levels*, p24 antigen status, Platelet counts*.
Treatment data	Yearly concentrate usage*, Date of starting zidovudine*
Non-routine ad-hoc data	p24 antibody status, soluble CD8*, beta-2 microglobulin (see Chapter 6)

* Not analysed in thesis

The markers included are the CD4 and CD8 lymphocyte counts, beta-2 microglobulin, Immunoglobulin A (IgA) and p24 antigen and antibody levels. These markers have been chosen as published studies have previously suggested that they may provide useful information on disease progression. A brief description of the laboratory methods for the measurement of these markers, along with details of tests for antibodies to HIV and to cytomegalovirus are described in Appendix I - Laboratory Methods.

In the cohort, p24 antibody status has been measured once on each patient soon after seroconversion. Beta-2 microglobulin has been measured on a maximum of four occasions throughout infection. Both laboratory variables are therefore treated as covariates which are 'fixed' at the time of measurement. For these covariates, the question studied in this thesis is whether these markers predict the development of clinical disease in the long-term. This has important implications for understanding the pathogenic mechanisms by which HIV causes disease. The remaining markers (CD4 and CD8 lymphocyte counts, IgA levels and p24 antigen status) are measured repeatedly over time. These markers may be analysed either by selecting values at chosen time points, for example soon after seroconversion, or by including all available measurements as time-updated covariates in the analysis. These two methods of analysis address very different issues. When studying the relationship between the first CD4 count measured after seroconversion and disease progression, for example, the issue of whether a single CD4 count can predict the development of disease in the long-term is addressed. However, due to changes in the CD4 count over time, the degree to which a single CD4 count predicts is likely to be different over the short term than the long term; this can be assessed by including all values as time-updated covariates in the model. To a clinician who has a patient's most recent CD4 count available and would like to assess the patient's risk of disease before their next visit in 3-6 months, short-term prediction is likely to be of more relevance. Whilst both types of analyses have been carried out on the data from the cohort, only the time-updated analyses, using all available measurements, have been included.

2.4 Statistical methods

In general, the statistical methods used in this thesis are chosen because they make allowance for the wide variation in progression/survival times and also allow for the presence of right-censoring of survival times. Kaplan-Meier methods are used to visually display cumulative rates of progression to the clinical endpoints[42] and the log-rank test is used to test the univariate effects of covariates on progression rates for

statistical significance. Cox proportional hazards models[43] are then used to assess the independent effects of covariates on patient survival/progression times.

2.4.1 Endpoints and censoring

The majority of the analyses in this thesis involve the study of progression from HIV seroconversion to three main clinical endpoints : the development of the first HIV-related event (either AIDS or one of the five pre-AIDS clinical conditions described in section 2.2), AIDS and death. Progression to each of the five pre-AIDS clinical conditions separately is considered in Chapter 3. Progression to two CD4-defined endpoints is also considered in Chapter 6.

By the 1st January 1995 patients had been followed for up to 15 years from seroconversion. However, for the purposes of this thesis, as the number of patients alive after 14 years is small and confidence intervals around progression rates at 15 years from seroconversion are wide, data on patients remaining alive and AIDS-free is right censored a year earlier at the start of 1994. All analyses will therefore report follow-up results at a maximum of 14 years after seroconversion.

For progression to AIDS, the first HIV-related event or one of the two CD4 endpoints, patient follow-up is right-censored at the time of death or at the start of 1994 if the patient remains alive without reaching the endpoint on that date. As the five pre-AIDS clinical conditions are not recorded after a diagnosis of AIDS, progression to these endpoints was additionally right-censored at the time of an AIDS diagnosis if the patient had not developed the condition by this time. For progression to death, patient follow-up was right-censored at the start of 1994.

In Chapter 6 two CD4 endpoints, defined as the dates when the CD4 count fell to 200 and 50 cells/mm^3, are used. These dates were estimated using linear interpolation between the dates of the last CD4 count above the level of interest and the first count below that level. In this cohort, where CD4 counts are measured every 3-6 months, this method of estimating the dates is thought to be acceptable and dates are not subject to any large error.

2.4.2 Cox proportional hazards models

This method (described in more detail in Appendix II - Statistical Methods) provides estimates of the relative hazard of progressing to each endpoint for each covariate of interest.

95% confidence intervals for the relative hazard are estimated as :

relative hazard $\pm$ 1.96 x standard error

Where time-updated covariates are used patients enter the risk set once their first measurement becomes available. Tests for non-proportionality are performed by the inclusion of an interaction term between the logarithm of time and the covariate of interest. If the inclusion of this term into the model results in a significant improvement in fit, then there is evidence of non-proportionality. All proportional hazards models are fit using PROC PHREG in SAS[41].

2.4.3 Multi-level modelling

In order to assess the pattern of change of laboratory markers over time within individual patients, multi-level modelling methods are used[44]. The history of the development of these methods is described in Appendix II - Statistical Methods. However, a brief introduction to the statistical ideas underlying these methods is given below.

Laboratory markers measured on patients in the cohort are hierarchical in nature. For example, within an individual, CD4 counts at different times are linked by some biological mechanism controlling the immune system. If a patient's CD4 count is very low at one time point, it is likely to be relatively low at the time of their next measurement. Measurements on two different individuals are not, however, likely to be related, although they may share common features, such as a general tendency to decline throughout infection. CD4 counts within an individual patient are, therefore, 'nested' within the CD4 counts of the whole population. This is a two-level hierarchy where the CD4 count at a particular time point may be a function of the individual's immune system at that time (at 'level 1' - the within-individual level) but may also share some characteristics at a population level with the CD4 counts of other patients (at 'level 2' - the between-individual level).

It is possible to use simple linear regression methods to estimate the initial CD4 count at seroconversion (the intercept) and the rate of change in this count over time (the slope) in a group of patients infected with HIV. Using these methods a separate intercept and slope is estimated for each patient. However, this method takes no account of the fact that CD4 counts from different individuals may tend to follow some general patterns. If this is the case, then information about the pattern of CD4 change in an individual can be obtained not only from his own CD4 counts, but also from the pattern of CD4 counts in other patients in the population.

For example, CD4 counts at seroconversion for each individual in the population are likely to come from some distribution - the distribution of CD4 counts at seroconversion amongst all HIV positive patients. If few CD4 counts are available for analysis on one particular patient, then the information can be 'borrowed' using knowledge about the distribution of CD4 counts at the time of seroconversion in the rest of the population. The shape of this distribution could take any form, but it is usual to assume that CD4 counts at seroconversion (or some transformation of these counts) in the population are normally distributed with a certain mean and variance. Similarly, the rate at which the CD4 count declines is also likely to have some average value with some individuals having more rapid declines and some having slower declines. Once the assumption of an underlying distribution of the values in the population is made, the estimation of the parameters becomes more efficient, as it is only necessary to estimate the distribution of each parameter in the population, rather than separate sets of parameters for each individual.

A further benefit of multi-level model methods is that they do not require that the random variation in the CD4 count is the same at all stages of infection, as is the case for linear regression methods. Multi-level modelling methods allow for the explicit modelling of variability over time.

For the simple situation described above, of a linear pattern of CD4 change over time multi-level modelling methods make the following assumptions. Each individual's estimated CD4 count at seroconversion is made up of a *fixed* part, which is shared by each individual and describes the mean CD4 count at seroconversion estimated from all patients in the study, and a *random* part, which describes that particular individual's deviation from the mean. These *random* deviations for the CD4 count at seroconversion coefficient are assumed to come from a normal distribution with zero mean, but with some variance. Hence, for each individual, the estimated CD4 counts at seroconversion vary around the *fixed* mean with the estimated variance of the *random* part. Similarly, each individuals' estimated rate of CD4 decline is made up of both a *fixed* and *random* part, again with the *fixed* part describing the overall mean rate of decline in the population and the *random* part describing the particular individual's deviation from the overall average. Multi-level methods provide direct estimates of not only the overall mean values and the variance of each random part, but also of the covariance between the parameter estimates.

Hence, the multi-level modelling formulation of a simple model to consider a linear rate of CD4 change over time would be :

$$y_{ij} = (intercept + v_i) + (slope + w_i) \times time_{ij} + \varepsilon_{ij}$$

where y_{ij} is the jth CD4 count for the ith person,

$time_{ij}$ is the time of the measurement of the jth CD4 count for the ith person,

intercept is the fixed part and v_i is the random part of the estimated 'CD4 count at seroconversion,

slope is the fixed part and w_i is the random part of the estimated rate of CD4 decline, and

ε_{ij} is a random error term after all other coefficients have been included in the model.

Multi-level modelling methods provide estimates of *intercept* and *slope,* the variances of v_i and w_i , the covariance between the two and the variance of ε_{ij}.

In this set-up both the CD4 count at seroconversion and the rate of CD4 decline are known as 'random' parameters at level 2, or at the between-patient level. This is because they are assumed to vary between individuals. In some situations it may be thought that a more suitable formulation is one in which values do not vary between individuals ie. a 'fixed' covariate. As their name suggests, no variance is estimated for these parameters and all patients have the same value for this parameter. For example, if the CD4 count at seroconversion were modelled as a 'fixed' covariate rather than a 'random' one, each individual would be given the value of *intercept* as their estimated CD4 count at seroconversion, ie. each individual would be estimated to have the same CD4 count at seroconversion.

Interactions can be incorporated between the random variation parameter, ε_{ij}, and time in order to allow this variation to increase or decrease over time. Further, if it is thought that some of the between-individual variation can be explained by differences in important covariates, such as age at seroconversion, then these covariates can be added to the model either as 'fixed' covariates at the population level in which the effect of the covariate on the CD4 count is the same for every patient, or as 'random' covariates at the individual patient level in which the effect can vary between patients.

For this thesis, the package ML3 has been used to estimate the parameters of the models[45]. Given the estimated parameters it is then possible to generate either hypothesis tests or 95% confidence intervals for the overall estimates of the mean parameters (Mean $\pm$ 1.96 x Standard Error of mean) or 95% ranges within which most of the individual estimates will lie (Mean $\pm$ 1.96 x $\sqrt{}$(Variance estimate)). The correlation between two estimated parameters can be calculated using the standard formula

$$\text{Correlation } (x,y) = \frac{\text{Covariance}(x,y)}{\sqrt{[\text{Var}(x)\ \text{Var}(y)]}}.$$

CHAPTER 3 : SEROCONVERSION DATES AND DISEASE PROGRESSION

3.0 Summary of contents

In this chapter I will describe the patients included in the Royal Free Hospital Haemophilia Cohort in terms of their demographics, their dates of infection and the occurrence of HIV-related conditions, AIDS and death. I shall assess the relationships between these disease end-points and the patients' state of immunosuppression, as described by their CD4 count.

3.1 Literature review

Factors potentially related to HIV infection were initially identified using what was thought to be the most appropriate study design, the case-control study. The distribution of these factors was compared in those patients with AIDS (the 'cases') and those without AIDS (the 'controls'). These studies had the advantages of being cheap and easy to perform, thus enabling information about the disease to be collected rapidly. With the discovery of the virus in 1983[7], however, came the awareness that the incubation period could be long and that patients could develop symptomatic disease prior to AIDS. Thus the need for cohort studies arose and these are now acknowledged to be the only reliable way of obtaining information on the natural history of the infection.

As infection with HIV is a relatively rare occurrence in most populations and because of its long incubation period, many researchers still find case-control or cross-sectional approaches attractive. However, it is almost impossible to identify temporal changes in infection and in markers in these studies, making it difficult to identify causal pathways linking HIV with disease.

3.1.1 Methodological issues

Currently most commercial tests for HIV infection detect antibodies to the virus, which are produced by the immune system beginning up to 6 months after infection. The time at which this happens is known as 'seroconversion'. Studies of the natural history of HIV infection are therefore constrained to follow individuals not from infection but from seroconversion. Whilst these studies may as a result underestimate the length of incubation period by a few weeks or months, the implications of this are limited when considering the long incubation period.

In order to detect a seroconverting individual it is usual to follow HIV negative individuals until the date of their first positive HIV test. Cohort studies which follow such individuals are known as *seroincident* cohorts. For patients who have serum samples regularly stored (for example haemophilic patients, or patients recruited into clinical trials which require the storage of serum samples), retrospective analysis of these samples can often identify a period within which the patient must have seroconverted to HIV and a date of seroconversion can be estimated. Whilst not strictly seroconverters, these patients are often retrospectively recruited as such for seroincident cohort studies.

Due to the problems of identifying seroconverting individuals, many cohort studies simply follow seroprevalent individuals - those individuals who are already HIV-positive at the start of the study (*seroprevalent* cohorts). In these studies, follow-up may simply be considered from the start of the study, thus mimicking a typical patient's presentation for care. Alternatively, an estimate of the likely time of seroconversion can be made for each individual which can then be combined with the follow-up information to provide an estimate of the incubation period[46,47]. The likely time of seroconversion may be estimated using information on the distribution of certain laboratory markers throughout infection to estimate how long a patient has been infected, or by using knowledge of the HIV epidemic in the region or exposure category of the patient. A further approach involves the simultaneous estimation of the distributions of both seroconversion dates and incubation periods[48,49].

Some hybrid studies also exist, which are made up partly of seroconverters and partly of seroincident patients.

3.1.2 The major cohort studies

Many of the major HIV cohort studies are based in the USA, including a number of large studies of homosexual men[50-53] and drug users[54-56]. The USA Army[57], Navy[58] and Air Force[59] provide useful opportunities to study HIV infection in groups of patients made up of multiple exposure groups. In Canada the Vancouver Lymphadenopathy AIDS Study[60] and the Toronto Sexual Contact Study[61] are of particular note. In Europe cohorts also exist of IVDUs[62-64] and homosexual men[65], along with cohorts of multiple exposure categories[66].

Haemophilia patients are widely studied in both the USA[67-69] and Europe[68,70]. Since 1983, an ongoing survey has been carried out by the United Kingdom Haemophilia Centres Directors Organisation. Information on positive HIV tests, AIDS-defining conditions and deaths are routinely collected on all haemophilic patients infected with

HIV in the UK. This 'cohort' has provided a number of useful estimates on the course of HIV disease in haemophilic patients[33,48]. However, as data collection is limited, studies from individual centres can provide more detailed information on the natural history of HIV infection in these patients. The Royal Free Hospital Haemophilia Cohort is one such study. Other small studies of patients with haemophilia in the UK have been carried out in Bristol[71], Edinburgh[72] and Leeds[73].

3.1.3 The pattern of HIV infection

3.1.3.1 CDC Stage A - Primary infection and asymptomatic infection

Between 20 and 90% of individuals are reported to develop some sort of illness at the time of seroconversion[74-77]. Differences between these rates can be mainly explained by differences in methods of ascertainment, definitions of illness, or exposure categories studied[78]. Commonly, individuals suffer from a glandular-fever like illness[75] which may last from a few days to several weeks[75,76]. The presence of a 'seroconversion illness' may identify those who will experience more rapid disease progression[76,78-80], although this has not been found in all studies[81].

After infection with HIV there is usually a long asymptomatic phase, sometimes accompanied by the emergence of persistently enlarged lymph glands[82,83]. Prior to the development of a commercial test for HIV, lymphadenopathy was thought to be associated with increased progression to AIDS[84]. However, there is now little evidence to suggest its development is associated with a poor prognosis[68,83,85,86].

3.1.3.2 CDC Stage B - symptomatic infection

Prior to AIDS, the development of skin complaints (especially seborrhoeic dermatitis), bacterial infections or constitutional symptoms (weight loss, diarrhoea and fever) are commonly some of the earliest signs of HIV infection[87,88]. Herpes zoster[89-91] and oral lesions (including oral hairy leukoplakia and oral candida)[92-94] are particular problems for HIV infection patients. Finally, the development of haematological complaints, such as thrombocytopenia, neutropenia and anaemia is often reported in HIV infection[95-100].

Several of these conditions, including herpes zoster[61,101,102], and oral lesions[61,88,93,103,104] are associated with rapid disease progression. Although their appearance may simply be a reflection of the severe immunosuppression experienced during HIV infection, studies taking this into account have shown that the association with disease progression remains. Skin complaints[92,103], bacterial infections[88,92,105,106] and

haematological complaints[98,100,104], however, do not appear to be related to disease progression once the patient's immunosuppression is taken into account.

3.1.3.3 CDC Stage C - AIDS

The clinical conditions which form the current AIDS definition are shown in Table 1.1. Kaposi's Sarcoma and PCP were the first recognised conditions of AIDS[1,2] and these remain the predominant initial AIDS defining conditions in homosexual men[107]. Estimates of the numbers with PCP as their first AIDS illness vary from 20% to 70%[89,108-116]. With the introduction of prophylaxis in the late 1980s, the incidence of PCP as an initial AIDS-defining condition appears to have fallen[112,115-119] resulting in an increase in other, previously less common, AIDS-defining conditions[120,121]. Kaposi's Sarcoma tends to occur at an earlier stage of HIV infection than other AIDS defining conditions[85,112]. Whilst Kaposi's Sarcoma occurs in 8% to 45% of homosexual men as an initial AIDS-defining condition[107,112,114,119,122] the condition is rare in other exposure categories[110,113,123,124], possibly because it may be caused by a sexually transmitted viral pathogen[125-127]. Oesophageal candidiasis is the initial AIDS-defining condition in 5% to 37% of patients[89,111,128,129]. Whilst mortality from an initial episode of one of these conditions is now quite low as the conditions are usually treatable[130,131], several other conditions including lymphoma[84,109,112,132-136], wasting syndrome[116,134,136], CMV disease[104,137-138] and MAI infection[129,139], tend to occur at a very late stage of disease when survival is poor.

3.1.4 The impact of HIV-related therapy

Anti-HIV therapy has to date been mainly centred around the development of antiretroviral drugs which inhibit the replication of HIV. The first antiretroviral drug licensed was zidovudine which has been shown to be effective in preventing the development of AIDS in patients with symptomatic HIV infection[140-142] and in delaying death in patients with AIDS[143]. Zidovudine may also protect against the neurological effects of HIV[144]. However, its value in delaying the development of AIDS in asymptomatic individuals is in doubt[145,146]. There is concern that the efficacy of zidovudine is short lived[147,148], that drug-resistant strains may emerge[149] and that the drug may have serious adverse effects when used for long periods of time[147,150]. Two other antiretrovirals, zalcitabine (ddC) and didanosine (ddI), have since been developed and are now licensed in this country for use as second-line therapies in patients in whom zidovudine has failed. It appears that these two drugs, either alone or in combination, may be more effective than zidovudine monotherapy in late stage HIV infection[151-153].

Prophylaxis against PCP with pentamidine, or more recently dapsone or co-trimoxazole has been available since the late 1980s. It has been shown to be effective both in primary and secondary prevention of the condition[154-157], delaying the onset of AIDS in some individuals[158]. This has resulted in an apparent increase in other conditions, both because patients now live longer in a more immunosuppressed state, and because patients develop other conditions in place of PCP[120,121]. There is a movement towards the use of prophylaxis for other conditions (including candidiasis, MAI, CMV disease and toxoplasmosis), although concerns about the development of resistance and adverse drug interactions has meant that the use of prophylaxis for some of these conditions is less widespread.

3.2 The cohort

The demographic details of the cohort are shown in Table 3.1. Patients were mainly from the non-manual social classes or were long-term unemployed. Almost all patients suffer from severe haemophilia A, although a small number of patients with mild or moderate haemophilia A, haemophilia B and vWD are also included. The age of the patients at the time of seroconversion ranged from 2 to 77 years (median 22.6 years).

In all further analyses, haemophilia type will be categorised as 'Severe A' or 'other', social class as 'non-manual' (I/II/III-N) or 'manual' (III-M/IV/V), and age at seroconversion will be split into four groups defined by the quartiles of the distribution. Patients with severe haemophilia A tended to be younger at the time of seroconversion than those with other disorders (median ages of 21.4 years and 26.8 years respectively, p=0.03, Wilcoxon test). There was also a tendency for older individuals to seroconvert earlier than younger (p=0.07, Kruskal-Wallis test). There were no other apparent associations between these demographic factors.

3.3 Seroconversion dates

As part of routine haemophilia care, a serum sample is taken and stored on all individuals at each visit to the centre. It has been possible to retrospectively test these stored serum samples for HIV seropositivity.

All patients in the cohort have at least one sample which gave a positive HIV serology. These samples were taken between October 1979 and December 1987 (median June 1983).

Table 3.1 : Demographic details of patients in cohort

		Number	%
Social Class	I/II (Professional/managerial)	36	32.4
	III-N (skilled non-manual)	24	21.6
	III-M (skilled manual)	5	4.5
	IV (unskilled manual)	7	6.3
	V (long-term unemployed)	38	34.2
	not known	1	0.9
Haemophilia	Severe A	101	91.0
	Mild/Moderate A	7	6.3
	B	1	0.9
	vWD	2	1.8
Age at seroconversion	0-10	13	11.7
	11-20	38	34.2
	21-30	32	28.8
	31-40	13	11.7
	41-50	5	4.5
	51-60	6	5.4
	61-	4	3.6
	Median age	22.6 years	
	Range	2.1-77.8 years	

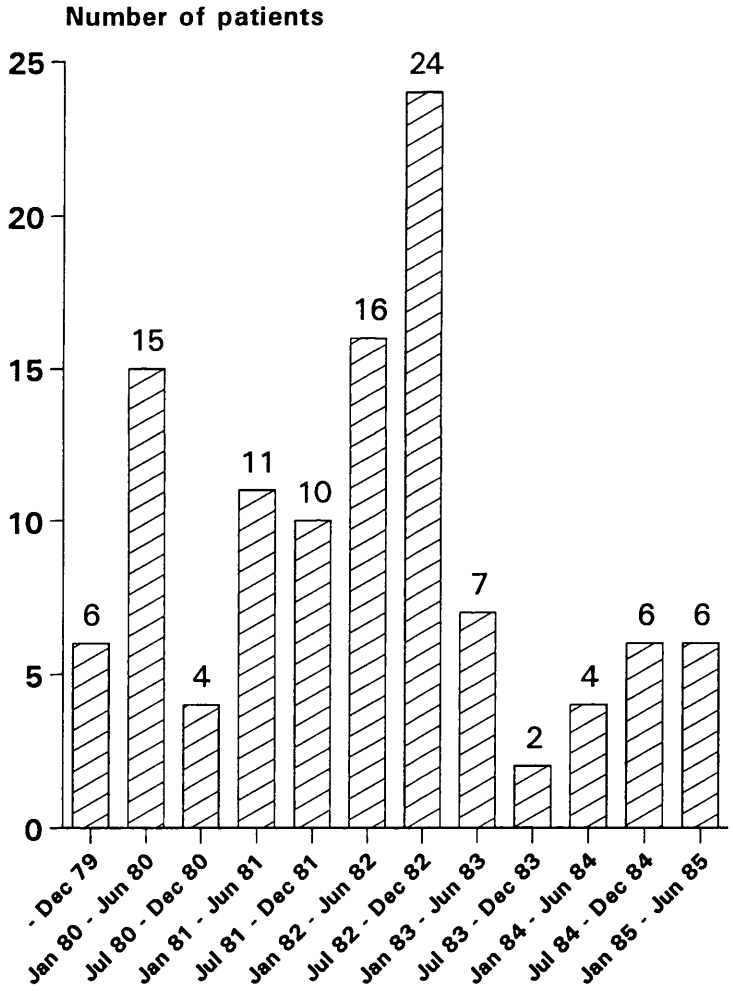
Sixty-three of the patients also had at least one sample which produced a negative HIV test result at some stage before their positive result. In these 63 patients, the dates of last negative tests range from March 1979 to March 1985 (median March 1982).

The date of HIV seroconversion was estimated to be half-way between the dates of the last negative and first positive HIV test results. The first documented seroconversion in a haemophilic patient in the UK was a member of this cohort in whom both negative and positive HIV test results were available from samples taken within a week of each other. The date of seroconversion of this patient, October 1979, has therefore been used as a marker of the introduction of HIV into blood supplies in the UK and in patients in whom no negative HIV test result was available (48 patients) dates of seroconversion were estimated using this as the date of the last negative test. By April 1985 all concentrates used to treat patients were heat-treated. Therefore, the assumption was made that seroconversions would take place within 3 months of this date at the latest (July 1985). In those patients with a positive test result after July 1985 (12 patients without a negative test, 3 patients with a negative test) the date of first positivity has been set at July 1985.

before July 1985

Estimated dates of seroconversion for all 111 patients are shown in Figure 3.1. The last estimated date of seroconversion was in May 1985, a patient in whom the last negative test result was from March 1985. The impact of the 12 patients without a last negative test result and in whom the first positive test was taken after July 1985 is clearly seen, with a distinct group of patients whose estimated date of seroconversion is August 1982.

Figure 3.1 : Estimated dates of seroconversion for all patients in the cohort



As the estimated date of seroconversion is taken to be midway through the interval between the last negative and first positive test results (or assumed dates), estimates are subject to a maximum possible error equal to half the width of this 'seroconversion interval'. In the 63 patients with a last negative HIV test, the maximum possible error ranged from 3.5 days to 1.29 years (median 23 weeks).* Among all patients, 80% are subject to an error of less than 2.5 years (median seven months).

* Among the 48 patients without a last negative test, the maximum possible error ranged from 24 days to 2.85 years (median 2.37 years).

3.4 Progression of HIV disease

3.4.1 Pre-AIDS HIV-related conditions

Over the follow-up period a total of 93 patients in the cohort developed at least one of the five conditions studied whilst AIDS-free, or developed AIDS, with the most common first events being bacterial infections and skin complaints (Table 3.2). Kaplan-Meier estimates suggest that patients develop their first indication of HIV infection (either one of the above conditions or AIDS) a median of 6.8 years after seroconversion (Figure 3.2). By 11.8 years after seroconversion, 50% of HIV infected individuals who have remained AIDS-free would have developed bacterial infections connected with their HIV infection, and by 12.5 years, 50% would have developed skin complaints (Figure 3.3). However, even after 14 years, only 11.9% of patients alive and free of AIDS would have previously had herpes zoster and 10.4% thrombocytopenia (Table 3.2).

Table 3.2 : HIV-related conditions witnessed in cohort, Kaplan-Meier estimates of the proportion of individuals (and 95% confidence intervals) developing each condition by 14 years after infection.

Event	No.	%	% with condition by 14 years	95% confidence interval
Bacterial infections	45	42.3	75.3	55.7 - 94.9
Skin complaints	41	39.6	59.6	42.9 - 76.4
Oral candida	23	22.5	28.5	17.5 - 39.4
Herpes zoster	11	11.7	11.9	5.0 - 18.8
Thrombocytopenia	9	8.1	10.4	3.8 - 17.0
Any HIV-related condition	82	73.9	94.3	84.9 - 100.0
AIDS	47	42.3	56.5	39.5 - 73.6
Any HIV-related event	93	83.8	95.5	87.9 - 100.0
Death	45	40.5	46.9	35.6 - 58.2

CD4 counts measured within 6 months of the development of each HIV-related event are shown in Figure 3.4. The first event tends to occur while the CD4 count is around 340 cells/mm^3, although patients with a first episode of herpes zoster, for example, tend to have a relatively high CD4 count and those with oral candida tend to have lower counts. As only the first episode of each condition prior to AIDS is recorded, the CD4 counts shown will be higher than those among *all* patients developing such conditions.

Figure 3.2 : Kaplan-Meier plot showing the cumulative progression rate to the development of the first HIV-related event (bacterial infection, skin complaint, herpes zoster, thrombocytopenia, oral candida or AIDS) according to the number of years from seroconversion

Figure 3.3 : Kaplan-Meier plot showing the cumulative progression rate to the development of each HIV-related condition prior to AIDS according to the number of years from seroconversion

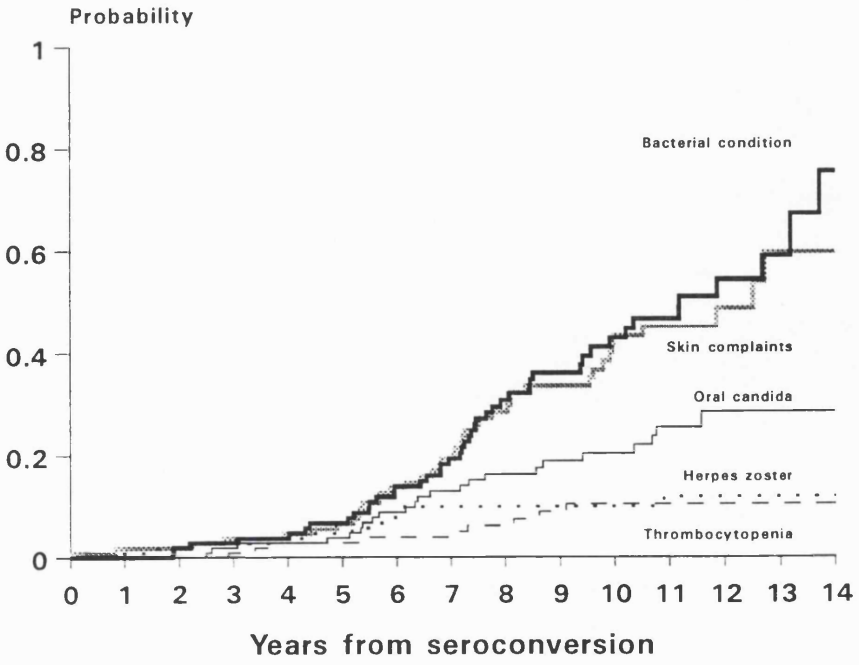
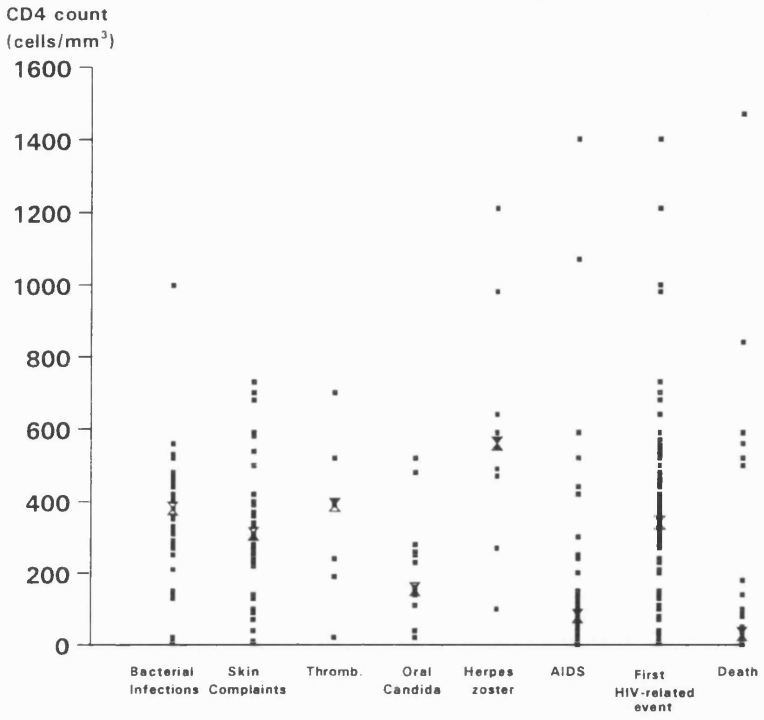


Figure 3.4 : Distribution of CD4 counts in the six months prior to the development of each HIV-related event (X - median count)



Figures 3.5 and 3.6 show Kaplan-Meier plots of the cumulative progression rates to each of these conditions, according to the patients lowest CD4 count measured prior to the event. This method estimates the risk of developing each condition before the CD4 count falls below any level, taking account of the CD4 count measured in *all* patients, not only those in whom the condition develops[159]. Using this approach, the first HIV-related event tends to occur once the CD4 count has fallen below 220/mm^3 on average, (median) although individual condition types show much more variability. Table 3.3 contains a summary of the progression rates to each condition whilst the CD4 count remains above certain levels.

3.4.2 AIDS

A total of 47 patients in the cohort have developed AIDS, representing a Kaplan-Meier progression rate of 56.5% by 14 years after HIV seroconversion (95% confidence interval 39.5% to 73.6% Figure 3.7, Table 3.2) and a median time to the development of AIDS of 13.1 years. Two-yearly progression rates to AIDS are summarised in Table 3.4. The median CD4 count measured in the 6 months prior to AIDS is 80 cells/mm^3 (Figure 3.4) although four patients developed AIDS whilst their CD4 count was at levels of 500 cells/mm^3 and above.

Figure 3.5 : Kaplan-Meier plot showing the cumulative rate of progression to the development of the first HIV-related event (bacterial infection, skin complaint, herpes zoster, thrombocytopenia, oral candida or AIDS) according to the minimum CD4 count measured

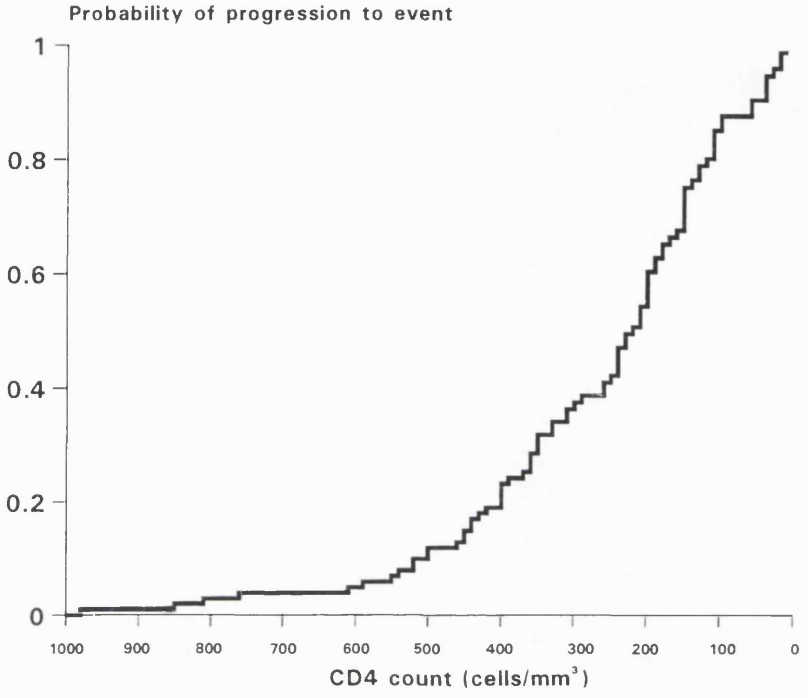


Figure 3.6 : Kaplan-Meier plot showing the cumulative rate of progression to the development of each HIV-related condition prior to AIDS according to the minimum CD4 count measured.

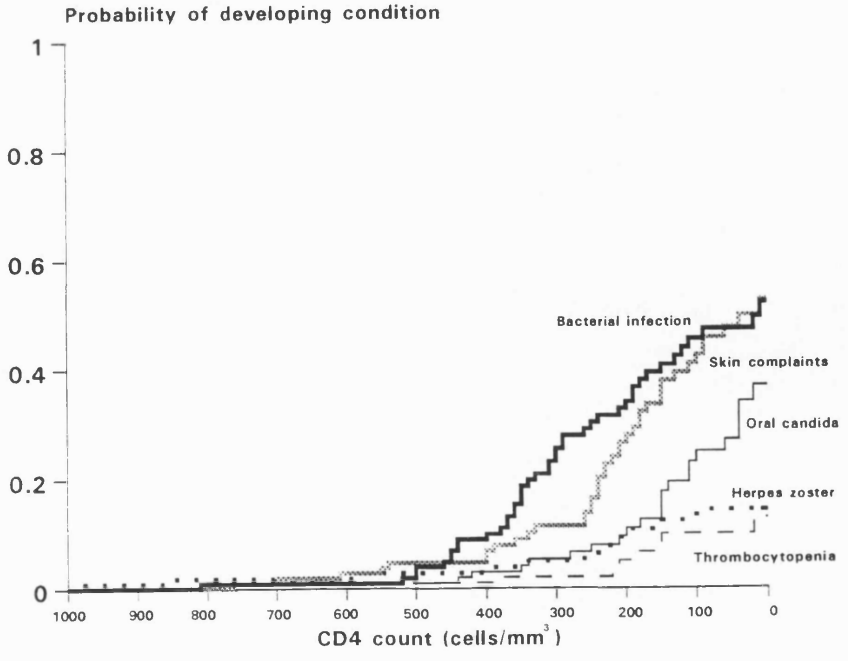


Table 3.3 : Kaplan-Meier estimates of the proportion (%) of cohort developing each condition according to the minimum CD4 count measured

Condition	Progression by a CD4 count of (cells/mm^3) : 500	400	300	200	100	0
Bacterial infections	3.9	9.9	25.4	33.9	45.4	52.1
Skin complaints	4.7	6.8	11.3	27.9	42.5	52.6
Oral candida	1.0	3.0	5.3	10.9	24.9	36.9
Herpes zoster	2.8	3.8	4.9	10.7	14.2	14.2
Thrombocytopenia	1.0	1.0	2.0	5.0	9.9	12.8
Any HIV-related condition	11.9	21.2	35.9	58.0	81.3	89.1
AIDS	1.9	4.9	7.0	11.1	29.3	100.0
Any HIV-related event	11.9	23.2	37.5	60.3	87.5	100.0
Death	4.6	6.6	7.6	11.6	17.7	87.2

Figure 3.7 : Kaplan-Meier plot showing the cumulative rate of progression to AIDS according to the number of years from seroconversion

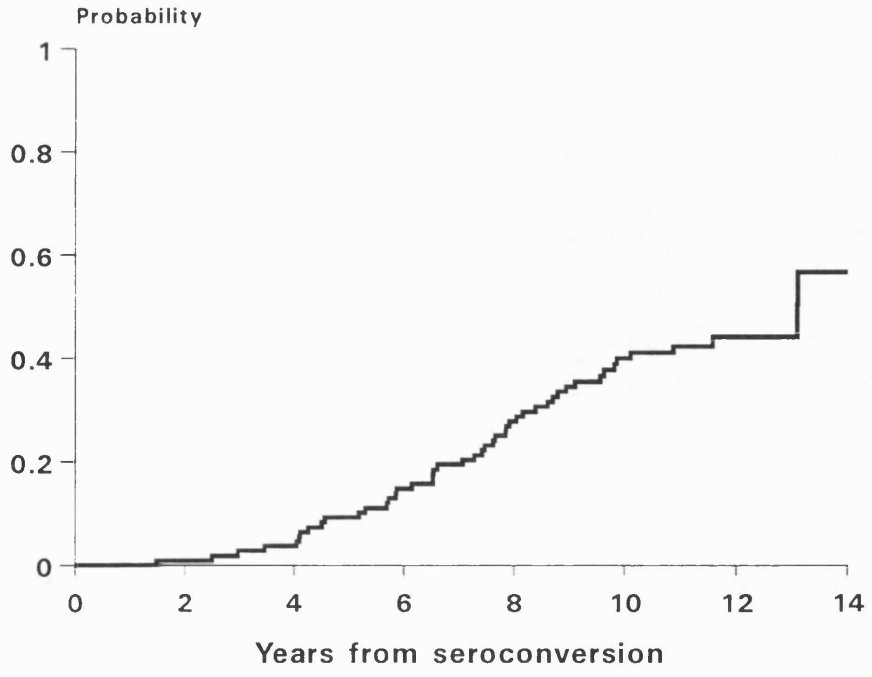


Table 3.4 : Two-yearly Kaplan-Meier progression rates to AIDS and death

	Progression to :	AIDS	Death
By :	2 years	0.9%	2.7%
	4 years	3.7%	5.4%
	6 years	14.7%	10.8%
	8 years	27.8%	21.6%
	10 years	39.9%	33.1%
	12 years	44.1%	43.9%
	14 years	56.5%	46.9%

When CD4 count information on all patients is considered (Figure 3.8), however, it is clear that the risk of developing AIDS is very low until the count falls below 150 cells/mm^3 at which stage the risk increases dramatically. By the time of the development of AIDS the count has fallen to 30 cells/mm^3 on average (median count)

The initial AIDS-defining conditions witnessed in the cohort are shown in Figure 3.9. PCP is the most common condition, with 18 of the 47 patients developing this as their initial AIDS-defining condition. Lymphoma (5 patients), oesophageal candida and wasting syndrome (4 patients each) were all seen in a small number of patients as an initial AIDS-defining condition.

To date there have been 44 subsequent AIDS-defining conditions among the 47 men. Whilst PCP was still the most common subsequent condition, MAI (9 episodes) and CMV disease (5 episodes), are seen much more frequently once the patient has already developed AIDS.

3.4.3 Death

45 of the patients in the cohort have died, a Kaplan-Meier death rate of 46.9% by 14 years after HIV seroconversion (95% confidence interval 35.6% to 58.2%) (Figure 3.10, Tables 3.2 and 3.4). 38 patients had a diagnosis of AIDS at the time of death, the remaining 7 patients died prior to AIDS (liver failure/cirrhosis [3 patients], suicide, cerebral haemorrhage, vomiting following a fit and cancer). Both suicide and liver failure[160,161] may be HIV-related. Hence, of these 45 deaths, 42 (93%) may be said to be HIV-related.

Figure 3.8 : Kaplan-Meier plot showing the cumulative rate of progression to AIDS according to the minimum CD4 count measured

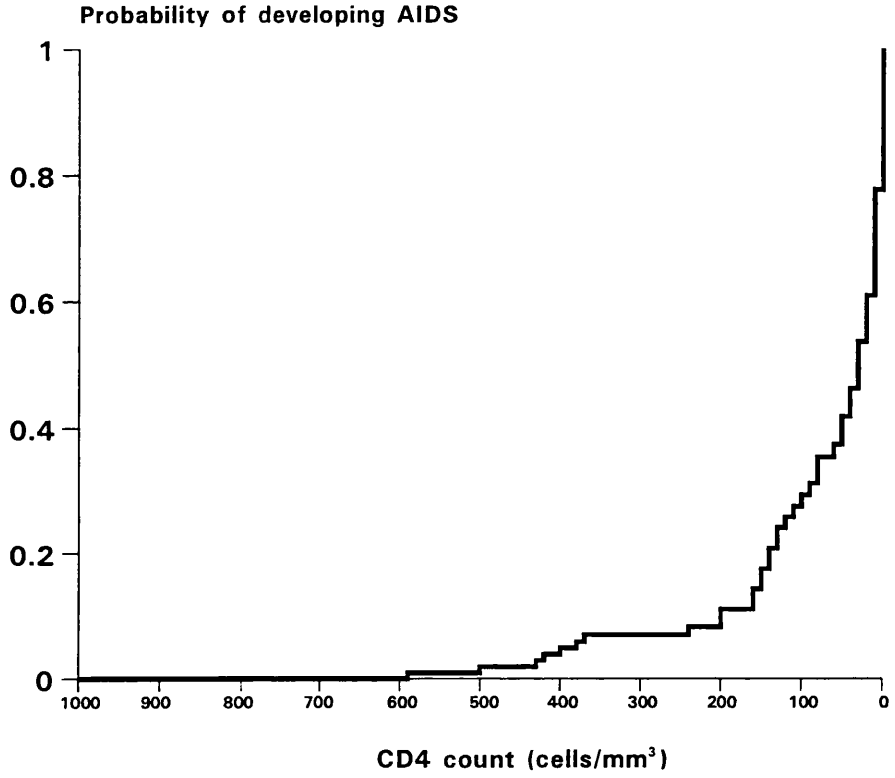


Figure 3.9 : Initial and subsequent AIDS-defining conditions witnessed in the cohort

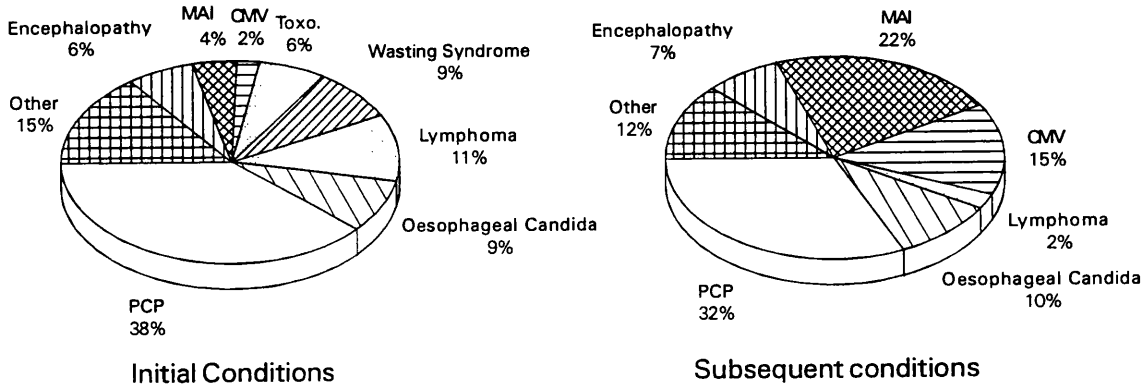
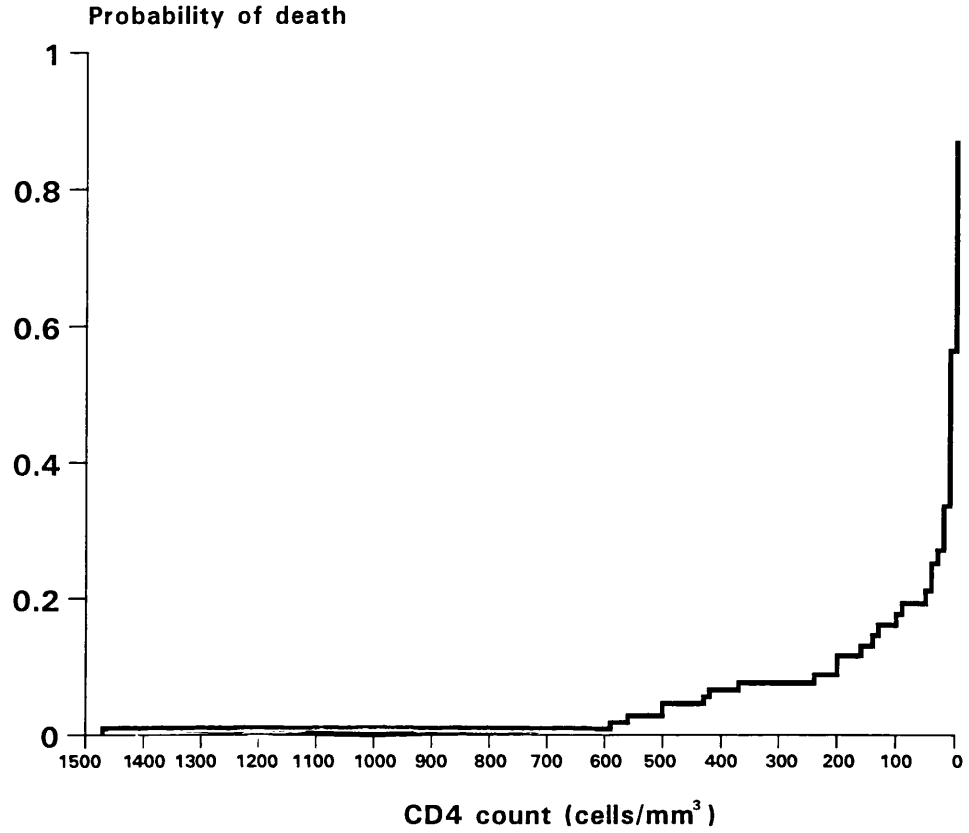


Figure 3.10 : Kaplan-Meier plot showing the cumulative rate of progression to death according to the number of years from seroconversion

Pre-terminal CD4 counts in the patients were very low, with CD4 counts prior to death having a median of 30 cells/mm^3 (Figure 3.4). When using CD4 information on all patients, the results suggest that the risk of death is very low until the CD4 count drops below 50 cells/mm^3 (Figure 3.11).

In those patients who have developed AIDS and who have died, the median survival time from AIDS diagnosis is only 5 months (range 0 to 5.07 years). Whilst this appears to be very short, 10 of the patients (mainly those who were diagnosed early in the epidemic when clinicians had little experience of AIDS) received their AIDS diagnosis posthumously, or at death. In patients with a non-zero survival time, the median survival was higher at 15 months. These median survival times are likely to be an underestimate of the true median survival after an AIDS diagnosis as they are only based on patients who have died.

Figure 3.11 : Kaplan-Meier plot showing the cumulative rate of progression to death according to the minimum CD4 count measured



The death rate after AIDS in patients with PCP as their initial AIDS-defining condition (18 patients) is much lower than in those with other conditions (29 patients, p=0.01, Table 3.5). The death rate was also significantly higher in patients who were older at diagnosis, than in younger patients (p=0.03). Higher death rates were also seen in those diagnosed early in the epidemic, in those with a lower CD4 count and in those who had used zidovudine prior to the development of AIDS, although these differences were not significant.

3.5 The effect of pre-AIDS HIV-related conditions on progression of HIV disease

In order to study whether the development of pre-AIDS conditions were associated with more rapid disease progression, each condition was included in a proportional hazards regression model as a time-dependent covariate. For each condition, a covariate was set to zero until the patient developed the condition, when the covariate was set to 1 thereafter.

Table 3.5 : The death rate after AIDS diagnosis, stratifying by initial AIDS-defining condition, calendar year of diagnosis, age at diagnosis, CD4 count at diagnosis and zidovudine usage prior to diagnosis.

		AIDS cases	Deaths	Patient-years of follow-up	Death rate per 100 patient years	p-value
Initial AIDS-defining condition	PCP	18	14	39.07	35.83	
	Other conditions	29	24	29.62	81.03	0.01
Calendar year of AIDS diagnosis	Prior to 1989	25	24	41.51	57.82	
	1989 onwards	22	14	27.18	51.51	0.73
Age at AIDS	≤ 32	23	16	41.00	39.02	
	>32	24	22	27.69	79.45	0.03
CD4 count at AIDS	> 80 /mm^3	19	14	37.38	37.45	
	$\leq$ 80 /mm^3	22	18	29.52	60.98	0.17
Zidovudine usage prior to AIDS	No	31	26	52.30	49.71	
	Yes	16	12	16.39	73.22	0.28

Table 3.6 shows the relative hazards associated with the development of each of the conditions. The development of bacterial infections, skin complaints and oral candida are all associated with a more rapid progression to AIDS.

There are a number of possible explanations for these findings. Pre-AIDS HIV-related conditions may occur as a consequence of immunosuppression and do not have any further effect on disease progression. Alternatively, they may have a direct detrimental effect on the patient's condition, leading to further disease progression. By adjusting for the underlying level of immunosuppression, it is possible to assess which of these two explanations is more likely. This analysis will be discussed in Chapters 5 and 6.

Table 3.6 : Relative hazards (and 95% confidence intervals) associated with the occurrence of pre-AIDS conditions for the development of AIDS. The occurrence of each condition is treated as a time-updated covariate in the Cox proportional hazards model.

Progression to :	Relative hazard	95% confidence interval
Bacterial infections	2.16	(1.11 - 4.19)
Skin complaints	2.37	(1.21 - 4.64)
Oral candida	4.42	(2.16 - 9.04)
Herpes zoster	0.65	(0.16 - 2.68)
Thrombocytopenia	1.26	(0.39 - 4.12)
First condition	4.01	(2.00 - 8.06)

3.6 Discussion

3.6.1 The study of haemophilic patients

In the UK, patients with haemophilia seroconverted to HIV very early in comparison to patients from other exposure categories. The availability of stored serum samples for patients in the cohort means that the date of seroconversion can be estimated accurately for many of these patients and therefore haemophilic patients are an ideal group to use for studying the natural history of HIV infection. Further, drop-out from the study is low as patients depend on treatment for their haemophilia. However, haemophilic patients differ in a number of demographic and clinical respects to HIV positive patients infected through other transmission routes. These differences may be relevant when comparing results on disease progression from different studies.

3.6.1.1 Age differences

The age spread of HIV positive patients with haemophilia is much wider than that seen in other groups of patients. The youngest patient in the cohort was only 2 years old at the time of seroconversion and the oldest 77 years. In contrast, cohorts of drug users tend to be younger[162] and cohorts of homosexual men tend to be older[163] than this group of patients, and there are unlikely to be many very old or very young individuals in these cohorts.

3.6.1.2 Social class differences

Individuals infected with HIV are likely to suffer a downward drift in economic well-being as increasing illness renders them unable to work[164]. However, cohorts may differ in social class *at seroconversion* before any effect of HIV disease progression is apparent. Because of the risk of bleeding, haemophilia patients are often restricted to non-manual jobs and in severe cases of disability, some patients cannot work at all. Consequently there is a social class pattern in the cohort which is in contrast to that seen both amongst the general non-haemophilic population, where the vast majority of individuals are in the skilled manual and non-manual social classes[165].

Social class may be associated with differences in smoking habits, diet, alcohol consumption, family support or quality of accommodation, all of which may be directly related to HIV progression. However, it is likely that the most important way in which social class may have an effect on HIV disease progression is through differential access to health care. Whilst in the UK, access to health care remains largely independent of income, in most states of the USA this is not the case. In cohorts where patients are seen regularly for treatment irrespective of their financial status, effects of social class differences may not be apparent. However, the relationship between social class and disease progression is likely to be more pronounced in the general population or in cohorts where clinical care is not routinely carried out as part of the study, with patients of a lower social class having less access to care than those of higher social class.

3.6.1.3 Differences in other characteristics

Both continued sexual behaviour[106,166] and continued injecting drug misuse[167] have been suggested by some authors as a factor in more rapid disease progression. The number of sexual partners and frequency of unprotected sexual intercourse is likely to be lower in haemophilic men than in cohorts of homosexual men and IVDUs. Injecting drug misuse is not a major concern in this cohort. However, haemophilic patients were potentially exposed to viral and microbacterial contaminants when treated with intermediate-purity concentrates. As the use of these concentrates has been shown to lead to a depression of the immune system[168,169], their use may possibly speed up the progression of HIV disease in these patients.

3.6.2 Estimation of seroconversion dates

The mid-point method has been used to estimate the dates of seroconversion in this cohort. This is a simple method which makes very few assumptions about infection dynamics. Other methods for the estimation of seroconversion dates which allow for an increasing risk of infection over time have been suggested. Darby[33,48], using a method suggested by Brookmeyer and Goedert[170], used a two-stage method where seroconversion was assumed to occur uniformly over three time intervals. The date of seroconversion was then estimated as the expected value from the distribution of seroconversion dates conditional on the seroconversion interval for the individual patient. In a comparison of this method, the midpoint method and a method based on the assumption that the distribution of seroconversion times came from a truncated Weibull distribution (again allowing for an increased risk of infection over time), Chiarotti *et al*[70,171] suggested that the choice of the estimation method had only a small effect on the overall results. If the use of the midpoint method leads to any such systematic bias in seroconversion date estimates, the error is unlikely to be more than a few months, an error which is of little importance for a virus with an average incubation period of 13 years or more and which is unlikely to have any large impact on clinical practice or on our knowledge of HIV pathogenesis.

When using this midpoint method, it is assumed that high and low risk individuals are tested for HIV positivity at the same frequency[46], implying that blood samples are drawn at roughly the same frequency for each patient. Individuals with severe haemophilia may attend the centre more frequently for clinical review and may therefore have blood stored more frequently than those with mild or moderate haemophilia. It is possible, therefore, that dates of infection will be estimated sooner and more accurately in severe haemophilic patients. Any relationship between the incubation period and severity of haemophilia which results from this differential estimation of seroconversion dates may lead researchers to erroneously conclude that there is a direct causal link between haemophilia status and the development of AIDS.

July 1985 was chosen as the last possible date for HIV seropositivity. It is possible that a small number of patients either received contaminated products after this date (because they had a supply of product at home which they used) or some patients infected by the last few batches of non-heat-treated concentrate in March 1985 had not seroconverted by July 1985. However, even if the assumed last possible date of HIV seropositivity were changed to October of that year (allowing 6 months for seroconversion), seroconversion dates would only be altered by a maximum of 1.5

months in a very small number of individuals. Hence this choice of date is unlikely to have a large impact on estimated progression rates.

3.6.3 The incidence of clinical disease prior to AIDS

A comparison of the results from the many studies which have considered the incidence of clinical disease prior to the development of AIDS is difficult due to differences in the measure used (incidence/prevalence), in study designs (cross-sectional/longitudinal) and in the times from seroconversion when patients are studied. The cumulative incidence of any condition has been quoted to lie between 50% 5-6 years after seroconversion[172] and 74% when the cumulative incidence of conditions was considered over an arbitrary six month period of infection[82]. Both authors included lymphadenopathy as one of the conditions studied. As this is not thought to be prognostic for the development of AIDS[61,68,82,83], it was not recorded in this cohort of patients, and therefore the results are not comparable.

Reported prevalence rates of seborrhoeic dermatitis from cross-sectional studies vary from 8% to 34%[87,173-175]. Oral candida has been shown to occur in 3-6% of HIV positive patients in cross-sectional studies[87,175,176] although Matis *et al* noted that its prevalence varied according to the stage of disease, with only 4% of non-AIDS patients but 35% of those with AIDS having developed the condition[173]. In a recent longitudinal study, Lifson *et al* suggested that 26% of individuals would develop oral candida by 5 years after seroconversion[94]. Quoted rates of herpes zoster from cross-sectional studies range from a prevalence rate of 1.5%[175] to an incidence rate of 29.4 per 1000 patient-years[90]. Longitudinal studies have suggested cumulative rates of 8.8% by 2 years after seroconversion[91] and 30% by 12 years[90]. Thrombocytopenia has been reported to occur in 11-15% of HIV positive patients in cross-sectional studies[97,100], although results from a cohort study of haemophilic patients[177] suggest a much higher rate of 43% by 10 years after seroconversion. In the Royal Free Hospital Cohort, thrombocytopenia is defined as a platelet count < 50 cells/mm^3. Both Allain's[97] and Eyster's studies[177] used a definition of a platelet count less than 100 cells/mm^3 which may explain some of the differences in the rates. Clearly there may be a wide difference in the prevalence of these conditions in the absence of HIV infection, and the prevalence rates of these conditions may differ by the exposure category studied[178] or the geographic area. The results presented in this thesis represent maximum estimates of the cumulative incidence of these conditions which result from infection with HIV.

3.6.4 CD4 counts and the development of clinical conditions

Oral candida has been shown to be associated with low CD4 counts in a number of studies[82,88,179-182] with a median CD4 count at the time of diagnosis of 300-400 cells/mm^3 [88,94]. Bacterial infections[88], skin complaints[183] and thrombocytopenia[82,99] are all increasingly prevalent at lower CD4 counts, findings which are broadly consistent with the results in this thesis.

Two methods were used to assess the relationship between the CD4 count and the development of HIV-related events. Firstly, the patient's most recent CD4 count measured in the six months prior to developing the condition was studied. As patients are seen every 3-6 months, most patients are expected to have at least one CD4 measurement within this period. However, some patients, possibly those who were less ill and who may have had higher CD4 counts, were excluded from the analysis. Hence, the median CD4 count for the patients who attended during the six months prior to the condition may underestimate the 'true' CD4 level. A problem with this approach is that information is only used on those who actually develop the condition, and therefore no assessment of the risk associated with a declining CD4 count can be made. The Kaplan-Meier approach[159] uses information on the minimum CD4 count measured in all individuals although the minimum CD4 count measured may itself be a biased estimate of the true underlying level of immunodeficiency.

3.6.5 The development of AIDS

On average AIDS develops 13.1 years after seroconversion in this cohort. Figure 3.12 and Table 3.7 contain summaries of progression rates found in a selection of published studies using similar methods to those in this thesis. Estimates of the HIV incubation period from published studies of non-haemophilic patients vary widely, although much of this variation can be explained by different follow-up periods. The median incubation periods reported of 8-10 years[189,191,193,195,197] are still, however, a couple of years shorter than that found in this cohort.

There does not appear to be any large differences in progression rates of non-haemophilic cohorts, although progression rates from haemophilic cohorts do appear to be slightly lower (Figure 3.12). In the two studies which report progression rates separately for adults and children, an age difference can clearly be seen[108,177].

Because of the impact of prophylaxis against some of the major opportunistic infections on the incubation period to AIDS[140-142,158], progression rates may be lower in more

recent studies which have followed patients up after the introduction of such therapies. The Royal Free Haemophilia cohort, which follows patients for up to 15 years after seroconversion, is one of the longest followed groups of HIV infected patients. Chiarotti *et al*[171] have suggested that increased follow-up leads to a longer estimate of the median incubation period, possibly explaining the lower progression rates in these patients. The authors do not suggest reasons for this, but the availability of prophylaxis may contribute to this finding.

Figure 3.12 : Progression rates to AIDS from published studies

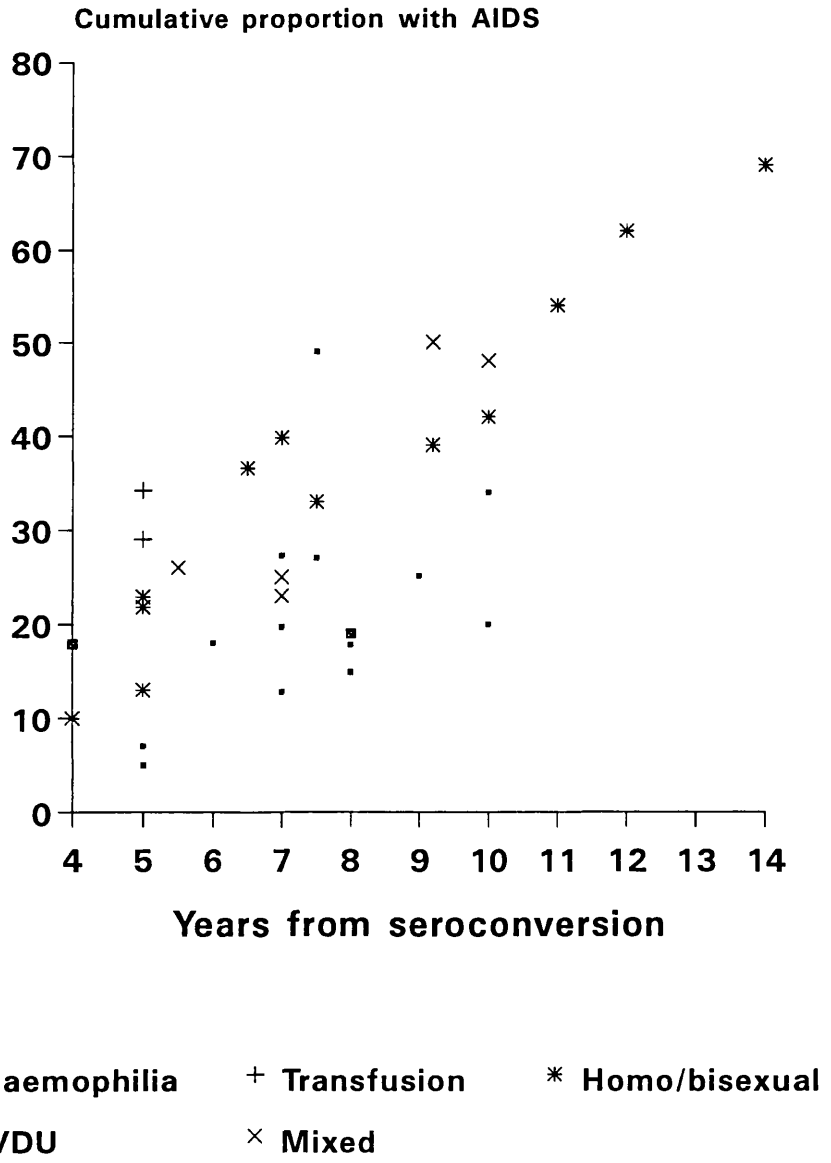


Table 3.7 : Summary of cumulative progression rates to AIDS from other published studies of HIV positive individuals

Study	Ref No.	Year of publication		Cumulative percentage progressing to AIDS	Median incubation period
Haemophilia patients					
Eyster	67	1987		18% by 6 years	-
Giesecke	172	1988		5% by 5 years	-
Darby	33	1989		7% by 5 years	-
Jason	184	1989		27% by 7.5 years	-
Goedert	68	1989		25.1% by 9 years	-
Biggar	108	1990	adults	27.3% by 7 years	-
			children	19.7% by 7 years	-
Ragni	185	1990		49% by 7.5 years	-
Schinaia	186	1991		12.8% by 7 years	-
Eyster	177	1993	adults	34% by 10 years	-
			children	20% by 10 years	-
Chiarotti	171	1994		14.9 - 17.8% by 8 years	-
Transfusion recipients					
Giesecke	172	1988		29% by 5 years	-
Msellati	187	1990		34.2% by 5 years	-
Homo/bisexual men					
Jason	184	1989		33% by 7.5 years	-
Hessol	188	1989		39% by 9.2 years	-
Munoz	189	1989		21.8% by 5 years	10.7 years
Schechter	84	1989		13% by 5 years	-
Biggar	108	1990		39.8% by 7 years	-
Rutherford	53	1990		54% by 11 years	-

Table 3.7 (continued)

Study	Ref No.	Year of publication		Cumulative percentage progressing to AIDS	Median incubation period
Schechter	79	1990		36.6 % by 6.5 years	-
Hessol	190	1990		-	6.8 years
Lifson	191	1992		-	9.7 years
Phair	106	1992		10% by 4 years	-
Buchbinder	192	1994		69% by 14 years	-
Veugelers	193	1994		22.9% by 5 years	8.3 years
Hogg	194	1994		42% by 10 years	-
Hessol	195	1994		62% by 12 years	10.2 years
Intravenous drug users					
Rezza	66	1989		17.9% by 3.5 to 4 years	-
Flegg	196	1994		19% by 8 years	-
Mixed cohorts					
Moss	85	1989		48% by 10 years	-
Sinicco	78	1993		26% by 5.5 years	-
Hendriks	197	1993		50% by 9.2 years	9.2 years
Cozzi Lepri	198	1994	IVDUs/Hetero	23% by 7 years	-
			- men		
			women	25% by 7 years	-

The diagnosis of AIDS is a collection of clinical conditions of differing severity and diagnostic complexity which may occur whilst the individual is only mildly immunosuppressed or may not be diagnosed until post mortem. Many conditions may be hard to diagnose in IVDUs[178]. Hence, differences in the estimated incubation period between studies may reflect differential diagnoses in different exposure categories. Further, conditions related to HIV infection which result in significant mortality may not always be included in the AIDS definition (e.g. liver failure due to co-infection with HCV), possibly resulting in a lower progression rate to AIDS in haemophilic patients.

In this cohort almost half of the patients presented with PCP as their initial AIDS-defining condition (18/47). This proportion is higher than in other studies although confidence limits around the estimated proportions are wide (24% to 52%) and include estimates from most other cohorts[89,108-116]. Initial AIDS-defining conditions may differ between exposure groups[110,128,178,199], and gender[113,200], racial[118] and geographic differences[122] have also been suggested. As Kaposi's Sarcoma is almost non-existent in haemophilic patients, the proportion presenting with PCP may be expected to be larger than in studies including homosexual men. However, since the incidence of Kaposi's Sarcoma as an initial AIDS-defining condition appears to be declining in other exposure categories[110,117,119], this difference may be less marked in the future.

CMV and MAI, seen rarely as an initial AIDS defining condition in this cohort, but quite frequently as a subsequent diagnosis, are also seen late on in infection in other studies[129,137,201].

3.6.6 CD4 counts at the time of AIDS

At the time of an AIDS diagnosis, CD4 counts were 80 cells/mm^3 on average. In other studies, median CD4 counts at diagnosis range from 100 to 190 cells/mm^3 [52,88,135,180] with around 85% having a count below 200 cells/mm^3 [69,199]. In addition to Kaposi's Sarcoma, lymphoma, extrapulmonary tuberculosis and HIV encephalopathy have been shown to occur at higher CD4 counts than other conditions[110,202]. If the frequency of these diagnoses varies between studies, then CD4 counts at the time of diagnosis may also be expected to vary[89,110]. Partly as a result of the use of prophylaxis and antiretroviral therapies which delay the onset of AIDS[140,142,154-157], CD4 counts at the time of AIDS diagnosis are thought to be dropping over time[110,120,136]. However, some of this effect may also be because in cohorts where patients are infected around the same time, those who develop AIDS at higher CD4 counts will develop AIDS sooner than those developing AIDS at low CD4 counts. Consequently, the CD4 count at AIDS will

appear to drop over time. The effect of prophylaxis and antiretroviral therapies on the incidence rates of clinical events in this cohort is of interest and has been studied, although is not included in this thesis. Two published papers which consider the effect of therapies on the incidence of clinical conditions[203,204] are included in Appendix III and suggest that whilst there has been little change in the incidence of conditions at high CD4 counts over time, there has been a reduction in the incidence of new AIDS-defining events (particularly PCP), oral candida and herpes zoster at low CD4 counts.

3.6.7 Survival

Whilst the incubation period from seroconversion to death is not affected by inconsistent diagnoses, it has been studied by far fewer authors than the AIDS incubation period. The median period between seroconversion and death has been reported to range between 10.6 and 12.3 years[195,205] with between 8 and 18% of individuals dying by 5 years after seroconversion[187,193] and a 33% death rate by 10 years after seroconversion[194]. As few clinicians continue to monitor CD4 counts after AIDS, preterminal CD4 counts are rarely reported.

Median survival times after AIDS are reported to be between 5 and 20 months[111,114,115,124,132,133,194,198,206-210]. Three year survival is poor[202,211], although survival does appear to be increasing[132,202,206,210,212], due to both improved clinical care and earlier diagnosis of AIDS. There is little evidence that survival is improving in this cohort, although the number of cases diagnosed is small and follow-up continues on many of the patients diagnosed since 1989. Survival may be related to the initial AIDS-defining condition[115,116,213]; those with Kaposi's Sarcoma or oesophageal candida have good survival[12,136,214] whereas those with wasting syndrome, lymphoma and MAI have poorer survival[131]. As in this cohort, a diagnosis of PCP is usually associated with longer survival[213,215] and improvements in survival have mainly been seen in patients with PCP[114,136,212], indicating that the treatment for PCP is improving. In this cohort, improved survival rates were seen in those who were younger at diagnosis, those with higher CD4 counts and those who had not received zidovudine prior to their AIDS diagnosis. Whilst these differences were not significant the differences were large and in the direction expected given other published research. In general, older individuals have a poorer expected survival than younger individuals[111,114,132,133,206,207,216-218] although this may be due to the fact that different age groups present with a different range of AIDS-defining conditions[207] which may have different prognoses. A higher CD4 count at diagnosis is usually associated with a better prognosis[115,207,218,219]. The association between zidovudine use and survival has also been shown in other

studies[202,209] and could reflect differences in the clinical status of patients at AIDS diagnosis - those who required zidovudine prior to AIDS may have been more ill than those who did not receive the drug. Alternatively, the effect of zidovudine may be of limited duration, and those patients who have received zidovudine prior to AIDS may have already experienced any benefit in delaying progression to AIDS.

In this cohort, ten patients received their AIDS diagnosis at or after death as a result of information available from post-mortem. In general, patients with zero survival reported in other studies tend to be patients who have less access to care and who therefore present late in infection[220]. However, in this cohort, all patients have equal access to care and these patients are simply those who were diagnosed early in the epidemic when the experience of AIDS was limited. There is the potential for bias when including these patients in the analysis of survival as they do not represent the true natural history of HIV infection, but an artefact of delayed diagnosis. However, even if these cases are excluded, the results are essentially unchanged.

3.6.8 The effect of clinical conditions on disease progression

Some oral manifestations of HIV infection (oral candida, oral hairy leukoplakia) have been shown to be associated with progression to AIDS in a number of studies[61,93,103,221]. In those who develop oral candida, AIDS develops between 219 and 790 days later[93,182,222] with 62% developing AIDS by 2 years after oral candida[177]. Consistent with these reports are the findings in this study that oral candida is associated with a more rapid progression to AIDS. There are reasons why one may expect an association between oral candida and progression to AIDS; oral candida may spread to the oesophagus to form oesophageal candida[223], or oral candida may lead to difficulty in eating and the development of wasting syndrome[224], both of which are AIDS-defining conditions. However, it may be possible that the association between oral candida and disease progression in this cohort reflects the fact that individuals with these conditions are more immunosuppressed and will progress to AIDS more rapidly than those without the condition. This will be discussed further in chapters 5 and 6.

Thrombocytopenia has been reported to increase progression to AIDS in one study[67] but not in others[100], findings which again may be explained by differences in the definition of thrombocytopenia used. Similarly, herpes zoster has been found to be associated with a more rapid disease progression in one study[61] but not in others[90,91,225,226]. This has not been shown in this study, although the incidence of skin complaints and bacterial infections do appear to be associated with progression to AIDS, findings which have not

previously been shown in other studies. The presence of bacterial infections may indicate deficiencies in B-cells, which was one of the earliest noted abnormalities in patients with AIDS[227]. Whether these conditions simply reflect individuals who are experiencing more severe immunosuppression will be discussed in Chapters 5 and 6.

CHAPTER 4 : CO-FACTORS AND THEIR EFFECT ON HIV DISEASE PROGRESSION

4.0 Summary of contents

In this chapter, the relationships between five co-factors recorded in the cohort (calendar year of seroconversion, age, social class, haemophilia diagnosis and CMV serostatus) and disease progression will be investigated. For each of the factors studied, Kaplan-Meier plots are used to illustrate the relationship between the studied factor and clinical outcome and Cox proportional hazards models are used to quantify this relationship.

4.1 Literature review

A co-factor can be defined as a factor which influences disease progression in some way. The wide inter-person variability of the HIV 'incubation period' suggests that co-factors may exist which interact with HIV to increase the rate at which AIDS develops. It is important to identify such co-factors. Some are potentially modifiable and knowledge that these affect disease progression may be of benefit to patients. Others may not be modifiable, but knowledge of their effect on disease progression may provide information about HIV pathogenesis and prognosis.

4.1.1 Potential co-factors

Potential co-factors for HIV infection fall into four main categories : patient demographics, behavioural factors, haemophilia-related factors and viral or other infectious agents.

4.1.1.1 Demographics

Demographic factors include patient age, race, social class, educational background and gender.

In general older individuals are likely to experience more rapid disease progression and shortened survival[33,64,77,195,205,222,228-230]. However, some authors question whether age is an important cofactor[62,172,231,232] or whether age is more important during early HIV infection[233], or for progression to different AIDS conditions[234]. The role of age may be related to other potential co-factors, such as longer exposure to other viral agents, and in haemophilia patients, longer exposure to clotting factor concentrates.

Racial differences are commonly studied in cohorts from the USA where the number of individuals from different racial backgrounds is usually large enough for comparisons to be made. Blacks and Hispanics may have shorter incubation periods and survival from AIDS than whites[220,235,236] although this is likely to reflect poorer access to care[237]. When length of infection is controlled for, this effect often disappears[88,188] suggesting that these individuals tend to present at a later stage of infection.

Social class and educational background are usually related to disease progression through access to health care, diet, alcohol consumption and drug use. However, it has been suggested that there is some effect of social class on disease progression which cannot be explained by differences in either access to health care or educational background[238].

Gender differences have been suggested[239] with the suggestion of a more rapid progression to AIDS in women than men[187] and shorter survival after AIDS[215,220,235]. This may reflect poorer access to health care in women[240], but it may also reflect the possible effects of pregnancy[241], or domestic violence[242] on disease progression.

4.1.1.2 Behavioural characteristics

Patient behavioural characteristics include HIV exposure category, sexual activity, injecting drug misuse, smoking, diet and alcohol consumption.

Unfortunately, many cohorts are made up of patients from one exposure group only. Comparisons of exposure groups may involve comparisons of different cohorts, and thus confounding with other factors (for example geographic or age differences) is present. Differences in progression rates by exposure category have been suggested, although the results are rarely consistent[77,108,163,172,184,240,243-245]. These differences, if they exist, may be explained by differences in the distribution of other co-factors, such as age[233], or exposure to other infectious and viral agents.

Increased numbers of sexual partners, especially anal receptive partners, have been found to be associated with a more rapid progression to AIDS[84,106,166]. Continued injecting drug use has also been studied[167] although there is little consistent evidence that continuing injectors have a more rapid disease progression than ex-injectors[88,246].

Tobacco smoking has been shown to have a positive effect on the immune system of the smoker[247,248]. Whilst smokers experience a higher rate of most illnesses[249], smoking may be protective against both Kaposi's Sarcoma[107] and oral lesions[250]. However, the

effect of smoking on HIV disease progression has not yet been confirmed[231,251,252] and any positive benefit for smokers to the immune system is unlikely to last for long after infection[248].

As some AIDS-defining conditions are seen in HIV negative patients deficient in particular dietary nutrients[253], nutritional factors may be expected to have an effect on disease progression. Increased levels of vitamins B and C and niacin are associated with slower progression of disease whilst high levels of other nutrients, including zinc, are associated with more rapid progression to AIDS[254]. The effect of weight on disease progression has also been studied[251,255], although its study is problematic due to its role as both a co-factor and a marker of progression of HIV disease. The few published studies into alcohol consumption have shown no consistent effect on HIV disease[102,231].

4.1.1.3 Haemophilia-related factors

It is thought that exposure to clotting factor concentrates may have an effect on disease progression in a number of ways. Exposure to a larger quantity of clotting factor concentrates could result in a larger overall virus innoculum and possibly reinfection with different strains of HIV after initial infection. As a small effect of concentrates on the immune system is seen in uninfected haemophilic patients[168,169], continued exposure to clotting factor concentrates which contain other viral contaminants may have a detrimental effect on the immune system. Haemophilia severity may be related to exposure to clotting factor concentrates or exposure to a higher innoculum of virus at the time of infection. Concentrate usage and severity and type of haemophilia have all been suggested as co-factors[186] although studies which have considered these factors have not found any effect on disease progression[32,33,68,225,256].

4.1.1.4 Viral / infectious agents

There are many ways in which a virus may interact with HIV to speed progression to AIDS[257]. However, it is important to distinguish these from a virus acting in an opportunistic manner, with the HIV infected individual being more likely to become infected with the virus, or to experience a reactivation of a previous infection, as their immune system deteriorates. There is little agreement in published studies about the effects of viral agents *in vivo* however. Cytomegalovirus (CMV) is a herpesvirus which has been shown to transactivate HIV *in vitro*. Whilst AIDS has been shown to occur primarily in those with high levels of antibody to CMV[258], CMV serostatus may not be predictive for the development of AIDS[62,259-261]. Hepatitis B virus (HBV) has also been shown to transactivate HIV and some studies suggest a relationship between markers to

HBV and disease progression[166,252,262,263] although results in the literature do not consistently find any relationship with the development of AIDS[62,225].

The presence of sexually transmitted diseases has been suggested to hasten progression to AIDS[125,126]. Some viruses have been shown to lead to illness in patients with HIV, including human herpesvirus 6[264,265], and possibly a viral agent related to the development of Kaposi's Sarcoma[127]. These have been suggested as potential co-factors in HIV infection.

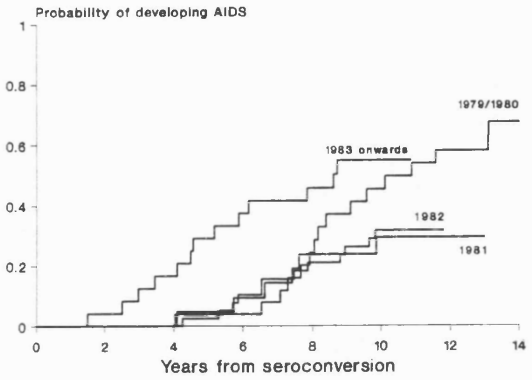
4.2 Calendar year of seroconversion

In the Royal Free Hospital Haemophilia Cohort, patients were stratified according to whether their estimated date of seroconversion was in 1979-1980 (25 patients), 1981 (21 patients),1982 (40 patients) or 1983 onwards (25 patients). Kaplan-Meier plots showing progression to the three endpoints stratified by year of seroconversion are shown in Figure 4.1 and progression rates are summarised in Table 4.1. Significant differences are seen in progression to AIDS (p=0.02) and to death (p=0.04), but only a marginally non-significant difference is apparent for progression to the development of the first HIV-related event (p=0.07, log-rank test). A higher progression rate is seen in those who seroconverted prior to 1981, and those who seroconverted in 1983 or later.

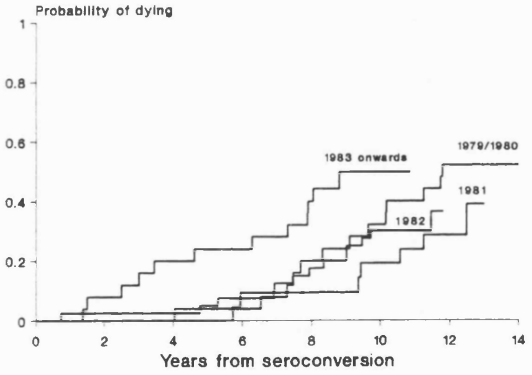
The year of seroconversion was added to a Cox Proportional Hazards model as a categorical variable. In comparison to those who seroconverted in 1979 and 1980, there was no evidence of any significant difference in the hazard of the development of AIDS in any of the three groups (Table 4.2). However, the relative hazards were consistent with the findings from the Kaplan-Meier analysis, with a lower risk in those seroconverting in 1981 and 1982 but a raised risk in those seroconverting later. The addition of interaction terms with the log of time to test the appropriateness of the proportionality assumption suggested that the effect of seroconverting in 1983 or later became less marked with increased time from seroconversion (p=0.008, Table 4.3). Results were similar for progression to death although there was no evidence of any significant change in the relative hazards over time. Progression to the first HIV-related event was significantly higher in those who seroconverted most recently although the hazards associated with seroconverting in 1981 and 1983 onwards both appear to decrease over time.

Figure 4.1 : Kaplan-Meier plots showing the cumulative proportion of infected patients progressing to (i) AIDS, (ii) death and (iii) the first HIV-related event, according to the number of years from seroconversion. Patients are stratified into four groups on the basis of the calendar year in which they were estimated to have seroconverted

(i) AIDS



(ii) death



(iii) first HIV related event

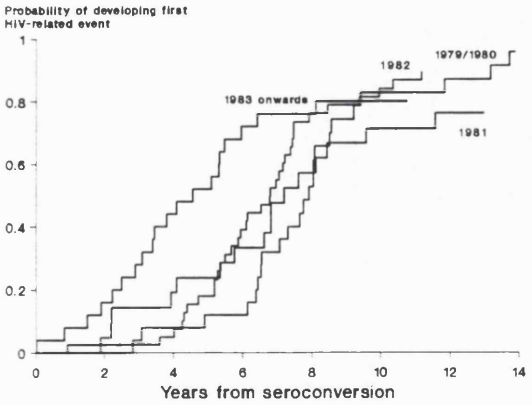


Table 4.1 : Numbers developing (i) AIDS, (ii) dying and (iii) an HIV-related event (skin complaint, bacterial infection, herpes zoster, thrombocytopenia, oral candida or AIDS) by 1st January 1994. Kaplan-Meier progression rates to AIDS, death and the first HIV-related event by 14 years, unless otherwise stated.

(i) Progression to AIDS

	AIDS			
	Number of patients	Number of events	Median time to progression (years)	Cumulative proportion developing AIDS
Calendar year of seroconversion				
1979-1980	25	16	10.87	67.3
1981	21	6	-	29.3 (13.0 yrs)
1982	40	12	-	31.7 (11.8 yrs)
1983-1985	25	13	8.60	54.9 (10.8 yrs)
p-value		0.02		
Age at seroconversion				
< 17	27	8	13.10	67.0 (13.4 yrs)
> 17, < 22.6	29	12	13.11	50.3
> 22.6, < 31	27	14	10.87	53.1
> 31	28	13	7.85	50.3 (13.9 yrs)
p-value		0.06		
Social class				
Non-manual	60	23	13.11	54.4
Manual	50	23	11.58	54.7 (13.9 yrs)
p-value		0.23		
Haemophilia type				
Severe A	101	43	13.10	58.2
Other	10	4	-	41.7
p-value		0.92		

(ii) Progression to death

	Death			
	Number of patients	Number of events	Median time to progression (yrs)	Cumulative proportion dying
Calendar year of seroconversion				
1979-1980	25	13	11.78	52.0
1981	21	7	-	38.8 (13.0 yrs)
1982	40	13	-	36.4 (11.8 yrs)
1983-1985	25	12	-	49.6 (10.8 yrs)
p-value		0.04		
Age at seroconversion				
< 17	27	4	-	24.0 (13.8 yrs)
> 17, < 22.6	29	9	-	31.4
> 22.6, < 31	27	13	12.49	59.1
> 31	28	19	7.89	71.4 (13.9 yrs)
p-value		0.0001		
Social class				
Non-manual	60	22	-	37.7
Manual	50	23	11.78	60.5 (13.9 yrs)
p-value		0.21		
Haemophilia type				
Severe A	101	41	-	46.9
Other	10	4	-	42.9
p-value		0.95		

(iii) Progression to first HIV-related event

	Number of patients	Number of events	HIV-related event Median time to progression (yrs)	Cumulative proportion developing HIV-related event
Calendar year of seroconversion				
1979-1980	25	23	7.92	95.7
1981	21	16	7.20	76.2 (13.0 yrs)
1982	40	34	6.81	89.4 (11.6 yrs)
1983-1985	25	20	4.56	80.0 (10.8 yrs)
p-value		0.07		
Age at seroconversion				
< 17	27	20	8.04	80.5 (12.7 yrs)
> 17, < 22.6	29	26	7.47	100.0 (13.7 yrs)
> 22.6, < 31	27	25	5.87	100.0 (13.2 yrs)
> 31	28	22	5.20	82.7 (13.9 yrs)
p-value		0.05		
Social class				
Non-manual	60	53	7.05	100.0 (13.7 yrs)
Manual	50	39	6.43	83.3 (13.9 yrs)
p-value		0.82		
Haemophilia type				
Severe A	101	84	5.87	100.0 (13.2 yrs)
Other	10	9	6.82	93.5 (13.8 yrs)
p-value		0.62		

Table 4.2 : Relative hazards and 95% confidence intervals from Cox proportional hazards model associated with progression to AIDS, death and the first HIV-related event

		AIDS			Death			HIV-related event		
		Relative hazard	95% Confidence Interval	p-value	Relative hazard	95% Confidence Interval	p-value	Relative hazard	95% Confidence Interval	p-value
Calendar year of seroconversion	1979-1980	1	-	-	1	-	-	1	-	-
	1981	0.47	0.18-1.23	0.13	0.58	0.23-1.45	0.24	0.94	0.49-1.80	0.85
	1982	0.53	0.25-1.15	0.11	0.73	0.33-1.60	0.43	1.39	0.80-2.41	0.24
	1983-1985	1.55	0.72-3.33	0.26	1.92	0.84-4.38	0.12	2.03	1.10-3.78	0.02
Age at seroconversion	(per 5 yrs)	1.20	1.10-1.32	0.0001	1.35	1.23-1.48	0.0001	1.13	1.06-1.22	0.0006
Social class	Non-manual	1	-	-	1	-	-	1	-	-
	Manual	1.42	0.80-2.55	0.23	1.45	0.81-2.60	0.21	0.95	0.63-1.44	0.82
Haemophilia type	Severe A	1	-	-	1	-	-	1	-	-
	Other	0.95	0.34-2.65	0.92	1.03	0.37-2.88	0.95	1.20	0.59-2.41	0.62

Table 4.3 : Tests of significance for non-proportionality (p-value for the inclusion of an interaction term with the logarithm of time from seroconversion into the Cox proportional hazards model)

		AIDS	Death	HIV-related event
Calendar year of seroconversion	1979-1980	-	-	-
	1981	0.11	0.84	0.04
	1982	0.16	0.26	0.41
	1983-1985	0.008	0.14	0.006
Age at seroconversion	(per 5 years)	0.02	0.77	0.16
Social class	Non-manual	-	-	-
	Manual	0.95	0.87	0.06
Haemophilia type	Severe A	-	-	-
	Other	0.42	0.96	0.95

4.3 Age at seroconversion

Age was initially stratified into four age groups, defined by the quartiles of the distribution of patient ages at seroconversion. The effect of age as a continuous variable was then studied using the Cox proportional hazards model.

Older individuals appeared to have a higher risk of AIDS in the first few years after seroconversion than younger individuals (Figure 4.2). However by 14 years after seroconversion progression rates were similar and differences between the groups were marginally non-significant (p=0.06, log-rank test, Table 4.1). Figure 4.3 suggested that there was evidence for lack of proportionality in the hazard rates over time. When fitted as a continuous variable in the Cox model, a five year increase in age at seroconversion resulted in a 20% increase in the hazard of AIDS (p=0.0001, Table 4.2). However, as there is significant evidence of non-proportionality in the estimate (Table 4.3) this should be viewed as an 'average' relative hazard; the relative hazard is higher at the time of seroconversion but declines over time.

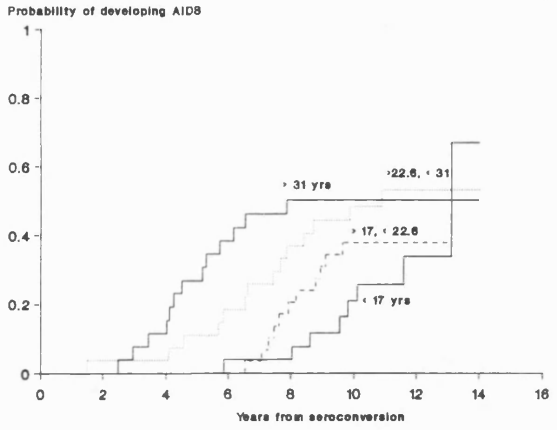
As expected, increased age was very strongly associated with survival (Figure 4.2). By 13.8 years after seroconversion only 24% of those under the age of 17 years at seroconversion are estimated to have died compared to 71.4% of those over the age of 31 years (p=0.0001, log-rank test, Table 4.1). Whilst there is a visual suggestion of non-proportional hazards, with the lines moving slightly closer together over time (Figure 4.3) there was no evidence of this when formally tested in the Cox Proportional hazards model (p=0.77 for interaction term). An increase of 5 years in age is associated with a 35% increase in the hazard of death in the Cox model (p=0.0001, Table 4.2).

Age was strongly associated with progression to the first HIV-related event (Figure 4.2). Median times to the onset of the first HIV-related events were 8.0 years in those under the age of 17 years at seroconversion compared to 5.20 years in those over the age of 31 years (p=0.05, log-rank test). Whilst there is strong evidence of non-proportionality when fitted as a categorical variable (Figure 4.3), when considered as a continuous variable in the proportional hazards model this effect disappeared (Table 4.3). A five year increase in age at seroconversion was found to be associated with a 13% increase in the hazard of developing the first HIV-related event (p=0.0006, Table 4.2).

The addition of an age-squared term did not improve the fit of any of these three models.

Figure 4.2 : Kaplan-Meier plots showing the cumulative proportion of infected patients progressing to (i) AIDS, (ii) death and (iii) the first HIV-related event, according to the number of years from seroconversion. Patients are stratified into four groups on the basis of their age at the time of seroconversion.

(i) AIDS



(ii) death

(iii) first HIV related event

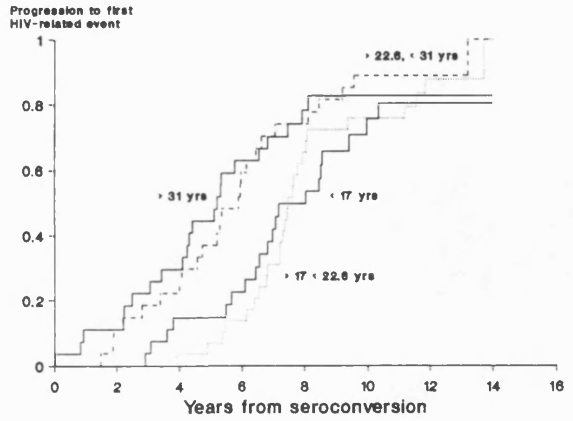
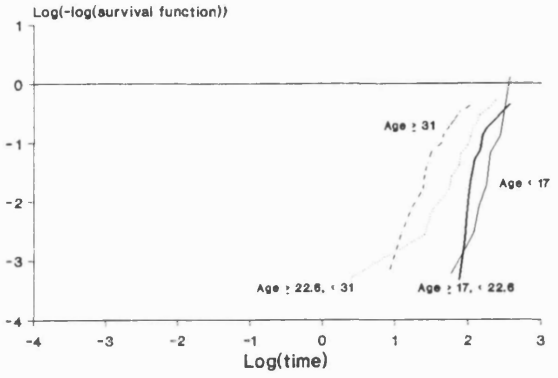
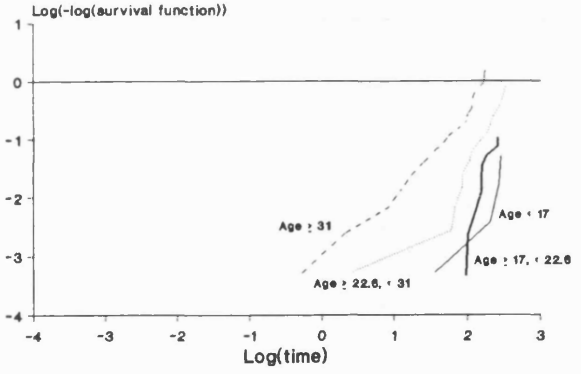


Figure 4.3 : A plot of the log(-log(estimated survival probability)) against the log of time for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event. If the assumption of proportionality is appropriate, the lines for each of the groups should be approximately parallel.

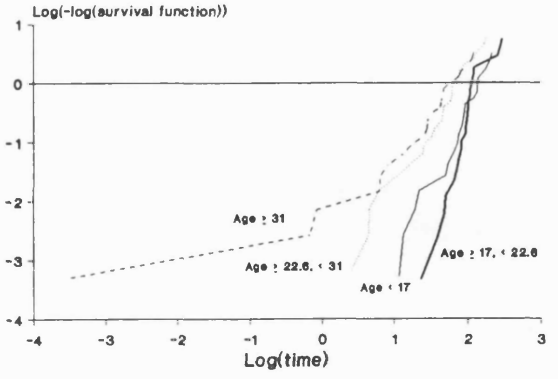
(i) AIDS



(ii) death



(iii) first HIV related event



4.4 Social class

For the purposes of this analysis, patients have been grouped into non-manual (I/II/III-N) and manual (III-M/IV/V) social classes. The median times to development of AIDS are 13.11 years and 11.58 years in non-manual and manual workers respectively, although overall there are no differences in progression rates to AIDS (p=0.23, log-rank test, Figure 4.4). By 14 years after seroconversion only 37.7% of non-manual workers are estimated to have died compared to 60.5% of manual workers by 13.9 years. Despite this difference in cumulative rates at 14 years there is no significant difference in the survival curves over the whole time period (p=0.21, log-rank test). Social class appears to have little effect on progression to the first HIV-related event, with median times of 7.05 years and 6.43 years in non-manual and manual workers respectively (p=0.82, log-rank test). The assumption of proportional hazards seems reasonable for progression to all three endpoints (Table 4.3).

4.5 Haemophilia Diagnosis

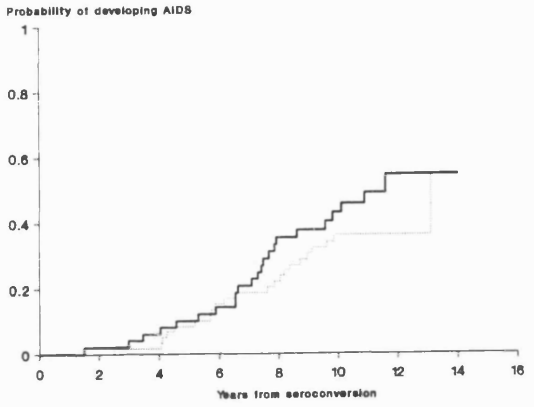
Because of the small numbers of individuals without severe haemophilia A the power to detect any difference if one exists is very small and Kaplan-Meier plots have not been included for this variable. Progression rates to AIDS, death and the first HIV-related event are similar in those who suffer from severe haemophilia A to others. In particular, 58.2% of those with severe haemophilia A would have developed AIDS by 14 years after seroconversion compared to 41.7% of those with other conditions (p=0.92, log-rank test). 46.9% of those with severe haemophilia A are estimated to have died compared to 42.9% of those with other conditions (p=0.95, log-rank test). Finally, median times to the development of the HIV-related first event are 6.82 years and 5.87 years in those with severe haemophilia A and those with other conditions respectively (p=0.62, log-rank test). No evidence of non-proportionality is seen (Table 4.3), although the power to detect this is very small.

4.6 CMV status

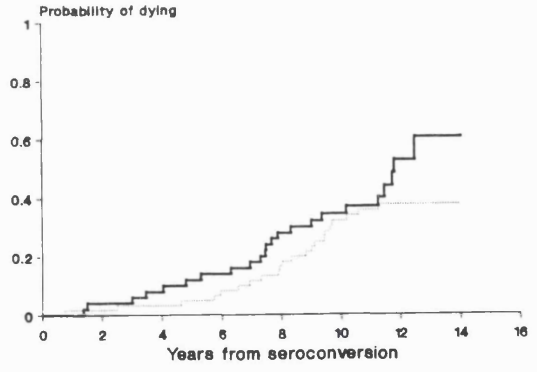
Antibodies to CMV were measured on early blood samples from 109 of the 111 (98%) patients in the cohort of whom 59 (54%) were found to be CMV-seropositive. A comparison of the CMV-seropositive and CMV-seronegative patients in the cohort with respect to their demographics is shown in Table 4.4.

Figure 4.4 : Kaplan-Meier plots showing the cumulative proportion of infected patients progressing to (i) AIDS, (ii) death and (iii) the first HIV-related event, according to the number of years from seroconversion. Patients are stratified into two groups according to whether they are from a manual (bold line) or non-manual (dotted line) social class

(i) AIDS



(ii) death



(iii) first HIV related event

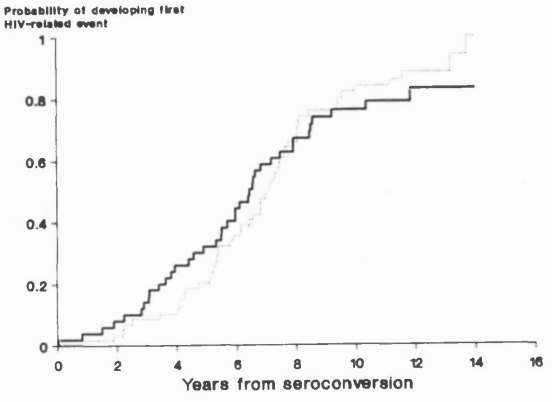


Table 4.4 : Comparison of CMV-positive and CMV-negative patients in cohort

		CMV-positive	CMV-negative	p-value*
	Number of patients	59	50	
Calendar year of seroconversion	1979-1980	15	9	
	1981	10	11	
	1982	20	19	
	1983-1985	14	11	0.75
Age at seroconversion	Median	25.6	18.7	
	Range	4.0 - 77.8	2.1 - 73.0	0.0006
Social Class	Non-manual	32	27	
	Manual	27	22	0.93
Haemophilia type	Severe A	52	46	
	Other	6	4	0.95

* Age at seroconversion - Wilcoxon test. All other variables - Chi-squared test.

CMV-seropositive patients were older at seroconversion but did not seroconvert to HIV any earlier or later, on average, than those who were CMV-seronegative, suggesting that CMV does not predispose patients to infection. No other associations between CMV status and demographic factors were apparent.

Kaplan-Meier plots showing progression to the three endpoints stratified by CMV status are shown in Figure 4.5. Individuals who are CMV-seropositive have a faster progression rate to AIDS and to death than those who are CMV-seronegative. Whilst 14 year progression rates are similar in the two groups, median times to development of AIDS are 9.86 years and 13.11 years in those CMV-seropositive and -seronegative respectively (p=0.03, log-rank test, Table 4.5). CMV serostatus is significantly associated with survival (p=0.01, log-rank test) with 60.4% of those who are CMV-seropositive but only 33.0% of those CMV-seronegative estimated to die by 14 years from seroconversion. Proportional hazards models suggest that individuals who are CMV-seropositive have a relative hazard of developing AIDS of 1.92 and of death of 2.23 (Table 4.5). CMV status does not, however, appear to have any effect on progression to more minor HIV-related events, with progression rates and median times to the first HIV-related event being similar in both groups (p=0.32, log-rank test). There is no evidence of non-proportionality on progression to any of the three endpoints.

The fact that CMV-seropositive patients tend to be older than CMV-seronegative patients could explain some of these differences in progression rates to AIDS and death. The use of proportional hazards models to estimate the relative hazard of CMV after adjusting for age differences (as a continuous variable), suggests that whilst some of the effect can be explained by age differences, there remains a raised, although non-significant, relative hazard to both endpoints (Table 4.5).

4.7 Discussion

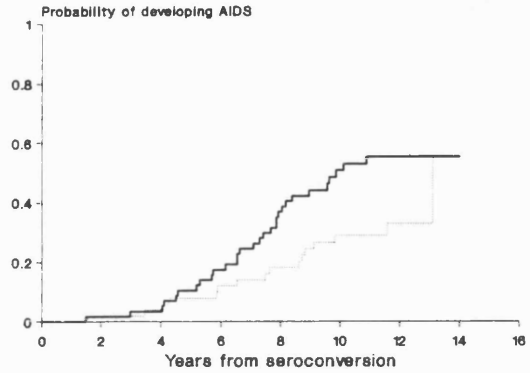
A number of factors which may be related to HIV disease progression and which were available from patient records have been studied in this cohort.

4.7.1 Calendar year of seroconversion

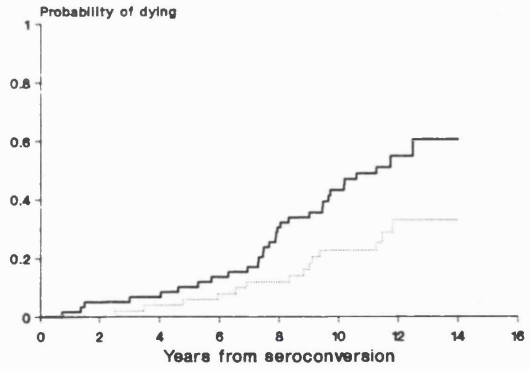
Interestingly, patients who seroconverted in 1979 and 1980 and those who seroconverted from 1983 onwards, had a higher risk of progression to all three endpoints. The most obvious explanation for increased risk of AIDS in those who seroconverted early is that clinicians had very little experience of HIV infection at that stage, HIV had not been identified and consequently patient survival was poor.

Figure 4.5 : Kaplan-Meier plots showing the cumulative proportion of infected patients progressing to (i) AIDS, (ii) death and (iii) the first HIV-related event, according to the number of years from seroconversion. Patients are stratified according to whether they are CMV-seropositive (bold line) or CMV-seronegative (dotted line)

(i) AIDS



(ii) death



(iii) first HIV related event

Table 4.5 : The effect of CMV serostatus on progression to AIDS, death and the first HIV-related event

Progression to:		AIDS		Death		HIV-related event	
		CMV positive	CMV negative	CMV positive	CMV negative	CMV positive	CMV negative
Number of events		30	17	31	14	51	42
Kaplan-Meier analysis							
Median progression time (yrs)		9.86	13.11	11.26	-	6.39	7.07
Proportion progressing*		55.5%	55.4%	60.4%	33.0%	100.0% by 13.7 years	100.0% by 13.2 years
p-value (log-rank test)		0.03		0.01		0.32	
Cox proportional hazards models							
Unadjusted	Relative hazard	1.92		2.23		1.23	
	95% confidence interval	1.06 - 3.50		1.19 - 4.21		0.82 - 1.86	
	p-value	0.03		0.01		0.32	
	p-value : test for non-proportionality	0.96		0.77		0.13	
Adjusted for age	Relative hazard	1.61		1.70		1.16	
	95% confidence interval	0.88 - 2.96		0.90 - 3.21		0.77 - 1.75	
	p-value	0.13		0.10		0.49	

* By 14 years after seroconversion unless otherwise stated.

Many patients did not have the opportunity to receive antiretroviral and prophylactic therapies. Further, these patients may have been those who received the most unheated clotting factor concentrates. After infection, these patients could have been exposed to infected concentrates for 4 or 5 years and may have been exposed to different viral strains of HIV. There is evidence that some strains of virus are more pathogenic than others[266], although it is not known whether early strains were more pathogenic. Unfortunately, we do not have information on the viral strain with which patients were initially infected and as computerised yearly concentrate usage records did not begin at the Centre until 1980, no information is available on the amount of concentrate received in the year of seroconversion in those who seroconverted earliest.

Such explanations for an increased risk in those infected early in the epidemic do not, however, lend themselves for the higher hazard seen in those infected from 1983 onwards. Improved diagnostic methods and a changing AIDS definition[9,10] may have resulted in an earlier diagnosis of AIDS, which would have most effect on those seroconverting most recently, although progression to death should be unaffected. Whilst biases in the estimates of seroconversion dates are small (see Chapter 3), more frequent blood testing from 1982 onwards means that the possibility of biased estimates of seroconversion dates which partly explain the differences in progression rates cannot be ignored. Further, this may simply represent a chance finding due to the small number of individuals seroconverting since 1983.

4.7.2 Social class

In this study, social class was not associated with disease progression. As discussed in Chapter 3, if the effect of social class on HIV progression is due to differences in access to care, then we would not expect to find large differences in progression rates in this cohort. Two other studies have shown effects on progression which are related to social class. Amongst the patients from the Vancouver Lymphadenopathy AIDS Study[194,238], those who progressed to AIDS or death rapidly were less likely to have finished secondary school and to have a higher social status. Downward social drift as a result of disease progression, could not explain these differences. Kerlikowske[205] also found that low household income was weakly associated with death.

4.7.3 Haemophilia type and severity

If continued exposure to intermediate purity clotting factor concentrates is related to HIV disease progression, then haemophilia type and severity may be expected to be related to disease outcome. Studies of Italian haemophilic patients[186,267] have repeatedly found that patients with haemophilia B had a faster progression rate to AIDS than those with

haemophilia A, although this difference was not statistically significant. In contrast, four studies[33,68,225,256] all found no difference in progression rates between patients with haemophilia A and B, and also found no effects of haemophilia severity on disease progression. In this study, no effects were found on disease progression, although the power to detect such differences was small. The finding of an increased rate of progression in patients with haemophilia B would be consistent with our findings that patients infected later in the epidemic had a faster progression rate to AIDS. In the UK, patients with haemophilia B tended to be infected later on in the epidemic, as they were not treated with concentrates imported from the USA. Unfortunately, haemophilia diagnosis and calendar year of seroconversion are confounding factors in this cohort. However, it may be possible to study the independent effects of these factors in some other haemophilia centres where commercial factor IX was used earlier.

4.7.4 Age at seroconversion

The finding that increased age at seroconversion was associated with disease progression was expected. Many other authors have also found age to be associated with disease progression[33,64,67,185,186,222,228,230]. Most of these studies included haemophilia patients who have a wide age range. Studies which have not found an effect of age on disease progression[62,188,231,232,268] are often made up of other exposure categories who have a narrower age range. It has been suggested that the increased risk due to age is mainly explained by a very high risk in individuals over the age of 40 at seroconversion[77,187] or possibly due to the very poor survival seen in babies if infected *in utero*[269]. Consequently, studies which follow young adults (e.g. IVDUs) may well not detect an age effect. Whilst results from other studies suggest that a quadratic relationship with age may be appropriate, a linear effect of age on progression appears to be adequate in this cohort with no evidence of an increased risk in the very young. This is consistent with findings from one other study[163]. However, this is perhaps not surprising as the children in the cohort were not infected *in utero*.

It is unlikely that age is a determinant of disease progression but it may be a marker for some characteristic which enables younger patients to cope with the pathological effects of HIV infection. For example, younger tissues are able to repair damage more rapidly. Alternatively, the immune system may deteriorate more rapidly in older individuals. This will be investigated further in Chapter 5. One of the assumptions of the proportional hazards model is that the effect of covariates on disease progression does not change over time (i.e. 'proportional' hazards). If this assumption is violated, as appears to be the case for age, then any adjustment for age to the effects of other factors in the model

may not be accurate. The finding that the detrimental effect of increasing age on disease progression diminishes with time has not been described in other studies. Mariotto *et al*[233] found, however, that there was a greater effect of age on progression from seroconversion to severe symptomatic disease, than from symptomatic disease to the development of AIDS. A recent study[234] suggested that age was only related to progression to neoplasms and not to other AIDS-defining conditions. This is surprising as the predominant neoplasm in their study was Kaposi's Sarcoma, and haemophilia patients, in whom the greatest age effect is often seen, very rarely develop Kaposi's Sarcoma.

4.7.5 CMV serostatus

The study of the effect of CMV infection on HIV disease progression is problematic as almost all homosexual men and IVDUs infected with HIV are co-infected with CMV. As CMV is transmitted by the cellular component of blood, it is not transmitted by clotting factor concentrates and the prevalence of antibodies to CMV in haemophilia patients is similar to that seen in the general population[270]. The effect of CMV status can therefore be studied in this cohort.

We have previously published papers suggesting that there is a detrimental effect of CMV-seropositivity on progression to AIDS[270,271]. However, the findings presented in this thesis benefit from an extra five years of follow-up with sufficient deaths to allow an analysis of survival to be carried out. Over time, the relative risk associated with CMV-seropositivity has reduced in size and become non-significant after adjustment for age differences. However, the risk remains raised and suggests an almost doubling in the hazard of AIDS. A number of other studies have reported findings about the effect of CMV positivity on HIV disease progression. Three fairly small studies[260,272,273] have considered the effect of CMV on progression to symptomatic HIV infection and AIDS and found no significant effect of CMV on progression. A recent study among haemophilia patients in the USA, however, offers the most convincing evidence for a lack of effect of CMV serostatus on disease progression[261]. The patients are of a similar age range to the patients in this cohort and antibodies to CMV were measured in the Department of Virology at the Royal Free Hospital under standardised conditions. Different patient recruitment and differences in the prevalence of other unidentified factors which may be confounded with CMV infection may explain the differences between these studies. As the finding of an increased risk associated with CMV seropositivity has been a consistent finding in this cohort, it seems likely that there may

have initially been some effect of CMV serostatus on disease progression, which was possibly limited in duration.

CHAPTER 5 : THE CD4 LYMPHOCYTE COUNT AND ITS RELATIONSHIP TO HIV DISEASE PROGRESSION

5.0 Summary of contents

In Chapter 4 I considered the role of co-factors in HIV infection. In Chapters 5 and 6 I shall consider the role of laboratory markers of progression. Markers of progression can be distinguished from co-factors in that they do not necessarily influence disease progression. However, they indicate how 'ill' a patient is and are therefore useful for disease staging. They may include laboratory markers or the presence of certain clinical conditions. In this chapter I shall consider the role of the CD4 count in HIV infection. I shall describe the pattern of the CD4 count throughout infection and will assess its value as a prognostic marker. Finally, I will begin to assess the effects of missing values and variability on the value attributed to the CD4 count as a prognostic marker. This topic will be further discussed in Chapter 7.

5.1 Literature review

Lymphocytes are central to the body's immune system. T-lymphocytes with the CD4 marker (CD4 lymphocytes) provide help for antibody production and co-ordinate immune responses to antigenic stimulation. They play an important role in the amplification of immune responses through the release of cytokine mediators which are important in the growth, function and differentiation of the cellular immune system[274-276]. It is the CD4 lymphocytes which appear to be the main target of HIV infection, and their loss appears to account for a major part of its immunosuppressive effect.

The CD4 count declines during HIV infection[260,277-280], with death usually occurring when the CD4 count is close to zero[208,275,281].

In uninfected individuals, a natural decline in CD4 counts is seen in children up to the age of 14[282]. Counts tend to be slightly depressed in uninfected haemophilic patients compared to the general population[73,74]. Other minor differences are thought to exist between different racial groups and by gender[283-285].

CD4 levels may drop rapidly in the months following infection with HIV[52,286]. They then usually return to near normal levels before dropping at an average rate of around 50 to 80 cells/mm^3 per year[280,287-291]. AIDS occurs on average once the CD4 count has fallen to around 50-150 cells/mm^3 [110,135,180]. Some authors have suggested that there is a

more rapid rate of CD4 decline immediately prior to AIDS[286,292]. Others have suggested that no such effect exists[260,293], and that possibly the reverse is seen, with a gradual slowing in the rate of decline at low levels[57]. There is a wide variability in individual rates of decline, and differences have been attributed to both exposure category[288] and age[260]. Whilst the CD4 count is the best marker of disease progression currently available to clinicians[51,180], it does not, however, fully explain differences in the rate of disease progression, and some individuals remain AIDS-free for long periods of time with low CD4 counts[294].

The CD4 count is a very variable measurement[92] being affected by both diurnal variation[295-297] and seasonal fluctuations[274]. Further, both storage methods[274] and exercise[298] can lead to changes in the count. This has led some authors to question whether the absolute CD4 count is the best marker to use[299].

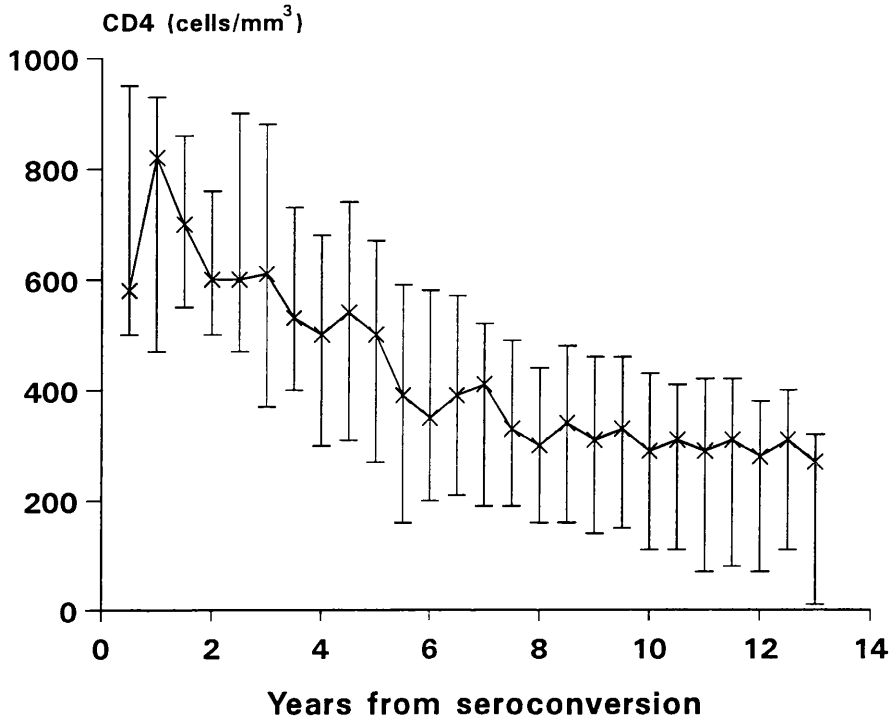
5.2 CD4 counts over infection

CD4 lymphocyte subsetting began at The Royal Free Hospital in December 1982 and routine blood tests are performed on patients every 3-6 months where possible. By the end of 1993, a median of 20 CD4 counts had been measured on each individual (range 1 - 63 counts per patient).

For each patient means of the CD4 counts measured within each 6 month period after seroconversion were calculated. Figure 5.1 shows the changing distribution of these means over time. A decrease in the average CD4 counts over time can clearly be seen. There is, however, a selective drop-out of patients who die with very low counts which results in a flattening in the CD4 level after 6 years or so.

Multi-level modelling methods were used to assess the rate and pattern of individual CD4 decline. Initially a simple linear model was fitted to assess whether a linear rate of decline in the CD4 count over time was acceptable. Both the CD4 count at seroconversion and the rate of CD4 decline were allowed to vary between patients (i.e. random at the between-patient level). A linear model suggested that the CD4 count at seroconversion was 809 cells/mm^3 (95% confidence interval 739 to 878 cells/mm^3) and counts declined at the rate of 64 cells/mm^3 per year (95% confidence interval 54 to 74 cells/mm^3 per year) on average. However, the standard deviations of both these parameters were large (348 and 49 respectively), suggesting that individual CD4 patterns varied considerably about this average slope.

Figure 5.1 : The medians and interquartile ranges of the means of all CD4 measurements taken within each six-monthly period after seroconversion



The addition of a random quadratic term resulted in a significant improvement in fit ($p < 0.00001$), indicating that rather than decline at a constant rate over time, the rate of CD4 decline gradually decreased as time increased. CD4 counts at seroconversion were now estimated to be 971 cells/mm^3 on average, the linear coefficient was estimated to be -121 and the quadratic coefficient was estimated to be 4.6 (Table 5.1). Again, high standard deviations of the three coefficients (459, 112 and 7 respectively) suggested large between-patient differences. Plots of the average curves generated from the mean values for both the linear and quadratic model for the untransformed CD4 counts are shown in Figure 5.2(i). The effect of the quadratic coefficient can clearly be seen in this plot. Whilst CD4 counts would fall below zero by around 12.5 years after seroconversion, on average, under a linear model, they would be expected to remain at higher levels above 200 cells/mm^3 by this time under the quadratic model.

One of the problems with modelling the untransformed CD4 count is that values estimated from the model can be negative. In order to avoid this situation, a further analysis of the pattern of CD4 decline over infection was performed after taking a square root transformation. As CD4 counts are more variable early after seroconversion, this also serves to partly stabilise the variation over time.

Table 5.1 : Final model coefficients from multi-level modelling of the CD4 count

	Fixed parameters		Random variances/covariances		
	Mean	95% confidence interval	Intercept	Time	Time^2
Untransformed CD4					
Intercept	971.3	871.5 to 1071.1	211,100	-	-
Time	-120.6	-147.0 to -94.2	-41,770	12,580	-
Time^2	4.6	2.7 to 6.4	2088	-757	55
Square root CD4					
Intercept	30.9	29.0 to 32.9	74.8	-	-
Time	-2.1	-2.8 to -1.4	-18.8	8.0	-
Time^2	0.01	-0.04 to 0.07	1.1	-0.5	0.05

The inclusion of a linear rate of decline for √CD4 over time (random at between-patient level) was highly significant ($p<0.00001$). The addition of a random quadratic coefficient for √CD4 also explained a significant amount of variation between patients ($p<0.00001$) although the overall mean coefficient was not significantly different from zero ($p=0.35$), suggesting that whilst √CD4 counts declined in a quadratic manner in some individuals, there was no consistent pattern to this.

Results from the final fitted model are shown in Table 5.1. The mean √CD4 at seroconversion was estimated to be 30.9 with the √CD4 count dropping at a mean rate of 2.1 per year and with a small positive quadratic coefficient of 0.01. Whilst the CD4 count is expected to be 955 cells/mm^3 at the time of seroconversion, therefore, by 12.5 years after seroconversion it is expected to have fallen to 39 cells/mm^3 on average (Figure 5.2(i)) . However, there is wide variation between the patients in the cohort with √CD4 counts at seroconversion ranging between 13.9 and 47.9 in 95% of patients (absolute counts of between 193 and 2290 cells/mm^3), and rates of √CD4 change ranging between a drop of 7.6 per year and an increase of 3.4 per year.

Much of the variation in √CD4 counts at the time of seroconversion may be explained by the fact that at this time CD4 measurements are only available for a small proportion of individuals and therefore the mean estimate is based on information only from these individuals. This wide variation in individual CD4 patterns is illustrated in Figure 5.2(ii).

Figure 5.2(i) : 'Average' patterns of CD4 change over infection generated from the models estimated using multi-level modelling methods. (i) Untransformed CD4 count, linear model - continuous line, (ii) untransformed CD4 count, quadratic model - dotted line, (iii) √CD4 count - dashed line.

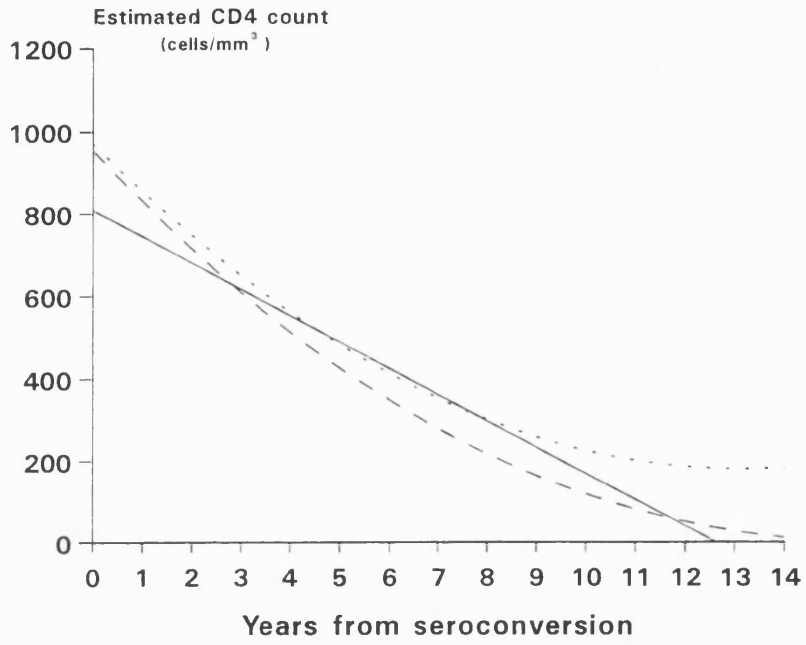
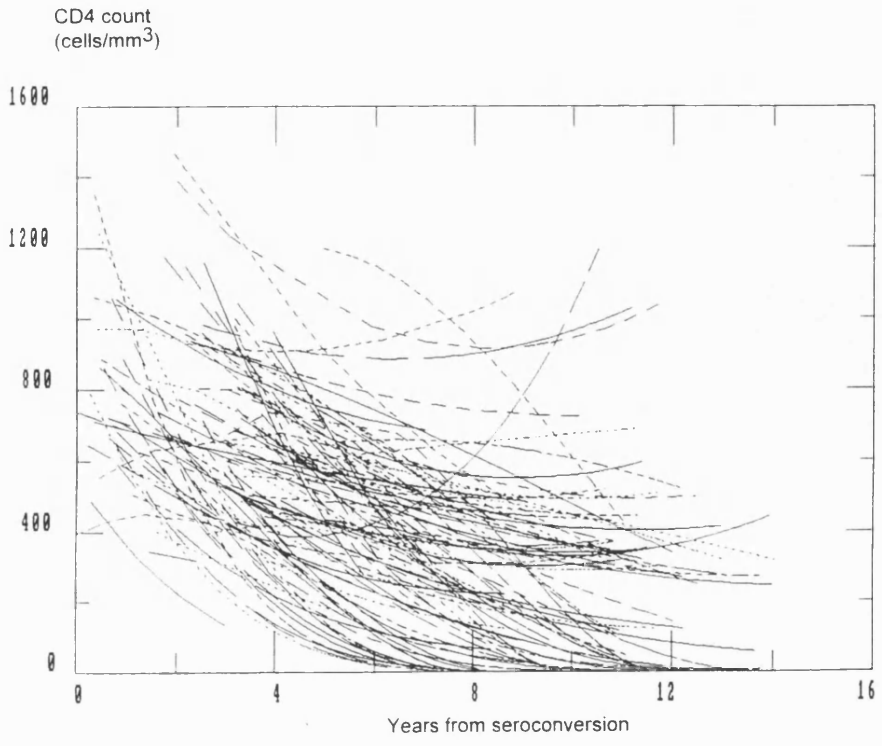


Figure 5.2(ii) : Estimated CD4 patterns for each patient in the cohort after modelling the square root of the CD4 count using multi-level modelling methods



There was a high level of correlation between the parameter estimates. For example, individuals with high CD4 counts at the time of seroconversion tend to have more rapidly declining CD4 counts (correlation = -0.77) and a higher quadratic coefficient (correlation = 0.57).

The within-individual variation for √CD4 was studied further and can be described by the equation

$$44.5 + (10.4 \times \text{time}) - (1.0 \times \text{time}^2) + (0.03 \times \text{time}^3)$$

So, for example, at seroconversion the within-individual variation for a patient's CD4 count is estimated to be 44.5. However, by 10 years after seroconversion this has increased to 78.5. Hence, after taking a square root transformation the amount of variation in the √CD4 measurement generally increases over the first 15 years after infection. In relative terms, although absolute counts become less variable over time, the relative variability in the CD4 count actually increases, i.e. a 50 cell/mm^3 variation when the CD4 count is only 100 cells/mm^3 represents a much larger, relative, variation than a 100 cell/mm^3 variation whilst the CD4 count is around 600 cells/mm^3.

Multi-level modelling methods were used to assess whether any of the demographic and clinical factors described in Chapters 3 and 4 (age group, calendar year of seroconversion, haemophilia type, and CMV serostatus) had an effect on either the CD4 count at seroconversion or the rate of CD4 decline. Increased age was associated with a lower CD4 count at the time of seroconversion (estimated values of 1079 cells/mm^3 in those aged < 22.6 years at seroconversion compared to 802 cells/mm^3 in those aged ≥ 22.6 years at seroconversion, p=0.00003) but no effect of age on the rate of CD4 decline was apparent. No other factor was associated with either an increased CD4 count at seroconversion or a more rapid rate of CD4 decline.

5.3 The prognostic value of the CD4 count

In order to assess short-term prognosis (i.e. over the typical length of time between CD4 measurements), the patients' most recent CD4 count was incorporated into a Cox proportional hazards model as a time-updated covariate. Results are shown at the top of Table 5.2.

Table 5.2 : Relative hazards and 95% confidence intervals from the Cox proportional hazards model for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event. The CD4 count has been included in the model as a time-updated covariate as an untransformed variable, and transformed using both the log and square root transformations.

Transformation	Progression to :	Relative hazard	95% confidence interval	p-value	Test of non-proportionality (p-value)	Wald Chi-square
None (per 100 cell/mm^3 drop)	AIDS	1.83	1.53 - 2.19	0.0001	0.005	43.02
	Death	1.68	1.40 - 2.00	0.0001	0.0001	32.33
	HIV-related event	1.27	1.14 - 1.40	0.0001	0.05	20.67
Log (per 1 log drop)	AIDS	1.43	1.31 - 1.56	0.0001	0.12	66.41
	Death	1.48	1.36 - 1.62	0.0001	0.59	76.70
	HIV-related event	2.76	2.09 - 3.64	0.0001	0.19	51.62
Square root (per 1 unit drop)	AIDS	1.78	1.56 - 2.03	0.0001	0.33	71.81
	Death	1.74	1.51 - 2.01	0.0001	0.02	55.96
	HIV-related event	1.42	1.26 - 1.61	0.0001	0.07	33.25

The value of the CD4 count as a short-term prognostic marker is clear with an 83% increase in the hazard of developing AIDS per 100 cell/mm^3 drop in the CD4 count (p=0.0001). The CD4 count similarly predicts death (p=0.0001) and the development of the first HIV-related event (p=0.0001) in the short-term.

Two assumptions have been made when fitting the CD4 count in the proportional hazards model as a time-updated covariate. Firstly, the relative hazard associated with a given drop in the CD4 count is assumed to remain constant over time ('proportional hazards'). Secondly, the CD4 count is assumed to be related to the hazard of AIDS in a log-linear way. For example, the relative hazard associated with a drop from 600 to 500 cells/mm^3 is the same as that for a drop from 100 to 0 cells/mm^3. As an individual is increasingly likely to experience disease progression once the CD4 count has fallen to very low levels, this assumption may not be appropriate. Transformation of the CD4 counts before entering into the model may address these issues.

In order to test the assumption of 'proportional hazards', an interaction term between the CD4 count and the logarithm of time was added to the model. If this is significant then it would suggest that the effect of a drop in the CD4 count is changing over time. With the untransformed CD4 counts there is substantial evidence of non-proportionality (Table 5.2) for progression to all three endpoints. Square root and logarithmic transformations of the CD4 count were taken, and the analysis repeated (Table 5.2). Whilst there was some suggestion of non-proportionality in hazards over time when the square root of the CD4 count was taken the log transformation appears to have been successful in removing all suggestion of non-proportionality. Further, the use of the log transformation appeared to give well fitting models (as shown by the high Wald Chi-squared values) for each endpoint. On the basis of these findings, all further analyses will be performed after taking the log transformation of the CD4 count.

5.4 CD4 decline as a prognostic marker

In order to assess whether the rate of CD4 decline is as or more important in determining patient prognosis, than a patient's most recent CD4 count, both the current and previous CD4 counts were included in the model as time-updated covariates. The addition of the previous CD4 count provided some additional prognostic information for the development of AIDS (Table 5.3). However, it added little prognostic information for progression to the development of the first HIV-related event and none for progression to death.

Table 5.3 : Relative hazards from the Cox proportional hazards model associated with a one unit drop in the log of the patient's previous CD4 count and with a 100 cell/mm^3 per year drop in the rate of CD4 decline for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event

	Progression to :								
	AIDS			Death			First HIV-related event		
	Relative hazard	95% confidence interval	p-value	Relative hazard	95% confidence interval	p-value	Relative hazard	95% confidence interval	p-value
Most recent CD4	1.13	1.02 - 1.25	0.02	1.45	1.27 - 1.66	0.0001	2.00	1.26 - 3.17	0.003
Previous CD4	1.11	1.00 - 1.23	0.05	1.04	0.89 - 1.21	0.66	1.58	0.94 - 2.64	0.08
Rate of CD4 decline	1.27	1.04 - 1.56	0.02	1.15	1.04 - 1.28	0.006	1.09	1.00 - 1.19	0.05
Rate of CD4 decline (all counts)	1.21	1.02 - 1.43	0.03	1.13	1.02 - 1.24	0.02	1.08	0.98 - 1.19	0.14
Most recent CD4	1.41	1.30 - 1.54	0.0001	1.47	1.35 - 1.61	0.0001	2.71	2.05 - 3.57	0.0001
Rate of CD4 decline (< 5 years)	1.22	1.03 - 1.45	0.03	1.13	1.03 - 1.25	0.01	1.08	0.98 - 1.19	0.14
Most recent CD4	1.41	1.29 - 1.54	0.0001	1.47	1.35 - 1.61	0.0001	2.69	2.03 - 3.55	0.0001
Rate of CD4 decline (< 3 years)	1.19	1.04 - 1.35	0.009	1.13	1.03 - 1.24	0.01	1.08	0.99 - 1.18	0.08
Most recent CD4	1.42	1.30 - 1.55	0.0001	1.48	1.35 - 1.61	0.0001	2.68	2.03 - 3.54	0.0001

Secondly, an estimate of the patients' previous rate of CD4 decline, based on all CD4 counts measured up to the current time point, was added to the model as a time-dependent covariate. A new CD4 measurement therefore has the effect of changing both the most recent CD4 count and the rate of decline in the model. Least squares regression estimates for the rate of decline have been used here as given the appropriate formula, a clinician may be able to calculate this if desired. In the absence of the absolute CD4 count, the rate of CD4 decline predicted the development of all three endpoints, although not as well as the CD4 count. These effects remained for progression to AIDS and death even after adjusting for the patients most recent CD4 count (Table 5.4). However, after adjustment for the CD4 count, the rate of CD4 decline did not provide any prognostic information for progression to the first HIV-related event. The relative hazard associated with the most recent CD4 count was only slightly reduced in these analyses. On the assumption that recent CD4 history is more important that longer term history, the analysis was repeated after using only CD4 counts measured over the previous five or three years for the slope calculation. The most recent CD4 count continued to have the most prognostic information. However, as the analysis was restricted to more recent CD4 counts only, the value of the slope became stronger for all three endpoints. The relationship between disease progression and the rate of CD4 decline will be discussed further in Chapter 6.

5.5 The relationship between CD4 count and other potential cofactors

As age at seroconversion and calendar year were shown to be associated with HIV disease progression, it is of interest to see whether their effect was mediated through the CD4 count. After adjusting for the patients' most recent CD4 count, only age at seroconversion remained significantly associated with more rapid progression to AIDS and death (Table 5.4). The calendar year of seroconversion was no longer independently associated with disease progression after adjustment for the CD4 count, suggesting that individuals who seroconverted either very early or very late have an increased risk of disease because their CD4 level falls at a more rapid rate.

The development of certain clinical conditions was also shown to be associated with a more rapid progression to AIDS in Chapter 3. In order to assess whether these conditions were simply a consequence of increased immunosuppression, or whether the development of such conditions was detrimental to the health of the patient, these conditions were also included in a model after adjustment for the CD4 count (Table 5.4).

Table 5.4 : Relative hazards from Cox proportional hazards models associated with the calendar year of seroconversion, age at seroconversion and the occurrence of pre-AIDS HIV-related conditions both unadjusted and after adjustment for the log of the CD4 count for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event.

		Progression to :					
		AIDS		Death		HIV-related event	
		Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Calendar year of seroconversion	1979-1980	1	1	1	1	1	1
		-	-	-	-	-	-
	1981	0.47	0.59	0.58	0.57	0.94	0.81
		(0.18 - 1.23)	(0.22 - 1.58)	(0.23 - 1.45)	(0.22 - 1.47)	(0.49 - 1.80)	(0.40 - 1.63)
	1982	0.53	0.46	0.73	0.61	1.39	1.45
		(0.25 - 1.15)	(0.20 - 1.04)	(0.33 - 1.60)	(0.25 - 1.45)	(0.80 - 2.41)	(0.82 - 2.59)
	1983 - 1985	1.55	1.12	1.92	1.20	2.03	1.27
		(0.72 - 3.33)	(0.48 - 2.61)	(0.84 - 4.38)	(0.44 - 3.24)	(1.10 - 3.78)	(0.63 - 2.57)
Age at seroconversion	(per 5 years)	1.20	1.17	1.35	1.33	1.13	1.03
		(1.10 - 1.32)	(1.06 - 1.30)	(1.23 - 1.48)	(1.19 - 1.49)	(1.06 - 1.22)	(0.95 - 1.12)
Bacterial infections		2.16	2.65	-	-	-	-
		(1.11 - 4.19)	(1.34 - 5.23)	-	-	-	-
Oral candida		4.42	1.15	-	-	-	-
		(2.16 - 9.04)	(0.41 - 3.24)	-	-	-	-
Skin complaints		2.37	1.96	-	-	-	-
		(1.21 - 4.64)	(0.97 - 3.95)	-	-	-	-
First HIV-related condition		4.01	2.75	-	-	-	-
		(2.00 - 8.06)	(1.30 - 5.81)	-	-	-	-

Both the development of skin complaints and oral candida appear to be a consequence of more severe immunosuppression in that they became non-significant after adjustment for the CD4 count. However, the development of bacterial infections heralds a poor prognosis, irrespective of the patient's CD4 count. In many individuals, bacterial infections may be the first indication of their infection. Consequently, the poorer prognosis associated with such conditions is reflected in the significant relative hazard associated with the development of the first HIV-related event. However, even when excluding bacterial infections from this analysis, the development of an HIV-related condition was associated with a relative hazard of 2.50 (p=0.01, data not shown).

5.6 Time between CD4 measurements

Where possible, CD4 counts are measured every 3-6 months. However, patients sometimes miss routine clinic visits and as CD4 counts are also measured when the patient is admitted to hospital there is the possibility that CD4 counts will be measured more frequently as the CD4 count itself declines and the patient becomes increasingly ill. Currently, the CD4 count has been fitted in the proportional hazards model in such a way that it is assumed to remain constant in the intervening period between visits. When CD4 visits are a long time apart, the CD4 count in the model may not truly reflect the CD4 count of the patient at any particular time point.

Table 5.5 shows the frequency with which CD4 counts are measured in the cohort. A total of 2201 CD4 measurements have been measured on average 2.3 months apart since 1982. However, there are considerable differences by calendar year and by the CD4 count of the patient. As expected, the count has been measured increasingly frequently over time and also as the CD4 count falls. At the time of an 'event' in a patient in the cohort (e.g. an AIDS diagnosis) CD4 counts of patients alive at the same time from seroconversion are more likely to be misclassified if this event occurred soon after infection, when counts were higher and measured less frequently, than more recently. This may have an effect on the estimate of the relative hazard associated with the CD4 count. One method of reducing the effect of misclassification due to differences in the frequency of measurements is to use some form of summary measure of the CD4 counts measured within certain time periods for each patient, rather than use the individual values themselves. All patients are therefore subject to the same level of misclassification.

Table 5.5 : Time intervals between consecutive CD4 measurements in the cohort

		Number of tests performed	Median time interval between visits	Range
Total		2201	0.19 yrs	0.00 - 6.93 yrs
Calendar year	up to 1985	165	0.60 yrs	0.02 - 4.50 yrs
	1985 - 1987	430	0.48 yrs	0.01 - 6.93 yrs
	1988 - 1990	898	0.15 yrs	0.00 - 2.00 yrs
	1991 - 1993	708	0.15 yrs	0.00 - 1.56 yrs
CD4 count*	> 500	156	0.58 yrs	0.02 - 4.13 yrs
	200-500	299	0.31 yrs	0.00 - 2.59 yrs
	<200	1746	0.16 yrs	0.00 - 6.93 yrs

* Classified according to the first of each pair of consecutive CD4 counts

Alternatively, some rule can be invoked whereby CD4 counts are only assumed to remain constant for a certain fixed period of time. After that time period, the individual is temporarily removed from the risk set until a new measurement becomes available.

Table 5.6 shows the effect of incorporating the CD4 count into the Cox model in a number of different ways. The first section of the table shows the results using the routine CD4 measurements. Analysis 1 shows the results when all available counts are included (as in Table 5.2) for comparison. Analysis 2 shows the results where patients are excluded from the risk set six months after a CD4 measurement if they have not had another measurement within this time period. In this case patients re-enter the risk set once their next measurement becomes available. Excluding patients in this way has little effect on the estimates of the relative hazard for progression to any endpoint.

The second section of the table shows the results obtained when six-monthly mean values are used in place of the routine measurements. If a patient has no CD4 counts measured within a particular six-month period, their value is initially assumed to remain unchanged from that in the previous period (analysis 3). The use of six-monthly means in this way leads to an increase in the relative hazards for all three endpoints from that obtained using routine measurements.

Table 5.6 : The effect of different inclusion methods for the CD4 count in the Cox proportional hazards model for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event. CD4 counts are included in the model after a log transformation.

Analysis			Progression to : AIDS	Death	HIV-related event
1	Routine CD4 counts	All patients included	1.43 1.31 - 1.56	1.48 1.36 - 1.62	2.76 2.09 - 3.64
2		Exclude patients from risk set after 6 months if no CD4 count available	1.39 1.26 - 1.52	1.46 1.32 - 1.61	2.66 1.95 - 3.64
3	Six monthly means	Keep CD4 mean at previous value if missing	1.69 1.48 - 1.94	1.61 1.41 - 1.83	2.94 2.27 - 3.80
4		Exclude patient from risk set if CD4 mean is missing	1.73 1.45 - 2.05	1.55 1.33 - 1.80	2.90 2.19 - 3.85
5		Estimate missing values by linear interpolation	1.69 1.48 - 1.94	1.61 1.41 - 1.83	2.97 2.29 - 3.85
6		Estimate missing values by linear interpolation and exclude patients at the end if last values are missing	1.78 1.49 - 2.12	2.04 1.49 - 2.77	3.01 2.30 - 3.93
7	First value in six month period	Include all patients	1.36 1.25 - 1.49	1.31 1.18 - 1.44	1.27 1.13 - 1.43
8		Exclude patient from risk set if first value is missing	1.41 1.25 - 1.60	1.46 1.24 - 1.72	1.28 1.10 - 1.50
9		Estimate missing values by linear interpolation	1.36 1.25 - 1.49	1.30 1.18 - 1.44	1.27 1.13 - 1.43
10		Estimate missing values by linear interpolation and exclude patients at the end if last values are missing	1.42 1.26 - 1.61	2.20 1.50 - 3.23	1.28 1.10 - 1.49

Either excluding the patient from the risk set for any six month period in which he has no values (analysis 4) or estimating these missing values by linear interpolation between the six-monthly values which are available before and after the period of interest and assuming that after the last CD4 measurement the CD4 average remains constant (analysis 5) both have little further effect on the relative hazard estimate. The only large effect on the relative hazard is seen for progression to death when values are interpolated and patients are permanently excluded from the risk set in the six monthly periods after their last measured CD4 count (analysis 6). This exclusion of patients has, however, only a small effect on the relative hazards for progression to either AIDS or the first HIV-related event, suggesting that there may be a number of individuals who do not have CD4 counts measured during very late stage HIV disease and that for these individuals the last measured CD4 count does not accurately reflect their CD4 count at the time of death.

In the final section of Table 5.6 results are shown where the first CD4 count within each six-month period is used instead of six-monthly means (analysis 7). In patients with only one measurement every six months, these two values will be the same. For progression to all three endpoints, the relative hazard estimate is substantially reduced from that obtained when using routine values. The temporary exclusion from the risk set of patients with missing values (analysis 8) or the estimation of these missing values by linear interpolation (analysis 9) has little further effect on the relative hazard estimate. Again, for progression to death, the exclusion of patients in the six monthly periods after their last measurement (analysis 10) leads to a much increased relative hazard estimate.

5.7 Variability in the CD4 measurement

Unfortunately, the CD4 count is not a perfect measure of the state of the immune system in HIV infection. Figure 5.3 shows the measured CD4 counts for one patient in the cohort. Whilst not a 'typical' patient, the plot illustrates that CD4 counts may be far more variable soon after infection than in later years. This may be partly due to improvements in laboratory methods over time, but may also reflect increased biological variability in the first few years after infection, and less potential for variability at low CD4 counts when the count cannot fall below zero.

One method to reduce the effect of variability is to smooth the CD4 values before including them in the model. Table 5.7 shows the effect of smoothing the CD4 counts in a number of ways.

Figure 5.3 : Measured CD4 counts in one patient in the cohort plotted against time from seroconversion

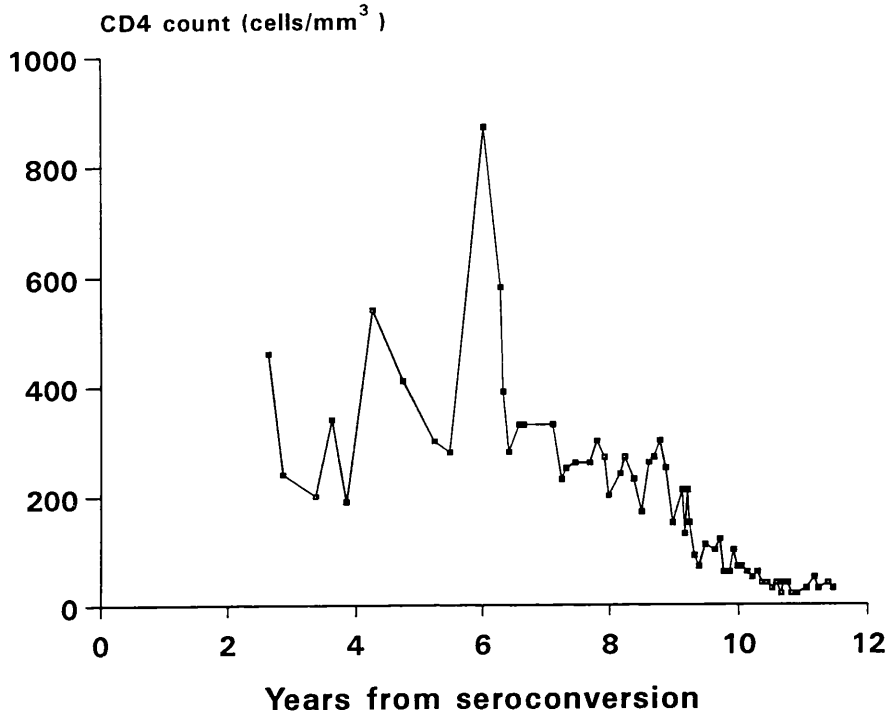


Table 5.7 : The effect of different smoothing methods on the relative hazard estimate for the CD4 count in the Cox proportional hazards model. CD4 counts have been included after a log transformation.

	Progression to :		
	AIDS	Death	HIV-related event
'Raw' CD4 counts	1.43 1.31 - 1.56 0.0001	1.48 1.36 - 1.62 0.0001	2.76 2.09 - 3.64 0.0001
Running mean of last 3 values	1.20 1.12 - 1.28 0.0001	1.14 1.05 - 1.23 0.002	1.37 1.19 - 1.57 0.0001
Running median of last 3 values	1.37 1.26 - 1.50 0.0001	1.43 1.31 - 1.56 0.0001	1.37 1.19 - 1.57 0.0001
Running minimum	1.19 1.12 - 1.27 0.001	1.11 1.04 - 1.18 0.003	1.09 1.03 - 1.14 0.001

In all cases, the use of a smoothed value leads to an decrease in the relative hazard estimates, suggesting that important information may be lost when smoothing. This will be discussed in more detail in Chapter 7.

5.8 Discussion

5.8.1 Summary of main results

Although individual patterns vary widely, on average CD4 counts in patients in the Royal Free Hospital Haemophilia Cohort drop rapidly in the first few years after seroconversion and then gradually 'flatten out' as the count gets lower. The CD4 count appears to be a good marker of HIV disease progression, being highly predictive of short-term development of AIDS, death or the first HIV-related event. Further, it appears that the rate of decline adds prognostic information for disease progression, with those individuals with the fastest rate of decline having a worse prognosis than individuals with the same CD4 count but a slower rate of decline. The development of clinical conditions prior to AIDS, particularly bacterial infections, appears to herald a poor prognosis, irrespective of the patient's CD4 count.

5.8.2 The pattern of CD4 decline over infection

One of the problems with estimating patterns of CD4 change on a group basis is that the selective drop-out of patients with the lowest CD4 counts makes the decline appear to be less rapid than it actually is. Thus any analysis of CD4 decline must be made on an individual patient basis rather than at a group level.

Multi-level modelling methods[44] offer an attractive way to model patterns of CD4 change. Whilst ordinary least squares regression methods can be used to model CD4 decline by estimating a different slope and intercept for each individual, it is not an efficient method and it does not use information about the 'population'. Least squares regression estimates for individuals with few CD4 counts may be influenced by an 'extreme' CD4 count. Multi-level modelling methods are less influenced by these values as the population values are taken into account. Other methods of modelling CD4 counts have been suggested, including time series methods[288] and Markov models[300], although in general all methods produce similar results.

In this cohort, whilst the absolute CD4 count appears to become less variable over time, in relative terms this still represents an increase in variability as the CD4 count drops. As they allow for the explicit modelling of variation, multi-level models do not insist on

prior transformation to stabilise this variance (as in least squares regression). However, this can be done if desired, and the square root transformation (taken to ensure the count remains above zero) removes some, but not all, of the dependence of the variation on time. Other authors have suggested that the cube root[301], fourth root[302] or log[303] transformations are the most effective at stabilising the variation. In attempting to stabilise or model the variation, I have assumed that it is a function of time from seroconversion. It is more likely that the variation may be directly a function of both the calendar year or the underlying average CD4 count, and only indirectly a function of the time from seroconversion in this cohort. I am, therefore, attempting to model the variation on a 'surrogate' scale and it is not surprising, therefore, that the relationship with time is rather complex.

One of the underlying assumptions of both the simple multi-level modelling approach and linear regression methods, is that the within-individual errors at different timepoints are uncorrelated[304]. Where CD4 counts are measured very close together in time, errors from the fitted model may well be correlated and this assumption will be violated. An autoregressive term can be included in the model to allow for this[304]. It is thought that in this cohort sufficient time usually elapses between each measurement for errors to be uncorrelated. I do, however, plan to study this assumption in more detail in the future.

In this cohort there appears to be a quadratic pattern to CD4 decline over time, with CD4 counts 'flattening out' as they get lower. We[277] and others[305] have previously reported that the rate of decline was approximately linear over time. However, more recently a number of authors have also suggested a quadratic pattern with counts flattening out over time[57,300,301,306,307]. This move towards a quadratic decline is not totally unexpected. Improved clinical care now means that patients live for longer with very low CD4 counts. Further, the introduction of very high purity clotting factor concentrates for haemophilia treatment (the use of which has been reported to have the effect of slowing the rate of CD4 decline[308,309]), may also result in a flattening of CD4 counts in haemophilic patients. Although there is wide variation between patients, there is no consistent evidence in this cohort that counts drop more rapidly with disease progression as has been suggested[67,292,310,311].

The results in this chapter are consistent with findings from other published reports. Most other studies have estimated linear rates of decline, which have been reported to be between 6 and 116 cells/mm^3 per year[52,192,260,280,287-289,291] depending on whether the patients have subsequently developed AIDS or not. The high variability in the

patterns of CD4 change between patients has also been shown by Hughes *et al*[306] who found that 10th and 90th percentiles for the rate of change in their study were a 40% decline to a 22% increase in the count each year.

5.8.3 Relationship of co-factors to CD4 decline

The estimated CD4 count at seroconversion is higher in younger individuals than in older patients in this cohort, although CD4 counts subsequently decline at a similar rate in all patients. As CD4 counts drop naturally from birth to around 14 years of age[282], this is not unexpected. No other demographic factor studied in the cohort appears to be related to either the initial estimated CD4 count at seroconversion or to the rate of CD4 decline.

Other studies have found that the CD4 count was not associated with age, sex or race[58,285]. However, information on the CD4 count at seroconversion is often limited and the pattern of CD4 count change following infection[52] means that CD4 counts measured over this period may be high (just before the drop), very low (just after the drop) or moderate (after counts have subsequently recovered). The estimated CD4 count at seroconversion will therefore be an average estimate of counts over this period. Further, as the date of seroconversion is often estimated, CD4 counts thought to be measured after seroconversion may actually be measured before seroconversion. Without very close CD4 measurements throughout seroconversion, it is not possible to improve on these estimates.

The CD4 counts of homosexual men may decline less rapidly[288] or more rapidly[312]than IVDUs. Alcabes *et al* found that the rate of decline was affected by the frequency of drug injecting[313] providing evidence for a faster rate of decline in drug users than in patients from other exposure categories. Munoz *et al*[288] found that non-whites had a faster rate of CD4 decline than other individuals, although after adjustment for other factors this became non-significant. CD4 counts have been suggested to be higher in women than men[284,285], although these differences may be explained by the fact that the women in these studies were recruited earlier in infection than the men.

5.8.4 The prognostic value of the CD4 count

The prognostic value of the CD4 count has been well recognised for some time[51]. In cross-sectional studies CD4 counts were found to be lower in patients with lymphadenopathy[53] and AIDS[60,79,225,314] than in asymptomatic patients. Often CD4 counts are categorised and the relative hazards quoted assess the effect of moving from

one CD4 category to another. Unfortunately, in doing this much information is lost and reported relative hazards are not comparable to those from this study. In studies which report relative hazards for non-categorised CD4 counts, relative hazards for the CD4 count as a fixed covariate at some, often arbitrary, baseline range from 1.35 to 2.5 per 100 cell/mm^3 drop[61,226,251,268,315-317]. The routine measurement of CD4 counts means that it is now possible to assess the role of the CD4 count as a time-updated covariate. Phillips *et al*[318] reported a relative hazard of 2.47 per 100 cell/mm^3 drop in the CD4 count as a time-updated covariate in a cohort of patients of different exposure categories. Among non-haemophilia patients infected with HIV at the Royal Free Hospital, the relative hazard per unit drop in the log of the CD4 count as a time-updated covariate is 1.53 (Ms A Mocroft, personal communication). Raboud *et al* found a relative hazard of 1.67 per 100 cell/mm^3 drop in the Toronto Sexual Contact Study[317] although CD4 counts in this model were lagged by one year. These results are consistent with those from this thesis.

The use of splenectomy as a treatment for thrombocytopenia has been shown to result in an increase in the CD4 count[319,320]. However, it is not yet clear whether the CD4 count in these patients gives an accurate measure of their immune status. In this cohort, five patients have undergone splenectomy, and therefore have high CD4 counts as a result. These patients have not been excluded from the analysis, although it is unlikely that results will be much changed if they were. There remains some debate as to whether splenectomy itself has an effect on the prognosis of the patient[98,123,321,322]. The use of zidovudine has also been shown to lead to a small increase in CD4 levels. Again, treated patients are not excluded, although analyses carried out censoring the data before the introduction of zidovudine in 1987 (data not shown) do not lead to substantially different results. Results from the Concorde trial[146] and trials of very high-purity clotting factor concentrates[323] suggest that therapeutically induced rises in the CD4 count may not necessarily result in a clinical benefit to patients.

5.8.5 Other markers of progression

The finding that the rate of CD4 decline adds additional prognostic information to that provided by the most recent CD4 count alone is of interest. Saah *et al*[221] showed that both the CD4% and the rate of CD4% decline were associated with the development of AIDS. However, Selwyn *et al*[88] suggested that once the absolute CD4 count was controlled for the rate of CD4 decline was no longer associated with progression to AIDS. Boutitie *et al* reported that neither of the two previous CD4 counts added any prognostic value after adjustment for the most recent count[301]. Further, adding the rate

of CD4 decline to a model including only the most recent CD4 count did not improve the fit of the model. However, in this study, only three CD4 counts were available for each patient and these were not truly 'time-updated'. It may be argued that much of the early CD4 information for a patient is redundant and only the slope over recent years is of interest. When the rate of CD4 decline was calculated only over the immediate three years prior to the time point of interest, the value of the slope becomes stronger, arguing that early CD4 history is of less importance than recent history.

The finding that the development of an HIV-related clinical event, especially bacterial infections, indicated a poor prognosis, even after taking account of the underlying level of immunosuppression suggested that these events themselves may be detrimental to the health of the patient. The presence of bacterial infections may reflect a poor immune function as a result of defects in B cells[227] which is not fully controlled for by taking account of the CD4 count.

The studies described predominantly use the development of AIDS as a clinical endpoint. Few studies have considered survival and hardly any have considered the development of HIV-related clinical conditions as an endpoint. Both Whittle[324] and Ehmann[325] showed that the CD4 count was associated with survival. Four year survival rates have been reported to range from 95% in those whose CD4 counts were higher than 500 cells/mm^3 to 32% in those whose CD4 counts were in the range 50-99 cells/mm^3 [325]. In contrast to the findings in this thesis, Smith *et al*[73] found that the CD4 count did not distinguish between those patients who developed symptomatic disease and those who did not.

5.8.6 The use of the proportional hazards model

The fit of the proportional hazards model is rarely questioned in published research. Coates *et al*[61] found that no transformation of the CD4 count resulted in an improvement to the fit of the model over that when untransformed CD4 counts were included. In this study a log transformation of the CD4 count appears to be successful in removing non-proportionality and improves the fit of the model over that of the untransformed values. It seems intuitive that a 50 cell/mm^3 drop to the CD4 count has a greater impact when the CD4 count is already low. Under this model, a decrease from 400 cells/mm^3 to 200 cells/mm^3 has the same impact as a decrease from 100 cells/mm^3 to 50 cells/mm^3.

5.8.7 Problems with the use of the CD4 count

Irregular measurements are a common problem in observational cohort studies and the time intervals between measurements are rarely constant between patients or visits. The scope for analysing such data is more restricted than in the situation where visit dates are more regular (e.g. in clinical trial follow-up). When methods are used which use mean CD4 counts over regular six-monthly intervals, the relative hazard estimate is increased, suggesting that it is important to consider a patients CD4 count history over the past 6 months rather than simply considering only the most recent count when making clinical decisions.

Variability in the CD4 count is a well recognised problem by clinicians who tend to take a confirmatory count before making any clinical decision. Hoover *et al*[326] quantified the amount of variability around the true CD4 count and showed, for example, that a patient with a measured CD4 count of 500 cells/mm^3 may have a true count in the range of 297 to 841 cells/mm^3. Hughes[306] suggested that there was an average coefficient of variation of 25% for the absolute CD4 count. Whilst the variation decreased as the underlying CD4 count dropped, the within-subject variation was found to be higher in relative terms in those with lower CD4 counts.

The effect of measurement imprecision on estimates of fixed covariates in the Cox proportional hazards model has been studied by Nakamura[327] and Hughes[328], amongst others. Raboud[317] has also considered the effect of variability on the relative hazard estimates for the CD4 count from the Toronto Sexual Contact Study, showing that the relative hazard estimate is underestimated. However, her results suggested that smoothing the CD4 counts led to an increase in the relative hazard estimate, a finding which is not confirmed in this cohort of patients. This may be explained by two differences in the study design. In Raboud's study the coefficients were lagged by one year. Hence, CD4 counts measured very close to the time of AIDS are excluded from this analysis. The pattern of variability in these counts may be very different from those a year earlier. Further, in contrast with the Toronto study in which CD4 counts were measured every 3 months, counts may be measured a long time apart in some patients in the Royal Free Hospital Cohort. Smoothing these values may therefore involve the inclusion of CD4 counts which are out-of-date. In this analysis a six-monthly mean, which effectively smooths only up-to-date values, did result in larger relative hazards. This issue of variability and its effect on the relative hazard estimate will be discussed in more detail in Chapter 7.

CHAPTER 6 : THE PROGNOSTIC ROLE OF OTHER LABORATORY MARKERS

6.0 Summary of contents

In this chapter I shall consider the prognostic role of five other laboratory markers in HIV infection. These are the CD8 lymphocyte count, the beta-2 microglobulin (B2M) level, the Immunoglobulin A (IgA) level, the development of p24 antigenaemia and the patients p24 antibody status soon after seroconversion. I will describe the measurement of these five markers in the cohort and assess their role as prognostic markers, both before and after adjustment for the CD4 count. Finally I will assess which of the co-factors described in Chapter 4, and laboratory markers independently predict the development of HIV disease when all are included in a multivariate model.

6.1 Literature review

6.1.1 The CD8 lymphocyte count

The CD8 molecule is expressed on the surface of a subset of T lymphocytes with suppresser/cytotoxic activity and on some natural killer cells[329]. After an initial quick rise immediately after seroconversion[52,281], the CD8 lymphocyte count continues to rise more slowly during HIV infection[72] possibly dropping during late-stage disease[292,330]. Either the CD8 count or the ratio of CD4 to CD8 counts[83] can be used as alternative markers of progression to the CD4 count[84] although in general they do not appear to provide as much information as the CD4 count[268].

6.1.2 The immunoglobulins

Raised immunoglobulin levels in HIV infection reflect B-cell activation[331]. The most commonly studied immunoglobulin is IgA[51,232,331-334] although some authors have also studied levels of IgG, IgM and IgD[84,333]. In the absence of HIV infection, haemophilic patients have been shown to have raised levels of immunoglobulins[74] and racial differences are thought to exist with blacks having higher IgG and IgA levels than whites[335]. The finding that IgM levels in pre-exposure samples were higher in men who subsequently became infected with HIV and who progressed rapidly to clinical disease than in both men who remained uninfected and in men who became infected but did not progress rapidly[336] suggests that rapid progressors may have a tendency to mount a prolonged IgM response to many antigens.

Levels of immunoglobulins are generally higher in HIV positive individuals than in healthy control subjects[60,258]. During HIV infection IgA and IgG increase with disease progression, although this may be a response to the development of symptoms[72]. There does not appear to be a similar increase in the levels of IgM throughout infection. Symptomatic patients have higher levels of IgA than asymptomatic HIV positive individuals[60] and thus IgA levels may be of some prognostic value in HIV infection[61,73,84,222,331].

6.1.3 Other non-specific markers of immune activation

Other commonly studied non-specific markers of immune activation include B2M, neopterin, soluble CD8, interleukin-2 levels (IL-2) and soluble IL-2 receptor levels.

B2M is part of the human leukocyte antigen (HLA) class I molecule which is found on the surface of most nucleated cells, including B and T lymphocytes. It is shed during cell turnover[337,338] and, as levels of B2M are usually fairly stable, increases in B2M are thought to represent immune activation[339]. Raised levels are seen in autoimmune diseases, lymphoproliferative disorders, in renal transplant patients and during other viral infection[339-341]. Because of repeated exposure to antigenic stimulatory materials and other infectious agents[342], levels tend to be raised in IVDUs and haemophilic patients, irrespective of their HIV status. Levels have also been shown to be higher in children in Africa[343], possibly due to the high background levels of bacterial and viral infections endemic to the country. No differences have been found according to either sex or race.

Around the time of infection with HIV, B2M levels rise for a short time[344], before dropping almost to 'normal' levels[287]. Levels then rise during infection[287,311,344] and are a good indicator of an individual's risk of disease progression[51,73,191,334,344]. As they can be measured on stored serum and are less affected by measurement variability[345] their use has been favoured in developing countries where there is a lack of financial support for HIV care.

Neopterin is produced by macrophages when stimulated by T-cell generated gamma interferon[287,346]. Increased levels of neopterin therefore, also reflect immune activation[347]. Elevated neopterin levels are often seen in conditions involving activation of cellular immunity and in patients undergoing immunomodulatory treatments[341]. Levels are increased in uninfected haemophilic patients[348,349], homosexual men[348], attenders at genito-urinary clinics[346] and IVDUs[348]. During HIV infection levels of neopterin tend to mirror those of B2M[105,347,350] and the correlation between the two markers ranges from 0.33 to 0.74[52,287,351-355]. As a result, whilst neopterin may be as

good a marker of progression as B2M[356], little additional benefit is obtained by measuring both markers[331,357].

In vitro studies have suggested that soluble CD8 (sCD8) is released during activation and turnover of cytotoxic T cells[329] and its measurement may therefore serve as an index of CD8 cell activity[292]. The few studies carried out on sCD8 suggest that levels are raised during HIV infection[292,329,358] and that levels are correlated with other immune activation markers, including B2M and neopterin. The sCD8 level has also been shown to be related to progression of HIV disease[292,359-361].

Interleukin-2 (IL-2) is essential for T-cell proliferation[359] and is released during T-cell activation. The presence of soluble IL-2 receptor (sIL-2R) suggests an impairment in T cell function[331] and therefore raised sIL-2R levels would be expected in the presence of T cell activation[359]. sIL-2R appears to have prognostic value for progression of HIV disease[331,336,359,360,362], although significant correlations have been found between sIL-2R and the other immune activation markers[352,359].

6.1.4 HIV viral markers

HIV contains a core protein antigen, p24, which appears in culture supernatants of cells in which HIV is replicating. Hence the presence of p24 antigen in serum appears to be correlated with active HIV replication *in vivo*[363]. p24 core antigen is usually detectable at the time of infection, but then becomes undetectable[287,364], usually reappearing late in disease, although many individuals remain free of p24 antigen even during late stage disease[105]. Both progression to AIDS and the rate of CD4 decline appear to be more rapid in individuals who are p24 antigenaemic[51,62,68,332,359,364-366].

Antibodies to p24 antigen appear soon after infection in almost all patients. Their loss may be one of the earliest markers of HIV progression[65], usually heralding a clinical deterioration[62]. Progression to AIDS appears to be slowest in those with the highest titer of antibodies at infection[330]. Geographic differences exist in the levels of antibodies to p24 and of p24 antigen. In particular, patients from Africa have high levels of antibodies at all stages of HIV infection and rarely become p24 antigenaemic during infection[367].

Early in infection, the majority of individuals are infected with usually slow replicating non-syncytium-inducing (NSI) HIV variants[368]. At some stage in infection there may be a switch to faster replicating syncytium-inducing (SI) variants. This switch appears to be associated with a more rapid loss of CD4 cells and more rapid progression of HIV disease[266,369-371]. The total amount of virus present in the body, the type of viral strains

present and the number of different viral strains present all appear to be related to disease progression[105,372-374].

6.1.5 Haematological markers

In general, haematological markers tend to reflect the consequences of immune activation. Platelets are important for blood clotting and levels have been shown to be lower in HIV-infected individuals than in healthy controls[60,330,375]. The development of thrombocytopenia (a low platelet count) is of particular concern especially for haemophilic patients. In most cases bleeding episodes are not life threatening and treatment, if required, is usually effective[123,376,377]. Some studies have suggested that AIDS patients have lower platelet counts than asymptomatic AIDS-free individuals[225,260], although its value as a prognostic marker[268] has not been confirmed by other studies[84,134,180,232].

The development of anaemia has been associated with faster progression to AIDS[51]. Haemoglobin levels are lower in HIV positive individuals both with and without AIDS[60,79], and the haemoglobin level may be a prognostic marker for HIV disease progression[221,226,232,268,378] although this has not been confirmed by others[238]. Patients with SI viral strains were more likely to have low haemoglobin levels in one study[369,371], suggesting that low haemoglobin levels may be a consequence of disease progression. Further, as anaemia is one of the main side-effects of zidovudine[143,379,380], low haemoglobin levels may simply identify those with the lowest CD4 counts who are receiving zidovudine.

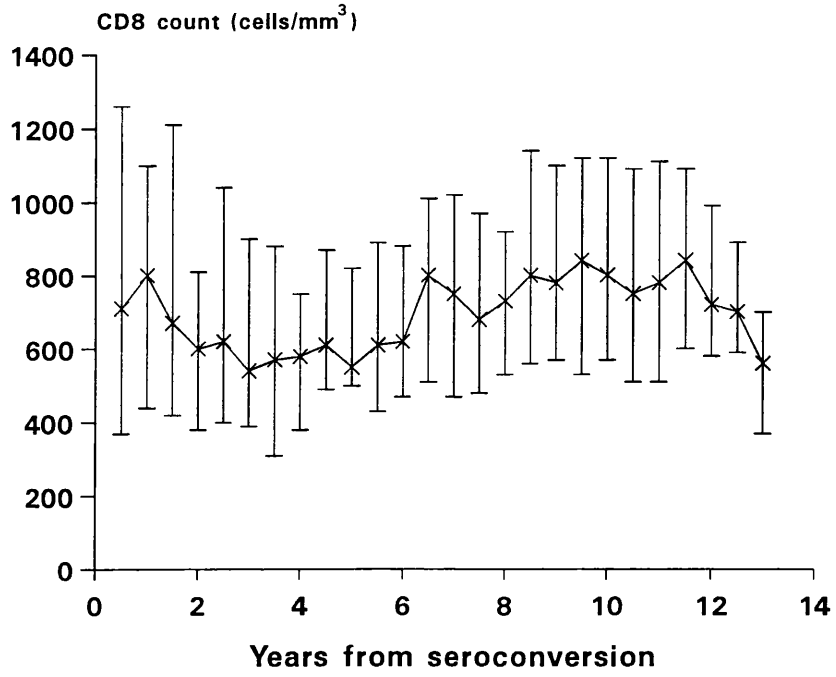
Other haematologic markers studied include the white blood count[60,192,238,358,381], the erythrocyte sedimentation rate[205,226,334] and hematocrit[205], all of which have been shown to have some prognostic power in some studies.

6.2 The CD8 lymphocyte count

6.2.1 The CD8 count throughout infection

CD8 lymphocyte counts have been measured since 1982 every 3-6 months. By the end of 1993 a median of 20 CD8 counts had been measured on each individual (range 1 to 64 counts). For each individual, the mean CD8 count measured in each six month period after seroconversion was calculated. Figure 6.1 shows the distribution of these means over time. At a population level, there is no evidence of any large change in the CD8 level over time of the patients over the cohort.

Figure 6.1 : The medians and interquartile ranges of the means of all CD8 measurements taken within each six-monthly period after seroconversion



Multi-level modelling methods were used to assess the pattern of CD8 change over infection. Initially the untransformed CD8 count was modelled in order to assess whether a linear rate of change over time was either necessary or adequate. After fitting a model with just an intercept term which was allowed to vary between individuals (i.e. constant CD8 over time) the addition of a 'random' linear coefficient resulted in a significant improvement in fit ($P<0.00001$), although overall there was no consistent evidence for either an increase of decrease in CD8 counts over time. The addition of a 'random' quadratic term to the model also resulted in a significant improvement in fit ($p<0.00001$) although again the estimate of the mean parameter was not significantly different from zero ($p=0.11$) suggesting that whilst CD8 counts may change in a quadratic manner in some patients, there is wide inter-patient variability to the individual patterns over time. The fitted coefficients from this model are shown in Table 6.1. The average pattern of decline as described by this fitted model is shown in Figure 6.2(i) (bold line). Under this model, CD8 counts tend to increase slightly after seroconversion before dropping off after around ten years or so.

Table 6.1 : Final model coefficients from multi-level modelling of the CD8 count

	Fixed parameters		Random variances and covariances		
	Mean	95% confidence interval	Intercept	Time	$Time^2$
Untransformed CD8 counts					
Intercept	592.8	450.7 to 734.9	310,900	-	-
Time	38.0	-5.0 to 81.0	-81,270	26,650	-
$Time^2$	-2.1	-5.4 to 1.3	4704	-1788	157
Square root CD8					
Intercept	21.6	19.2 to 24.0	73.7	-	-
Time	1.5	0.8 to 2.2	-17.7	5.9	-
$Time^2$	-0.2	-0.2 to -0.06	0.8	-0.4	0.04

In order to restrict the modelled CD8 counts to being positive, the analysis was repeated after square root transformation of the CD8 count. At seroconversion the $\sqrt{}$CD8 count was estimated to be 21.6, corresponding to a CD8 count at seroconversion of 467 cells/mm^3 (rather lower than that estimated using untransformed values). Again, whilst the mean values of the linear and quadratic coefficients were not significantly different from zero, the inclusion of these terms into the model as random coefficients significantly improved the fit of the model, suggesting that individual patterns varied considerably. Final model coefficients are shown in Table 6.1 and the average slope generated by these coefficients is plotted in Figure 6.2(i) (dotted line). Average values are estimated to be much lower at all stages of infection when the transformed CD8 counts are modelled than when untransformed counts are modelled. A much larger drop in CD8 counts, starting around 4.5 years after seroconversion, is also seen when modelling the transformed CD8 counts.

There is a large variation between patients. For example, as approximately 95% of the population will have estimates within $\pm$ 2 standard deviations of the mean, CD8 counts at seroconversion are expected to lie within 20 and 1503 cells/mm^3 in most individuals. Fitted slopes for individual patients are shown in Figure 6.2(ii). The standard deviations of the parameter estimates (8.6, 2.4 and 0.2 for the intercept, linear and quadratic terms respectively) suggest that again there is wide variation between individual patients.

Figure 6.2(i) : 'Average' patterns of CD8 change over infection generated from the models estimated using multi-level modelling methods. (i) Untransformed CD8 count - continuous line, (ii) √CD8 count - dotted line.

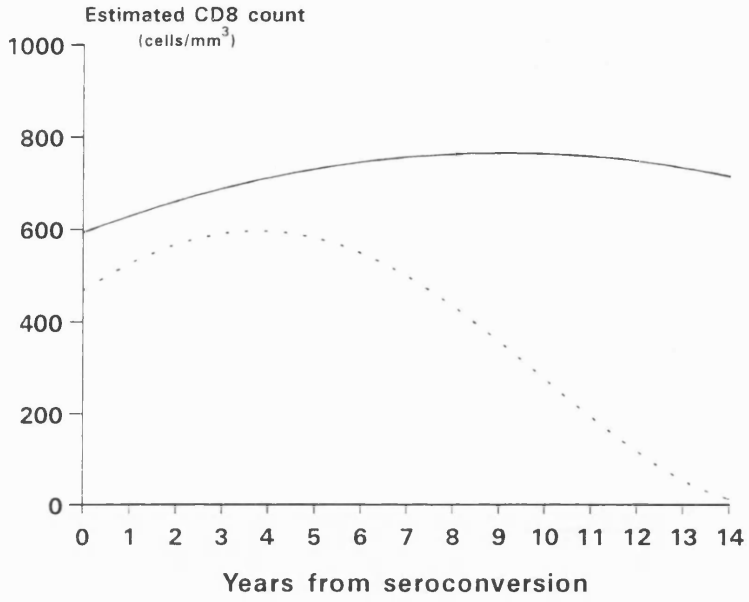
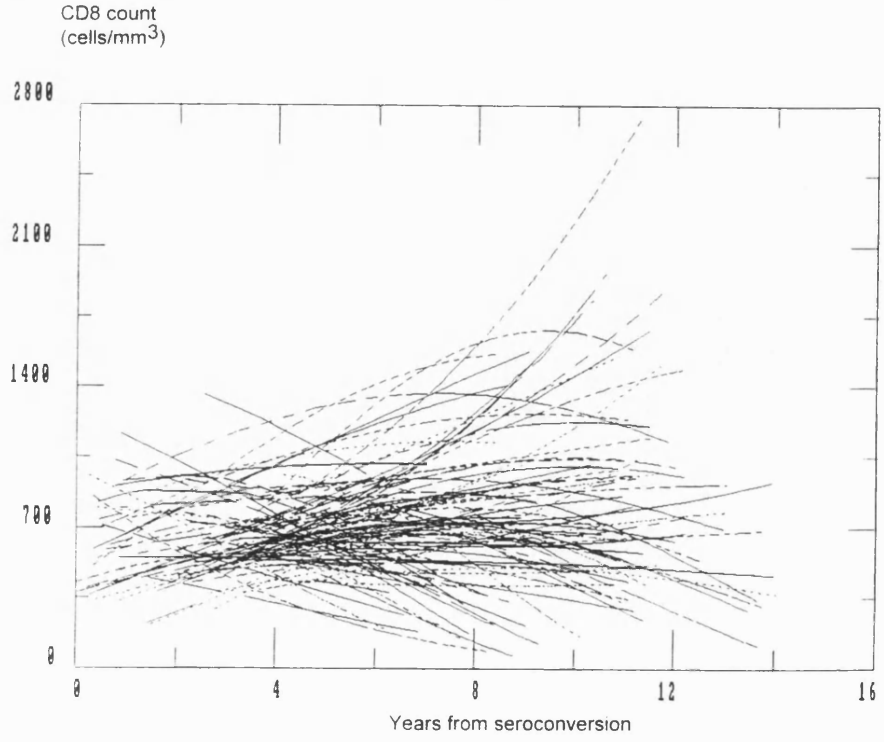


Figure 6.2(ii) : Estimated CD8 patterns for each patient in the cohort after modelling the square root of the CD8 count using multi-level modelling methods



The five demographic/clinical cofactors considered in Chapters 3 and 4 (age group, CMV serostatus, haemophilia diagnosis, social class and year of diagnosis) were studied to see whether there were any relationships between these factors and either the CD8 count at seroconversion or the subsequent change in CD8 count over time. All were included in the model as fixed covariates. There was some suggestion that those with severe haemophilia A had higher counts at seroconversion than those with other diagnoses (472 and 365 cells/mm^3 respectively), although this was marginally non-significant (p=0.08). The was also some suggestion that individuals of manual social class had a larger linear coefficient than those of non-manual social class (p=0.08).

6.2.2 Relationship with the CD4 count

For each patient, CD8 counts were split into those measured whilst the CD4 count was in the ranges 600-700, 500-600, 400-500, 300-400, 200-300, 100-200 and less then 100 cells/mm^3. The mean CD8 count measured in each of these CD4 ranges was then calculated (Figure 6.3). There is little evidence of any large change in the CD8 count as the CD4 count declines, although there is some suggestion that the CD8 count drops when the CD4 count falls below 100 cells/mm^3. There is wide variation in the CD8 counts measured in each CD4 level.

For each patient, the CD4 and CD8 counts were estimated yearly after seroconversion using linear interpolation between the counts immediately before and after the start of each year. At each time point, the correlation between the CD4 and CD8 counts was calculated (Figure 6.4). There is a high and fairly stable correlation between the CD4 and CD8 count over the first 13 years of infection.

6.2.3 The prognostic value of the CD8 count unadjusted for the CD4 count

Unadjusted for the CD4 count, the most recent CD8 count appears to have some prognostic value (as a time-updated covariate) for predicting both AIDS and death (Table 6.2), although the prognostic value for the development of AIDS is only of borderline significance (p=0.06). A 100 cell drop in the CD8 count is associated with a 9% increase in the risk of AIDS and a 29% increase in the risk of death. However, there appears to be no association between the CD8 count and the development of the first HIV-related event (p=0.71). The CD8 count appears to have some prognostic value for progression to a CD4 count of 50 cells/mm^3 (p=0.05). However, there appears to be no association with progression to a CD4 count of 200 cells/mm^3.

Figure 6.3 : The medians and interquartile ranges of the means of all CD8 counts taken while the CD4 count is in different ranges

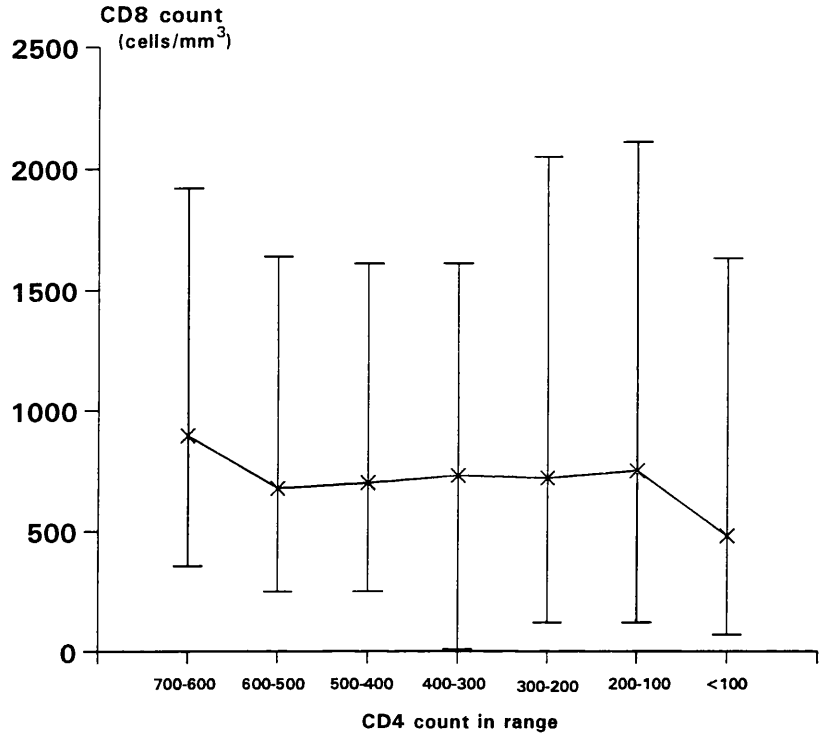


Figure 6.4 : Spearman rank correlation coefficients and 95% confidence intervals between CD4 and CD8 counts estimated at yearly intervals after seroconversion

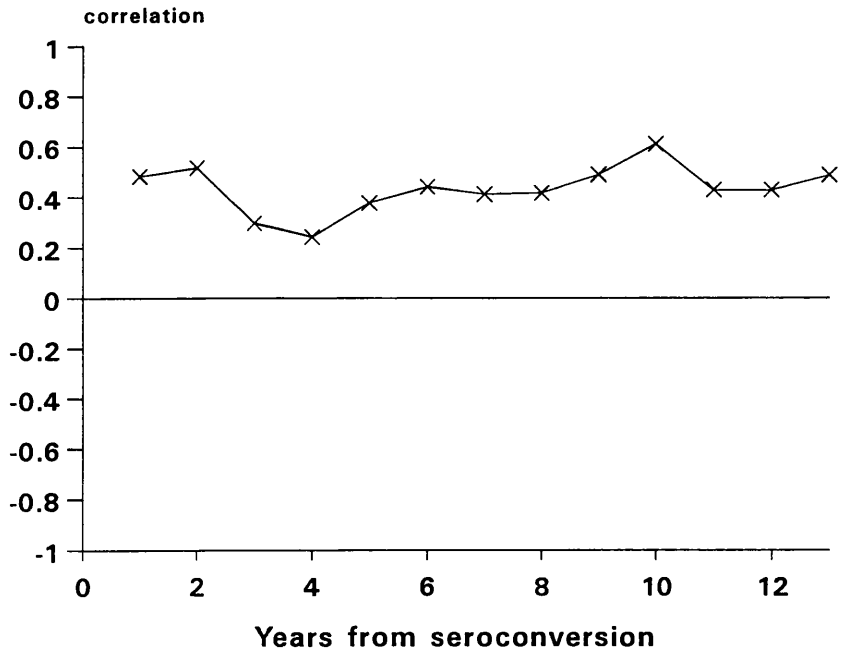


Table 6.2 : Relative hazards and 95% confidence intervals from the Cox proportional hazards model associated with a 100 cell/mm^3 drop in the CD8 count for progression to (i) AIDS, (ii) death, (iii) the first HIV-related event, (iv) a CD4 count of 200 cells/mm^3 and (v) a CD4 count of 50 cells/mm^3. Both the CD8 and CD4 counts have been included in the model as time-updated covariates and adjustments for the CD4 count have been made using log-transformed CD4 counts

		Progression to :				
		AIDS	Death	HIV-related event	CD4 200 cells/mm^3	CD4 50 cells/mm^3
Unadjusted	Relative hazard	1.09	1.29	1.01	0.99	1.13
	95% CI	1.00 - 1.19	1.16 - 1.44	0.96 - 1.07	0.90 - 1.09	1.00 - 1.28
	p-value	0.06	0.0001	0.71	0.86	0.05
	Test for non-proportionality (p-value)	0.12	0.001	0.44	0.61	0.07
Adjusted for CD4	Relative hazard	0.97	1.08	0.97	-	-
	95% CI	0.90 - 1.05	0.97 - 1.19	0.92 - 1.03	-	-
	p-value	0.46	0.15	0.34	-	-
	Test for non-proportionality (p-value)	0.71	0.09	0.66	-	-

6.2.4 The prognostic value of the CD8 count adjusted for the CD4 count

Because of their correlation, the finding that a drop in the CD8 count is associated with a raised risk of disease progression may simply reflect the prognosis associated with a drop in the CD4 count. In order to assess the independent effects of the CD4 and CD8 counts on HIV disease progression, both markers were incorporated into the Cox proportional hazards model. After adjusting for the CD4 count, the CD8 count appeared to add little prognostic information for progression to any of the three clinical endpoints (Table 6.2), confirming that a low CD8 count simply identifies those with the lowest CD4 count.

It has been suggested that the CD8 count increases through infection, but very late on in infection it drops again. The multi-level modelling results and the finding that the patient's most recent CD8 count is associated with a CD4 decline to 50 cells/mm^3 but not to 200 cells/mm^3 appear to be consistent with this hypothesis. If this is the case, then the effect of the CD8 count on disease progression may change over time in a closed cohort such as this. Whilst there is no evidence of non-proportionality in the unadjusted relative hazards for progression to either AIDS (p=0.12) or the first HIV-related event (p=0.44), there was strong evidence of non-proportionality for progression to death (p=0.001), suggesting a change in the association between the CD8 count and the risk of death at different times after infection. After adjustment for the CD4 count, however, this effect becomes non-significant (p=0.09).

6.2.5 Summary of CD8 results

Whilst there does not appear to be any strong consistent change in the CD8 count on a population level, at an individual level it appears that the CD8 count may drop during late stage disease. Unadjusted for the CD4 count, the CD8 count appears to predict death and, to a lesser extent, AIDS. However, much of this effect appears to be due to lower and more rapidly declining CD4 levels in these patients.

6.3 Beta-2 microglobulin

6.3.1 B2M measurements in the cohort

The routine measurement of B2M began in 1990. However, as many patients had died before 1990, routine measurements are not available for all patients. Hence, in 1992 it was decided to retrospectively measure B2M levels in stored serum samples from all members of the cohort. A maximum of 4 samples per patient were chosen : the first

sample after seroconversion, the nearest sample within 6 months of the date of the patients' CD4 count falling to 500 cells/mm^3, the nearest sample within 6 months of the date of the patients' CD4 count falling to 200 cells/mm^3, and in those who had developed AIDS, the last sample prior to AIDS diagnosis. It is these measurements which will be considered in this section.

6.3.2 B2M measurements in stored serum samples

Table 6.3 contains a summary of the B2M levels measured. In 1992 when it was decided to measure B2M levels in the cohort, CD4 counts had at some time fallen below 500 cells/mm^3 in 47 of the 111 patients and below 200 cells/mm^3 in 45 of the 111 patients. In the 33 patients who were known to have developed AIDS by this time, B2M was measured in 26 with available serum samples. There was significant correlation between the patients' ages and the B2M levels at three of the four time points.

6.3.3 Progression from a CD4 count of 500 cells/mm^3

Patients were stratified above and below the upper 33rd percentile of the B2M level at a CD4 count of 500 cells/mm^3 (3.0 mg/l) so as to ensure an approximately equal number of events in each group. Individuals with the highest B2M levels have a much faster rate of progression to all three clinical endpoints than do those with lower B2M levels (Figure 6.5, p=0.006, 0.0003 and 0.02 respectively for the endpoints of AIDS, death and the first HIV-related event, log-rank test).

The relative hazards of progressing to each clinical endpoint associated with a one unit increase in B2M are shown in the top half of Table 6.4. A high B2M level is significantly associated with more rapid progression to all three clinical endpoints. There is no evidence of any non-proportionality in the relative hazard for progression to AIDS or the first HIV-related event, although there is some suggestion that the relative hazard for progression to death changes over time (p=0.08). After adjusting for age there remains a significantly raised risk associated with an increase in B2M levels for progression to both AIDS and the first HIV-related event. The relative hazard associated with the B2M level for progression to death became non-significant.

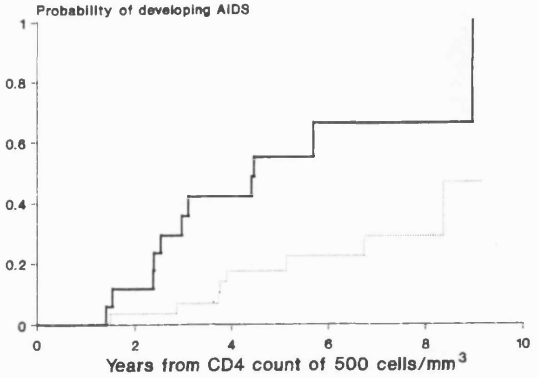
Increased B2M levels were associated with an increased risk of progression to both CD4 endpoints (Table 6.4), suggesting that any effect of B2M on clinical disease progression may be mediated partly through CD4 cell loss.

Table 6.3 : B2M levels and patient ages at four different time points in cohort

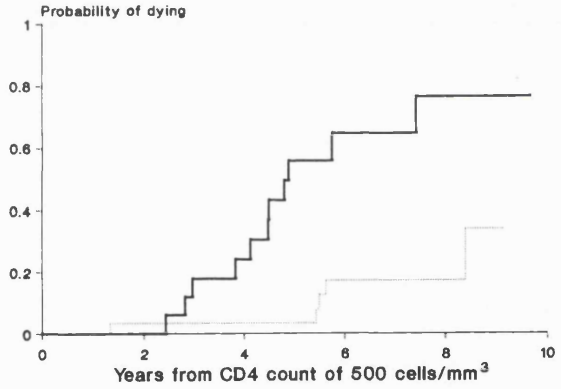
		Time point			
		After seroconversion	CD4 500 cells/mm^3	CD4 200 cells/mm^3	AIDS
Number of samples		110	47	45	26
Time of sample after seroconversion	Median	0.64 yrs	4.63 yrs	5.46 yrs	6.45 yrs
	Range	0.01 - 7.20 yrs	0.03 - 8.29 yrs	1.44 - 5.61 yrs	1.4 - 10.07 yrs
B2M	Median	2.3	2.7	3.4	4.1
	Range	1.4 - 6.5	1.5 - 6.5	1.8 - 6.5	2.4 - 9.6
Age	Median	24.3 yrs	28.0 yrs	31.4 yrs	32.1 yrs
	Range	2.6 - 77.8 yrs	6.4 - 66.4 yrs	16.8 - 74.4 yrs	19.4 - 81.2 yrs
Correlation coefficient (age and B2M)		0.37	0.35	0.47	0.20
	p-value	0.0001	0.02	0.001	0.34

Figure 6.5 : Kaplan-Meier plots showing the cumulative rate of progression to (i) AIDS, (ii) death and (iii) the first HIV-related event according to the number of years from the CD4 count reaching 500 cells/mm^3. Patients are stratified into two groups on the basis of their B2M level : B2M $\geq$ 3.0 mg/l - bold line, B2M < 3.0 mg/l - dotted line

(i) AIDS



(ii) Death



(iii) First HIV-related event

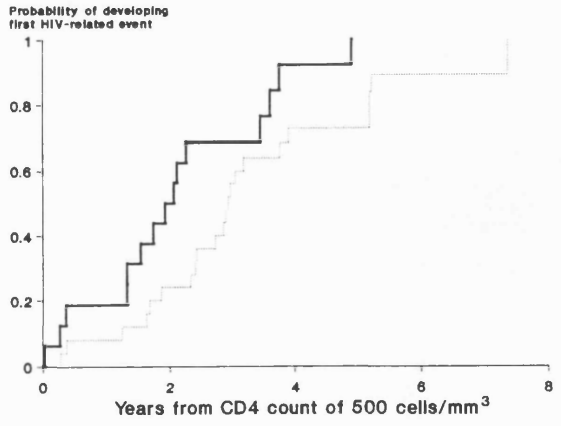


Table 6.4 : Relative hazards and 95% confidence intervals from Cox proportional hazards model associated with a one unit increase in B2M levels for progression to (i) AIDS, (ii) death (iii) the first HIV-related event, (iv) a CD4 count of 200 cells/mm^3 and (v) a CD4 count of 50 cells/mm^3. The CD4 count is included in the model as a time-updated covariate and is included after taking a log transformation

Progression from :			Progression to : AIDS	Death	HIV-related event	CD4 200 cells/mm^3	CD4 50 cells/mm^3
CD4 500 cells/mm^3	Unadjusted	Relative hazard	1.69	2.08	1.47	1.47	2.12
		95% CI	1.20 - 2.37	1.44 - 3.00	1.10 - 1.95	1.05 - 2.04	1.39 - 3.24
		p-value	0.003	0.0001	0.009	0.02	0.0005
		p-value for non-proportionality	0.26	0.08	0.42	0.35	0.95
	Adjusted for age	Relative hazard	2.37	1.50	1.69	1.39	2.14
		95% CI	1.46 - 3.84	0.92 - 2.45	1.11 - 2.58	0.91 - 2.13	1.36 - 3.37
		p-value	0.0005	0.10	0.02	0.13	0.001
	Adjusted for age and CD4 count	Relative hazard	2.15	1.09	1.51	-	-
		95% CI	1.28 - 3.60	0.64 - 1.84	0.97 - 2.34	-	-
		p-value	0.004	0.76	0.07	-	-
CD4 200 cells/mm^3	Unadjusted	Relative hazard	1.37	1.66	1.16	-	1.42
		95% CI	1.03 - 1.84	1.23 - 2.23	0.85 - 1.56	-	1.06 - 1.90
		p-value	0.03	0.0009	0.35	-	0.02
		p-value for non-proportionality	0.94	0.32	0.72	-	0.11
	Adjusted for age	Relative hazard	1.54	1.40	1.27	-	1.74
		95% CI	1.03 - 2.83	0.94 - 2.09	0.86 - 1.88	-	1.18 - 2.58
		p-value	0.03	0.10	0.23	-	0.006
	Adjusted for age and CD4 count	Relative hazard	1.42	1.05	1.05	-	-
		95% CI	0.90 - 2.21	0.66 - 1.65	0.69 - 1.62	-	-
		p-value	0.13	0.84	0.82	-	-

After further adjusting the relative hazard to take account of the most recent CD4 count, there remains a significant association between increased B2M levels and the development of AIDS. The relative hazard for progression to the first HIV-related event became marginally non-significant after adjustment.

6.3.4 Progression from a CD4 count of 200 cells/mm^3

Patients were stratified into two groups on the basis of the 33rd percentile of B2M levels at the time of their CD4 count falling to 200 cells/mm^3 (3.8 mg/l). Patients with the highest B2M levels have a faster rate of progression to death (Figure 6.6, p=0.0007, log-rank test) but no relationship is seen with progression to either AIDS (p=0.19) or to the first HIV-related event (p=0.70).

The relative hazards of progressing to the three clinical endpoints associated with a one unit increase in B2M are shown in the bottom half of Table 6.4. Unadjusted for age or the CD4 count, there was a significantly raised hazard of developing AIDS or dying associated with an increased B2M level. The use of B2M as a categorical variable in the Kaplan-Meier analysis is likely to explain the differing results for progression to AIDS. After adjusting for age differences, only the effect of B2M on progression to AIDS remained significant (p=0.03).

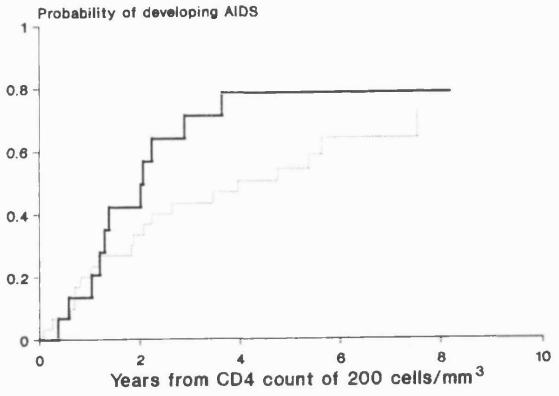
A one unit increase in B2M was associated with a 42% increase in the hazard of the CD4 count falling to 50 cells/mm^3, suggesting that B2M identifies those whose CD4 counts are likely to decline most rapidly. After adjusting for the CD4 count, the effect of B2M on all three clinical endpoints became non-significant (Table 6.4).

6.3.5 Summary of B2M results

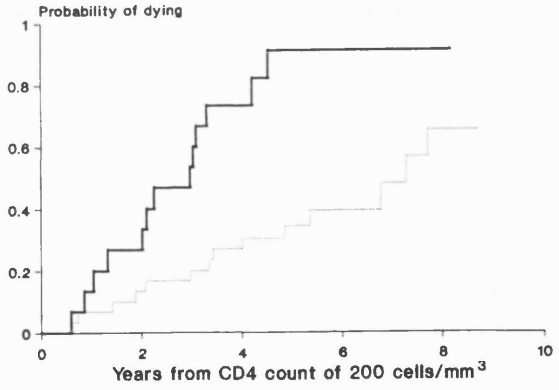
At high CD4 levels increased B2M levels appear to predict the development of clinical disease, even after adjusting for age differences and the CD4 count. At lower CD4 levels, however, this predictive ability becomes less apparent and disappears after adjusting for the CD4 count. The B2M level does not appear to be prognostic for death at any CD4 level.

Figure 6.6 : Kaplan-Meier plots showing the cumulative rate of progression to (i) AIDS, (ii) death and (iii) the first HIV-related event according to the number of years from the CD4 count reaching 200 cells/mm^3. Patients are stratified into two groups on the basis of their B2M level : B2M $\geq$ 3.8 mg/l - bold line, B2M < 3.8 mg/l - dotted line

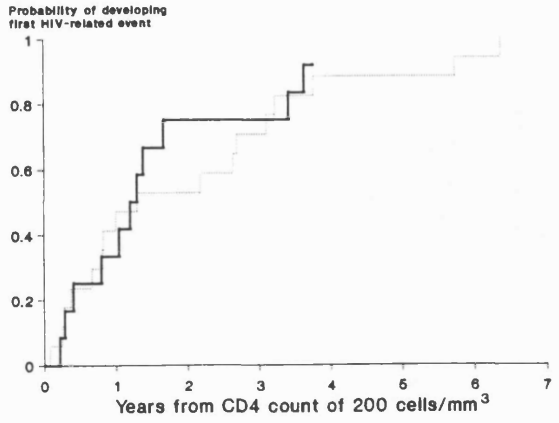
(i) AIDS



(ii) Death



(iii) First HIV-related event

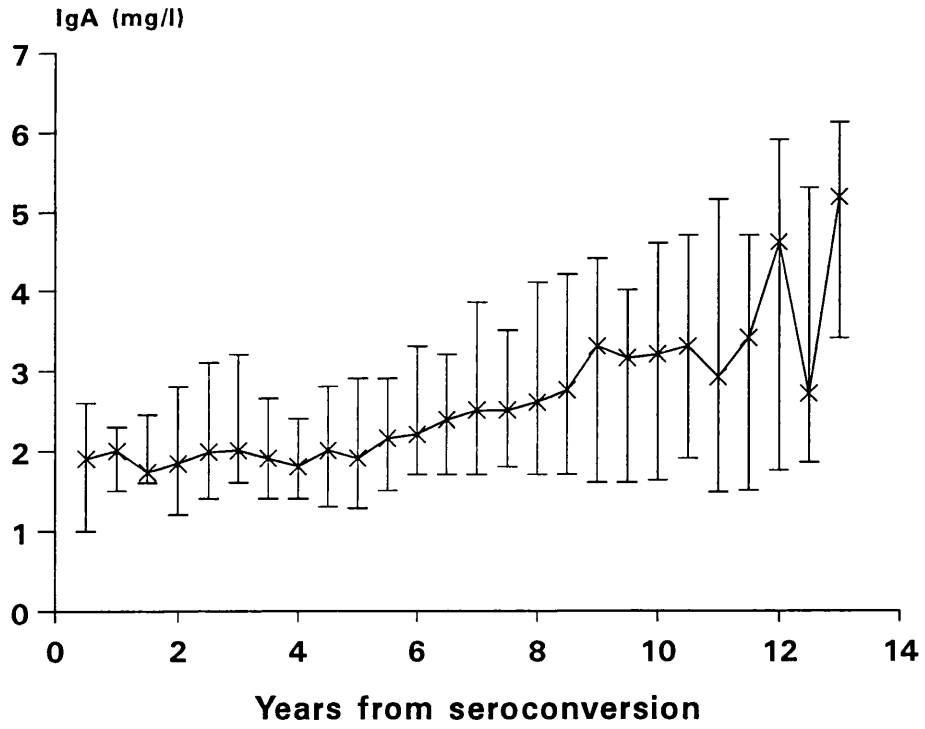


6.4 IgA

6.4.1 IgA measurements in the cohort

IgA has been routinely measured in patients in the cohort since 1985 (median 10 IgA measurements per patient, range 0-27 measurements). Figure 6.7 shows the distribution of mean IgA measurements in each six month period after seroconversion. On a population level, IgA levels tend to increase over time. However, the variation between patients becomes increasingly large over time.

Figure 6.7 : The medians and interquartile ranges of the means of all IgA measurements taken within each six-monthly period after seroconversion



Multi-level modelling indicates that there is a strong increase to the untransformed IgA values over time, ($p<0.00001$, Table 6.5). The inclusion of a 'random' quadratic term significantly improves the fit of the model ($p<0.00001$) although the mean parameter estimate is not significantly different from zero (Table 6.5, Figure 6.8(i)), suggesting that individual patterns vary widely. Overall, the IgA at seroconversion is estimated to be 1.5 mg/l, to increase at the rate of 0.2 mg/l per year on average, and to have a quadratic coefficient of 0.01 on average.

Table 6.5 : Final model coefficients from multi-level modelling of IgA

	Fixed parameters		Random variances and covariances		
	Mean	95% confidence interval	Intercept	Time	$Time^2$
Untransformed IgA					
Intercept	1.5	0.9 to 2.1	4.3	-	-
Time	0.2	0.009 to 0.4	-1.0	0.4	-
$Time^2$	0.01	-0.004 to 0.03	0.08	-0.03	0.003
Square root IgA					
Intercept	1.2	1.1 to 1.3	0.4	-	-
Time	0.07	0.02 to 0.1	-0.08	0.03	-
$Time^2$	0.002	-0.002 to 0.005	0.006	-0.002	0.0001

As IgA values were not measured until 1985, very few measurements are available to estimate the IgA level at seroconversion. This is evident in the extremely large standard deviations of the parameters. Hence estimates of the intercept should be interpreted carefully.

The analysis was repeated after taking a square root transformation of the IgA values. Again, a 'random' linear term improved the fit of the model ($p<0.00001$) as did a 'random' quadratic term ($p<0.00001$). However, again there was no consistent pattern to the quadratic coefficient and the mean estimate was not significantly different from zero ($p=0.21$). Overall, $\sqrt{}$IgA levels at seroconversion are estimated to be 1.2 on average, and to increase at the rate of 0.07 per year, with a quadratic coefficient of 0.002. The fitted curves from both the transformed and untransformed IgA levels are very similar (Figure 6.8(i)), both indicating a gradual increase in IgA over time and little evidence of any curvature to the IgA level. Fitted patterns of change for individual patients are shown in Figure 6.8(ii). The figure clearly shows the wide variation in fitted patterns over time, especially in values at the time of seroconversion.

An analysis of the relationships between the five cofactors described in Chapters 3 and 4 and both the IgA value at seroconversion and its subsequent change over time, suggested that levels tended to be higher in those over the age of 22.6 years ($p=0.00008$) than in those younger than 22.6 years (values of 1.82 and 1.02 mg/l respectively).

Figure 6.8(i) : 'Average' patterns of IgA change over infection generated from the models estimated from multi-level modelling methods. (i) Untransformed IgA - continuous line, (ii) √IgA - dotted line

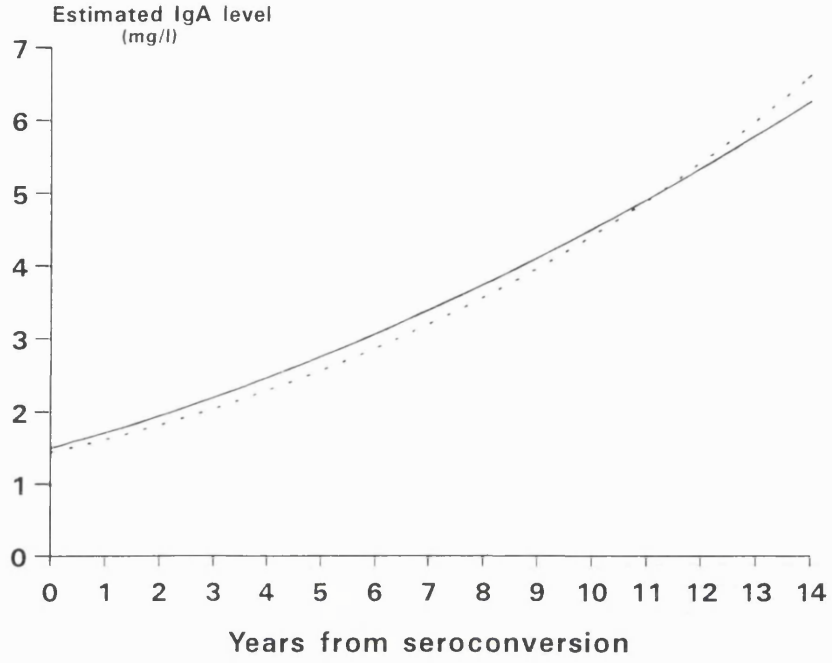
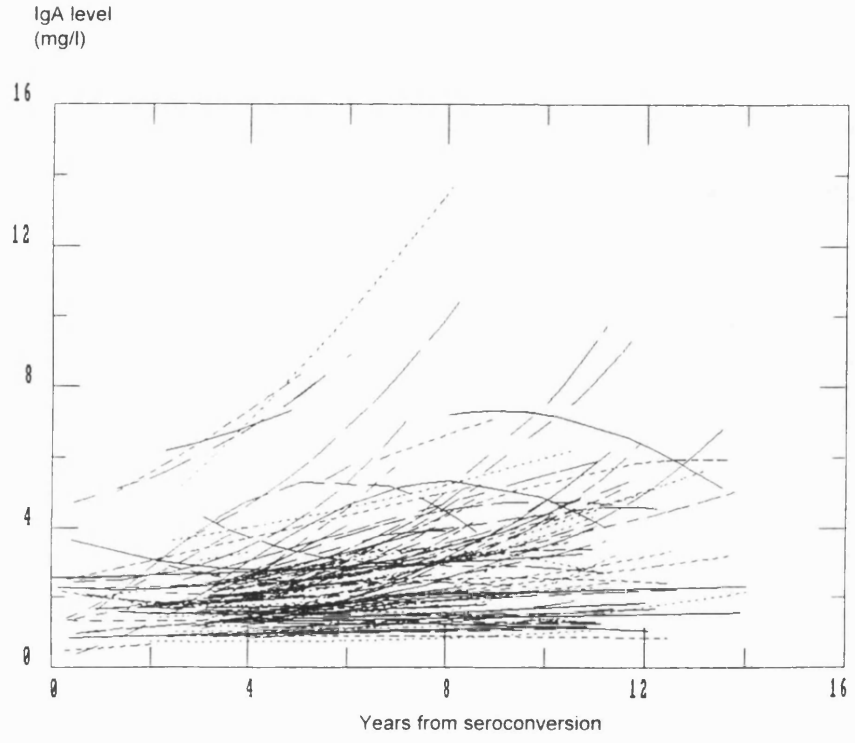


Figure 6.8(ii) : Estimated IgA patterns for each patient in the cohort after modelling the square root of the IgA level using multi-level modelling methods



IgA levels were also higher in those who were of a non-manual social class rather than of manual social class (1.64 and 1.22 respectively, p=0.04) and also in those who seroconverted most recently (1.01, 1.23, 1.46 and 1.71 in those who seroconverted earlier than 1981, in 1981, 1982 or after 1982 respectively, p=0.01). However, the lack of IgA measurements at seroconversion suggests that confirmation of these findings is required before interpreting these as novel findings.

6.4.2 Relationship with the CD4 count

Figure 6.9 shows the distribution of the mean IgA values whilst the CD4 count is measured in different ranges. There is a gradual increase in median IgA levels as the CD4 count drops. At very low CD4 counts (< 100 cells/mm^3) IgA levels become very variable with the 75th percentile reaching 12.9 mg/l. There is an increasing level of negative correlation between the IgA level and CD4 count as time from seroconversion increases (Figure 6.10), confirming the finding that the IgA value increases as the CD4 count falls.

6.4.3 The prognostic value of the IgA level unadjusted for the CD4 count

Table 6.6 shows the relative hazards associated with a one unit increase in the IgA level when it is included in the Cox model as a time-updated covariate. Unadjusted for the CD4 count, there is a strong association between the IgA measurement and progression to all three clinical events, suggesting that the IgA level is an important univariate prognostic marker. There is no evidence of any non-proportionality for progression to AIDS or death, although there is some evidence that the relative hazard for progression to the first HIV-related event changes over time.

6.4.4 The prognostic value of the IgA level adjusted for the CD4 count

An increase in IgA levels is associated with more rapid progression to both CD4 endpoints (Table 6.6). Consequently, some of the effect of the IgA level on progression to clinical endpoints may be explained by more rapidly dropping CD4 counts in those with the highest IgA levels. After adjustment for the CD4 count, however, the most recent IgA level continued to provide additional prognostic information for progression to both AIDS and to the first HIV-related event. However, the IgA measurement does not provide any additional prognostic information for death. Further, after adjustment, there is no evidence of non-proportionality in the relative hazard estimates for progression to any endpoint.

Figure 6.9 : The medians and interquartile ranges of the means of all IgA measurements taken while the CD4 count is in different ranges

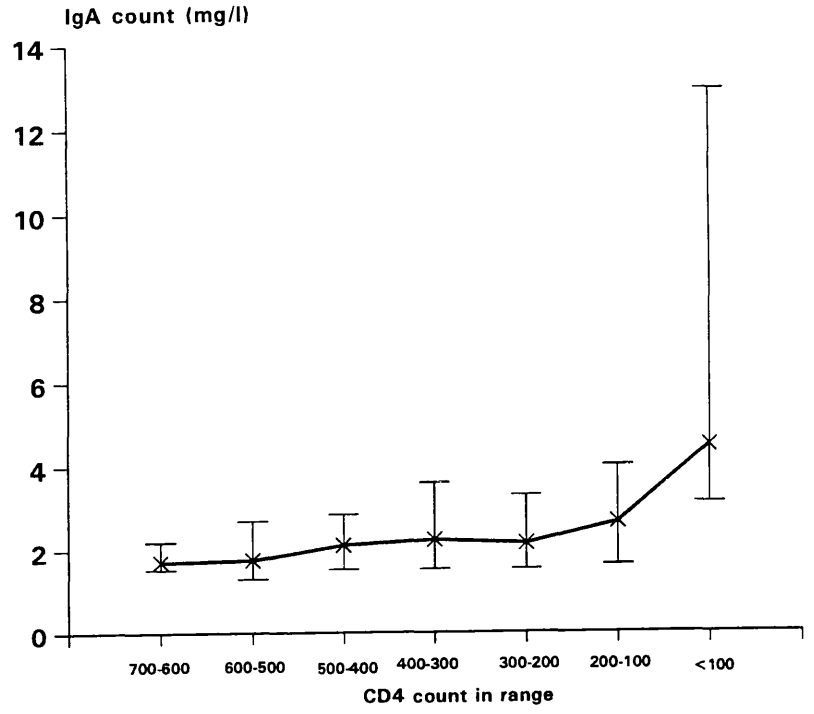


Figure 6.10 : Spearman rank correlation coefficients and 95% confidence intervals between CD4 counts and IgA measurements estimated at yearly intervals after seroconversion

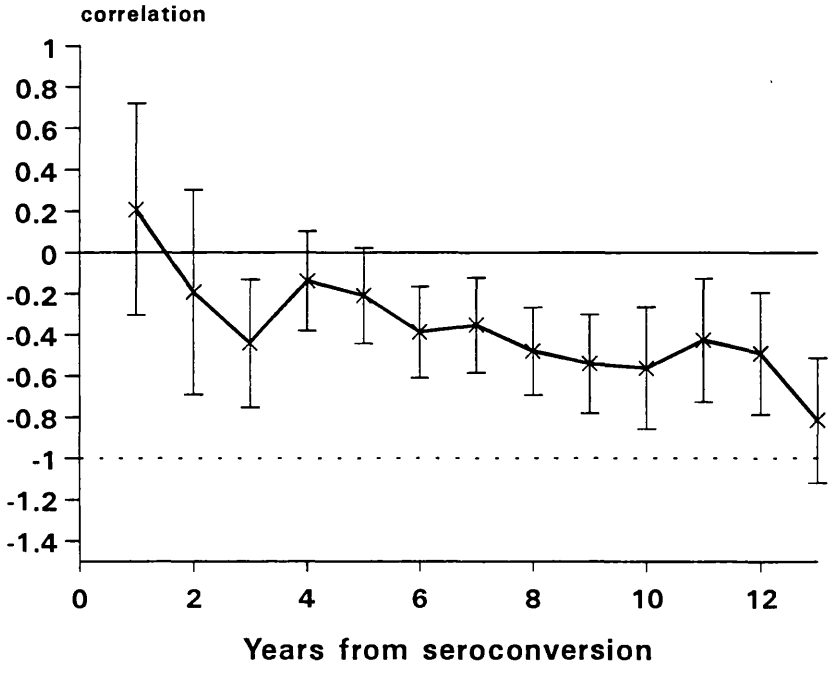


Table 6.6 : Relative hazards and 95% confidence intervals from the Cox proportional hazards model associated with a one unit increase in the IgA level for progression to (i) AIDS, (ii) death, (iii) the first HIV-related event, (iv) a CD4 count of 200 cells/mm^3, (v) a CD4 count of 50 cells/mm^3. Both IgA and the CD4 count are included in the model as time-updated covariates. The CD4 count is included in the model after a log transformation.

		Progression to :				
		AIDS	Death	HIV-related event	CD4 200 cells/mm^3	CD4 50 cells/mm^3
Unadjusted	Relative hazard	1.27	1.26	1.25	1.22	1.41
	95% CI	1.18 - 1.36	1.16 - 1.38	1.16 - 1.34	1.08 - 1.39	1.24 - 1.59
	p-value	0.0001	0.0001	0.0001	0.002	0.0001
	Test for non-proportionality (p-value)	0.22	0.77	0.02	0.10	0.67
Adjusted for CD4	Relative hazard	1.14	1.07	1.16	-	-
	95% CI	1.04 - 1.26	0.97 - 1.19	1.06 - 1.26	-	-
	p-value	0.007	0.19	0.0007	-	-
	Test for non-proportionality (p-value)	0.65	0.59	0.13	-	-

6.4.5 Summary of IgA results

IgA levels rise throughout infection, largely in tandem with the drop in CD4 counts. IgA levels provide strong prognostic information for the development of clinical HIV disease in addition to that provided by the CD4 count. However, their role is of less importance when considering death as an endpoint.

6.5 p24 antigenaemia and antibody

6.5.1 p24 antigen measurements in the cohort

The presence of the core antigen to HIV, p24, is measured on all routine blood samples taken on patients in the cohort and is recorded on the data set as either positive or negative.

By the end of 1993, 49 patients in the cohort had developed p24 antigenaemia between 3 weeks prior to and 12.89 years after their estimated date of seroconversion. By 14 years after seroconversion, an estimated 67.9% of patients are expected to develop p24 antigenaemia (median time to development 12.53 years, Figure 6.11).

In those who had counts available for analysis, CD4 counts at the time of p24 antigenaemia were in the range 10 to 1170 cells/mm^3 (median 330 cells/mm^3). When using CD4 information on all patients irrespective of whether the patient developed p24 antigenaemia or not, the CD4 count was found to fall to a median level of 60 cells/mm^3 before the development of p24 antigenaemia (Figure 6.12).

6.5.2 Effect of p24 antigenaemia on HIV disease progression

Unadjusted for the CD4 count, the development of p24 antigenaemia is associated with a large and highly significant increase in the risk of progression to all three clinical endpoints (Table 6.7). However, there is significant evidence of non-proportionality for progression to both AIDS and to the first HIV-related event, suggesting that the detrimental effect of p24 antigenaemia does not remain constant over time.

There is a significant association between the development of p24 antigenaemia and a drop in the CD4 count to both 200 and 50 cells/mm^3 (Table 6.7), suggesting that individuals who develop p24 antigenaemia have rapidly declining CD4 counts.

Figure 6.11 : A Kaplan-Meier plot showing the cumulative progression rate to the development of p24 antigenaemia according to the number of years from seroconversion

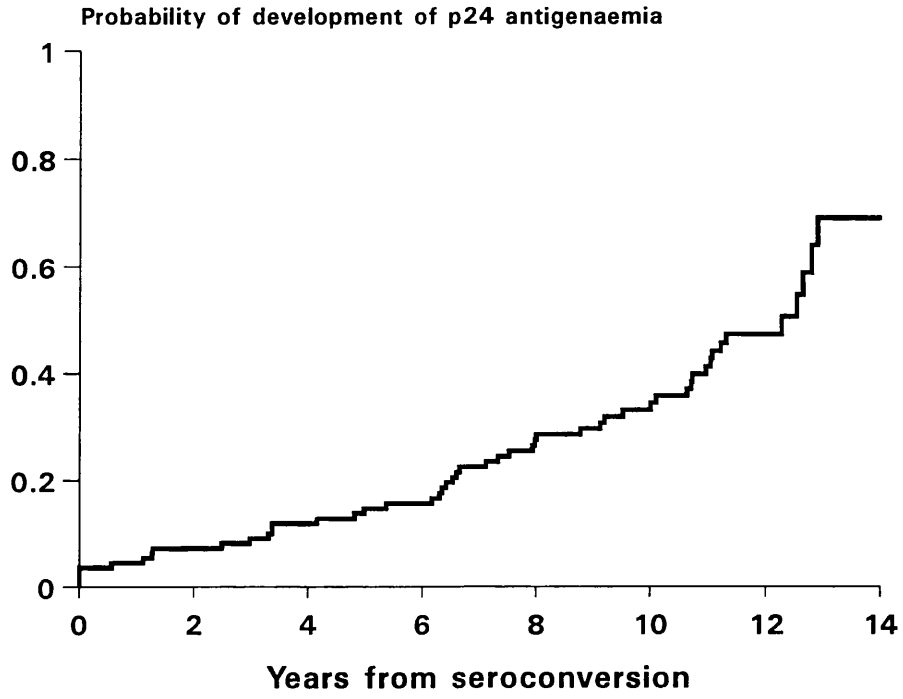


Figure 6.12 : A Kaplan-Meier plot showing the cumulative progression rate to the development of p24 antigenaemia according to the minimum CD4 count measured

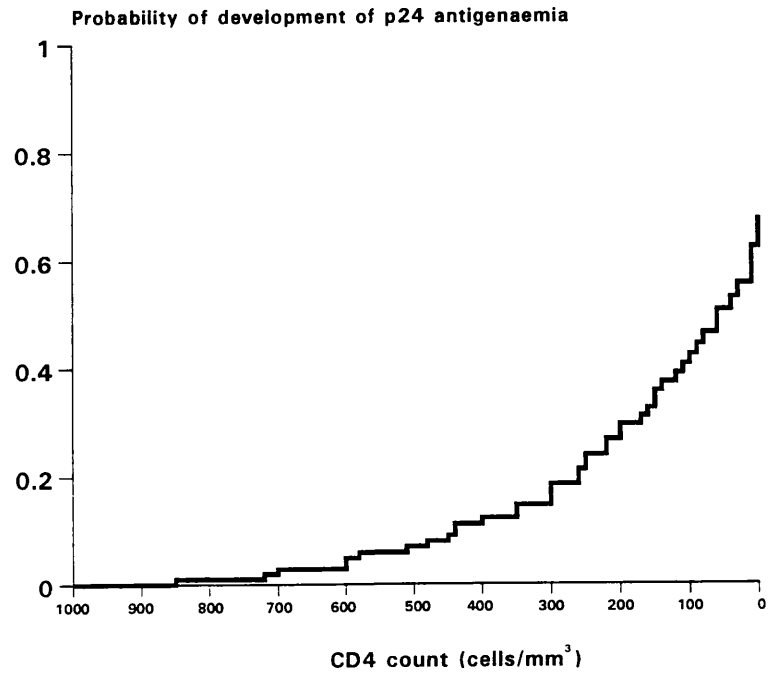


Table 6.7 : Relative hazards and 95% confidence intervals from the Cox proportional hazards model associated with the development of p24 antigenaemia for progression to (i) AIDS, (ii) death, (iii) the first HIV-related event, (iv) a CD4 count of 200 cells/mm^3 and (v) a CD4 count of 50 cells/mm^3. Both p24 antigen status and the CD4 count are included in the model as time-updated covariates. The CD4 count has been included after taking a log transformation.

		Progression to :				
		AIDS	Death	HIV-related event	CD4 200 cells/mm^3	CD4 50 cells/mm^3
Unadjusted	Relative hazard	5.27	4.11	3.73	3.35	6.01
	95% CI	2.92 - 9.52	2.25 - 7.53	2.23 - 6.23	1.85 - 6.06	3.25 - 11.10
	p-value	0.0001	0.0001	0.0001	0.0001	0.0001
	Test for non-proportionality (p-value)	0.01	0.16	0.0001	0.18	0.07
Adjusted for CD4 count	Relative hazard	3.15	1.85	2.45	-	-
	95% CI	1.66 - 5.98	0.95 - 3.62	1.40 - 4.27	-	-
	p-value	0.0004	0.07	0.002	-	-
	Test for non-proportionality (p-value)	0.01	0.22	0.64	-	-

After adjustment for the CD4 count as a time-updated covariate there remains a significant, although reduced, increase in the risk of progression to AIDS and the first HIV-related event associated with the development of p24 antigenaemia (Table 6.7). There also appears to be some relationship with survival, although this relationship is now marginally non-significant. There remains non-proportionality in the effect of p24 antigenaemia on the development of AIDS with the detrimental effect of p24 antigenaemia becoming less marked over time.

6.5.3 p24 antibody measurements in the cohort

The presence of antibodies to p24 has only been measured once in the cohort on an early sample soon after seroconversion. This sample was taken on average 33 weeks after seroconversion, but in some individuals the sample was not taken until up to 5.91 years after seroconversion. A total of 110 patients had samples tested of whom 88 were found to be positive for antibodies to p24. Table 6.8 contains a comparison of the patients found to be p24 antibody positive and negative in terms of their demographics. Patients who were p24 antibody positive were more likely to have severe haemophilia A than those who were p24 antibody negative. No other differences were seen in the demographics of the patients.

Table 6.8 : A comparison of the patients found to be p24 antibody positive and negative at the start of the study

		p24 Antibody status		
		positive	negative	p-value
Haemophilia Type	Severe A	83	16	
	Other	5	5	0.03
Social Class	Non-manual	44	14	
	Manual	44	6	0.17
Year of seroconversion	1979-1980	20	5	
	1981	17	3	
	1982	31	8	
	1983-1985	20	5	0.96
Age at seroconversion (years)	Median	21.28	25.04	
	Range	2.06 to 72.95	5.85 to 77.78	0.21

Patients who were initially p24 antibody positive had a less rapid progression to the development of p24 antigenaemia than those who were p24 antibody negative, as is shown in Figure 6.13 (p=0.004, log-rank test), although by 14 years after seroconversion progression rates were similar.

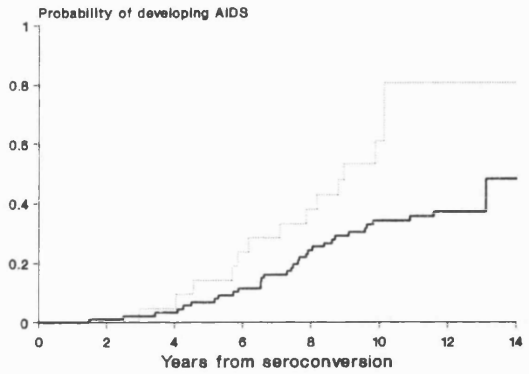
Figure 6.13 : A Kaplan-Meier plot showing the cumulative progression rate to the development of p24 antigenaemia according to the number of years from seroconversion. Patients are stratified according to their p24 antibody status shortly after seroconversion : p24 antibody positive - bold line, p24 antibody negative - dotted line

6.5.4 Relationship between p24 antibody status and HIV disease progression

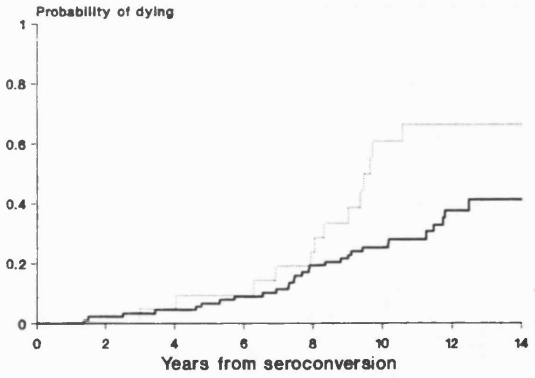
Patients who were p24 antibody negative at seroconversion had a faster rate of progression to both AIDS and death (p=0.003 and p=0.01 respectively, log-rank test, Figure 6.14). Indeed, the hazards of developing AIDS or dying were over twice as high if the patient was p24 antibody negative compared to p24 antibody positive (Table 6.9).

Figure 6.14 : Kaplan-Meier plots showing the cumulative progression rates to the development of (i) AIDS, (ii) death and (iii) the first HIV-related event, according to the number of years from seroconversion. Patients are stratified according to their p24 antibody status shortly after seroconversion : p24 antibody positive - bold line, p24 antibody negative - dotted line

(i) AIDS



(ii) Death



(iii) First HIV-related event

Table 6.9 : Relative hazards and 95% confidence intervals associated with an initial p24 antibody negative status for progression to (i) AIDS, (ii) death, (iii) the first HIV-related event, (iv) a CD4 count of 200 cells/mm^3 and (v) a CD4 count of 50 cells/mm^3. p24 antibody is included in the model as a fixed covariate at baseline although the patient only enters the risk set when the measurement becomes available, the CD4 count is included as a time-updated covariate after taking a log transformation.

		Progression to :				
		AIDS	Death	HIV-related event	CD4 200 cells/mm^3	CD4 50 cells/mm^3
Unadjusted	Relative hazard	2.06	2.37	1.48	1.74	2.16
	95% CI	1.08 - 3.95	1.22 - 4.58	0.89 - 2.47	0.94 - 3.23	1.08 - 4.33
	p-value	0.03	0.01	0.14	0.08	0.03
	Test for non-proportionality (p-value)	0.95	0.40	0.70	0.38	0.20
Adjusted for CD4 count	Relative hazard	1.13	1.79	1.08	-	-
	95% CI	0.54 - 2.37	0.90 - 3.57	0.63 - 1.88	-	-
	p-value	0.75	0.10	0.78	-	-
	Test for non-proportionality (p-value)	0.64	0.32	0.90	-	-

There was no evidence of any non-proportionality for either of these relative hazard estimates. There appeared to be no relationship, however, between initial p24 antibody status and the development of more minor clinical events.

Patients who were initially p24 antibody negative had a faster rate of progression to a CD4 endpoint of 50 cells/mm^3 (Table 6.9). There was also some evidence of a faster progression to a CD4 count of 200 cells/mm^3, although this was marginally non-significant. A multi-level analysis of the relationships between p24 antibody status and both the CD4 count at seroconversion and subsequent rate of CD4 decline, suggested that patients progressed to low CD4 counts more rapidly simply because CD4 counts at seroconversion were lower in those initially p24 antibody negative than in those p24 antibody positive (767 and 997 cells/mm^3 respectively, p=0.006). There was no evidence of any subsequent increase in the rate of CD4 loss in those initially antibody negative (p=0.37).

After adjustment for the CD4 count the relative hazards associated with p24 antibody status for progression to AIDS and death were reduced and became non-significant.

For these analyses the baseline date has been chosen to be the time of seroconversion rather than the time of the blood sample measured. Where samples were taken close the time of seroconversion, this is a reasonable assumption to make. However, as a number of these measurements were carried out some time after seroconversion, the analysis was repeated after excluding patients in whom p24 antibody levels were measured more than three years after the time of seroconversion. The relationship with progression to death remained significant in this more restricted analysis.

6.5.5 Summary of p24 antigenaemia and p24 antibody results

The development of p24 antigenaemia appears to herald a poor prognosis, especially with respect to the development of clinical HIV disease. However, it does appear that this effect may become less pronounced over time. Whilst the absence of antibodies to p24 appear to predict a poor prognosis, much of this can be explained by lower CD4 counts at seroconversion in these individuals.

6.6 The independent effects of all time-updated markers and age at seroconversion on progression to AIDS

After adjustment for the CD4 count, the prior rate of CD4 decline, the patient's IgA level and the development of p24 antigenaemia remain associated with the development of AIDS in this cohort of patients. Further, the development of bacterial infections prior to AIDS and age at seroconversion were both independently related to disease progression after adjustment for the CD4 count. It is of interest to see which of these laboratory markers and co-factors remain associated with the development of AIDS after adjustment for each other. Whilst there were some significant relationships between B2M levels and the development of clinical disease, B2M measurements are only available at selected time points. Hence, they cannot be included in any analysis in which markers are updated with time. Table 6.10 contains the results of a final analysis of the time-updated markers on progression to AIDS (all markers), death and the first HIV-related event (age and laboratory markers only). The CD4 count remains highly associated with progression to all three endpoints, even after adjustment for all other factors.

6.6.1 Progression to AIDS

Age at seroconversion remains associated with the development of AIDS, even after taking account of the all other laboratory and clinical markers, suggesting that the effect of age on disease progression is not mediated through immune system changes.

After adjustment for all markers, the development of p24 antigenaemia and the presence of a bacterial infection prior to AIDS are associated with a more rapid progression to AIDS. The IgA level becomes marginally non-significant after adjustment. After adjusting for all other markers, the previous rate of CD4 decline continues to have some prognostic importance, although it becomes marginally non-significant.

6.6.2 Progression to the first HIV-related event and death

For progression to the first HIV-related event, highly significant independent effects of all three laboratory markers are apparent, although interestingly no effect of either age at seroconversion or the rate of CD4 decline are seen. In contrast, for progression to death, only the patients age, their CD4 count and the rate of CD4 decline remain independently associated with disease progression.

Table 6.10 : Relative hazards and 95% confidence intervals associated with age at seroconversion (per 5 years), a one unit drop in the log of the CD4 count, a 100 cell/mm^3 drop in the CD8 count, a one unit increase in the IgA level, the development of p24 antigenaemia, a 100 cell/mm^3 per year drop in the rate of CD4 decline and the development of bacterial infections for progression to (i) AIDS, (ii) death and (iii) the first HIV-related event.

	Progression to :								
	AIDS			Death			First HIV-related event		
	Relative hazard	95% confidence interval	p-value	Relative hazard	95% confidence interval	p-value	Relative hazard	95% confidence interval	p-value
Laboratory markers and age									
Age at seroconversion	1.17	1.04 - 1.30	0.006	1.34	1.19 - 1.50	0.0001	1.01	0.92 - 1.10	0.87
IgA	1.11	1.00 - 1.23	0.05	1.04	0.94 - 1.16	0.46	1.14	1.04 - 1.24	0.005
CD4	1.27	1.13 - 1.42	0.0001	1.41	1.28 - 1.57	0.0001	2.21	1.59 - 3.08	0.0001
p24 antigenaemia	2.21	1.12 - 4.36	0.02	1.26	0.62 - 2.59	0.53	2.23	1.25- 4.00	0.007
Rate of CD4 decline	1.17	0.99 - 1.39	0.07	1.13	1.02 - 1.25	0.02	1.04	0.94 - 1.14	0.46
All markers									
Age at seroconversion	1.18	1.05 - 1.32	0.004	-	-	-	-	-	-
IgA	1.09	0.98 - 1.21	0.11	-	-	-	-	-	-
CD4	1.31	1.17 - 1.48	0.0001	-	-	-	-	-	-
p24 antigenaemia	2.14	1.09 - 4.21	0.03	-	-	-	-	-	-
Rate of CD4 decline	1.19	0.98 - 1.44	0.07	-	-	-	-	-	-
Bacterial infection	2.65	1.30 - 5.39	0.007	-	-	-	-	-	-

6.7 Discussion

6.7.1 Summary of main results

In this chapter I have studied three markers which are time-dependent (in that they change over time) and which can be analysed as such because repeated measurements are available on patients over time. Two further markers are fixed in that patients only have measurements at certain, chosen time points.

Of the time-updated measurements, both the IgA level and the development of p24 antigenaemia appear to offer additional prognostic information to that provided by the CD4 count. The CD8 count, on the other hand, simply identifies those with the lowest and most rapidly declining CD4 counts and as such does not provide any prognostic information once the patient's CD4 count is known.

Of the fixed covariates studied, the B2M level appears to be a useful marker of future disease progression at high CD4 counts, suggesting that this marker may be useful for establishing long-term prognosis early in infection. The loss of antibodies to p24, however, simply identified patients with the lowest CD4 counts soon after seroconversion and as such did not provide any additional prognostic information.

When time-updated covariates were adjusted for each other in a multivariate model which also included the patients' age at seroconversion and the development of bacterial infections, the CD4 count appeared to be the most consistent prognostic marker, being significantly associated with all three clinical endpoints. Both the IgA level and the development of p24 antigenaemia were associated with early clinical events, being associated with the development of the first HIV-related event and AIDS, but not with death. The patient's age, in contrast, was associated with only the more major events of AIDS and death.

The development of a bacterial condition prior to the development of AIDS continued to suggest that a patient's condition was likely to deteriorate, irrespective of their immune status. The rate of CD4 decline continued to add some additional prognostic information, although this became marginally non-significant for progression to AIDS.

6.7.2 CD8 lymphocyte counts

The finding that CD8 counts are raised during HIV infection has often been reported[52,60,72,75,258,281,372]. A drop at late stages of infection has been suggested by both Raska *et al*[330] and Brinchmann *et al*[281] who found that CD8 counts were lower in

AIDS patients than in HIV-negative controls. Buchbinder *et al*[192] found that healthy long-term survivors had higher CD8 counts than rapid progressors, further confirming the possibility of a drop in CD8 counts at late stages of disease. However, findings from two studies[292,329] suggest that any decline, if it exists, is likely to be small. Through the most part of infection, however, there appears to be no large change in CD8 counts[52,278]. I have found that there is a general trend for counts to increase over the first few years after infection, before dropping to low levels. Whilst multi-level models are an improvement on population-based methods for studying the pattern of CD8 change, there is still the possibility for the selective drop out of patients who die to have an effect. For example, if the CD8 counts of patients who die drop rapidly in the year prior to death, then the overall estimate of the CD8 count will be 'pulled down' as patients die. As no outcome is attached to patients in the multi-level modelling it has not been possible to consider whether there is a different pattern in those who die than in those who remain alive. de Stavola[382] has suggested a method for considering this by censoring patients at different time points and then considering the changing patterns in those alive and those dead at each time point. However, with the relatively small number of deaths in this study, it is unlikely that this would reveal any major differences.

The role of the CD8 count in assessing patient prognosis remains unclear. Some authors have suggested that it has additional prognostic value to the CD4 count[51,62], but others find that it has no independent prognostic effect[50,84,226,314] or that it may only be predictive of AIDS close to the time of diagnosis[68]. After adjustment for the CD4 count, the relative hazard associated with a 100 cell/mm^3 drop in the CD8 count has been reported to range between 0.93 and 0.96 when treated as fixed at some baseline[268,315] and 0.99 as a time-updated covariate (Ms A Mocroft, personal communication). Chevret[332] reported a non-significant increase in the progression rate to a CD4 count of 200 cells/mm^3 when the CD8 count rose above 1500 cells/mm^3.

In a recent paper from this cohort[383], the CD8 count was shown to be a useful marker of long-term disease progression and CD4 decline when measured soon after seroconversion, indicating that the immune activation seen very soon after the time of infection may be linked with the process of CD4 decline. This finding is of limited use for patients who do not usually present at the time of infection. In these patients, once their most recent CD4 count is known then it would appear that knowledge of their CD8 count does not add any further prognostic information in the short-term.

6.7.3 B2M

It has been suggested that B2M levels are generally higher in HIV positives than in HIV negatives[83,287,311,342,344,351,358,372]. One paper in particular has suggested that B2M increases at a fairly slow rate until a year before AIDS diagnosis when it then increases rapidly[384]. In contrast, a recent paper analysing routine B2M levels from five cohorts, including this cohort, suggested that B2M rises over the first 6 years of infection before dropping over later years[385]. However, this may be an artefact of the inclusion of data from cohorts who each measured B2M over different time periods. Unfortunately, routine follow-up data on B2M is not available for many patients in this cohort, so I am not able to consider this.

As a result of including only men with measurements taken within six months of their CD4 count falling to each level, the samples of men included in this analysis are small but homogeneous in terms of their CD4 count. At high CD4 counts the B2M level provides additional prognostic information for the development of AIDS to that provided by the CD4 count. At lower CD4 counts, however, the B2M level simply identifies those whose CD4 counts will drop more rapidly in the future and are, therefore, more likely to develop clinical disease.

Figures 6.5 and 6.6 provide a convenient tool for clinicians who may use these figures to assess long-term prognosis and who may, for example, choose to delay treatment in patients with low CD4 counts and low B2M levels whilst considering the early introduction of treatment in patients with high CD4 counts but high B2M levels. Although differences in B2M levels between exposure groups exist[342], this does not invalidate the use of B2M as a prognostic marker. However, the cut-offs quoted in this thesis may need to be validated in other exposure groups before use.

The reported value of B2M as a prognostic marker independently of the CD4 count varies in the literature. In two independent analyses of data from the Los Angeles centre of the Multicenter AIDS Cohort Study, both Fahey[331] and Hofmann[344] suggested that the predictive power of B2M and CD4 counts were of a similar size and were independent. This independence has also been shown by other authors[191,334,339] although some suggest that any predictive power disappears after adjustment for the CD4 count[180]. Interestingly, the former studies were carried out using fixed covariates only. Alcabes *et al*[180], on the other hand, used time-updated values, suggesting that the B2M level may indicate those whose CD4 counts will decline rapidly, a finding confirmed by Sheppard *et al*[291].

Some authors suggest that neopterin, which is highly correlated with B2M[324,331,332], has a stronger predictive power than B2M[232,331,357]. Neopterin is not measured routinely in this cohort and therefore it is not possible to assess which has the greater predictive power.

The introduction of zidovudine has been shown to have a beneficial effect on B2M levels in patients[386]. I have previously shown that these results are, however, essentially unchanged when patient follow-up was censored in 1987, prior to the availability of zidovudine in the UK[387] (See Appendix III - Published Papers).

6.7.4 IgA levels

One of the consequences of repeated exposure to the contaminants contained in intermediate purity products is that immunoglobulin levels are elevated in men with haemophilia, irrespective of HIV status[74]. Any generalisation of the results in this thesis to other exposure categories must allow for this. IgA levels are increased in HIV infection[60,79,333]. At the time of seroconversion levels may be higher in older patients, homosexual men compared to patients of other exposure categories and in men compared to women[385]. The finding of a higher IgA level in older individuals is confirmed in this thesis, although actual IgA levels at the time of seroconversion are few and the results may simply reflect differential availability of measurements for analysis. The rise in IgA levels appears to begin soon after seroconversion[73], suggesting that the IgA level could be a useful prognostic marker for early clinical events.

The finding that the IgA level is a strong predictor of the development of AIDS has previously been shown[232,333]. However, the finding that changes in the IgA level correlate with changes in the CD4 count, both in this study and in others[86] would seem to suggest that the effect of IgA may not be independent of the CD4 count. Further, we have recently shown[383] that early IgA levels soon after infection are related to the subsequent rate of CD4 depletion in patients in this cohort. Despite this the IgA level has been shown to predict the development of AIDS when it is included in multivariate models which also include the CD4 count both as fixed[84,221,331] or time-updated[61] covariates. For the patients in this cohort, the IgA level does provide additional prognostic information for the development of HIV-related clinical disease to that provided by the CD4 count.

6.7.5 p24 antigenaemia and antibody status

The development of p24 antigenaemia is a strong prognostic marker for progression to clinical endpoints in this cohort, confirming findings from the cohort published in 1991[388] in which the development of p24 antigenaemia was a strong univariate predictor of the development of AIDS. However, in this earlier study, the effect of p24 antigenaemia became non-significant after adjusting for the CD4 count. After a further three years follow-up, however, this does not now appear to be the case and after adjusting for the CD4 count and other laboratory/clinical markers, the development of p24 antigenaemia remains associated with more rapid disease progression.

The disappearance of antibodies to p24 is one of the earliest markers of disease progression, usually occurring before the development of p24 antigenaemia[65]. In an earlier study by the de Wolf *et al*[364], 13.8% of seroconverters developed p24 antigenaemia at entry to the study or soon after. Amongst a cohort of haemophilia patients[365], the prevalence of p24 antigenaemia was 21% by eight years after seroconversion, comparing to 29% by eight years in this cohort. Also in her cohort of haemophilia patients, Eyster showed that 63% remained p24 antibody positive by ten years after seroconversion[365].

It is well documented that the development of p24 antigenaemia is associated with more rapid progression to AIDS[51,62,68,73,330,331,365,366]. Despite the fact that individuals with p24 antigenaemia may experience a more rapid rate of CD4 depletion[332,366] than those who are p24 antigen negative, the effect of p24 antigenaemia on disease progression does appear to be independent of that of the CD4 count[229,365]. p24 antigenaemia is assumed to reflect viral replication[370,389] and therefore it would be expected that increased levels of p24 antigenaemia would be associated with a worst prognosis. This is confirmed by recent studies[390,391] in which increased viral load appears to be associated with disease progression.

The presence of antibodies to p24 is a good sign with a reduced hazard of AIDS in those with p24 antibodies[68,365,392]. In a direct comparison, the development of p24 antigenaemia appears to have greater prognostic power than the presence of antibodies to p24[393]. Unfortunately, as p24 antibody levels have only been measured once in this cohort a direct comparison cannot be made. Whilst having high specificity for the development of AIDS, neither marker is particularly sensitive for the development of AIDS[105,392,394] with many patients never having become p24 antigenaemic by the time

they develop AIDS and patients with high titres of antibodies at seroconversion eventually developing clinical disease.

The interpretation of results regarding p24 antigenaemia and p24 antibody from different studies are difficult due to racial differences in the levels of the markers[284,367]. Consequently, the value of the p24 antigen or antibody levels must be evaluated in terms of the racial characteristics of the patients studied.

CHAPTER 7 : THE EFFECTS OF MEASUREMENT VARIABILITY AND MISSING VALUES ON THE RELATIVE HAZARD ESTIMATE

7.0 Summary of contents

In Chapter 5, I suggested that variability in the CD4 measurement may lead to a reduction in the relative hazard estimate. In this chapter I will study the effects of measurement variability and missing values on the relative hazard estimate in more detail. Rather than using the data from the cohort, I will illustrate these effects using simulated data. This enables both the pattern of missing values and the amount of variation in the measurement to be controlled.

7.1 Introduction

It is well documented that the inclusion of imperfectly measured covariates in regression models can lead to biased estimates of the coefficients of covariates in the model, even if some covariates are measured without error[395-397]. When only one covariate is included in the model this problem is usually termed as 'Regression dilution'. In order to obtain unbiased estimates of the coefficients, some form of correction is usually made to the coefficient either during or after the modelling process[317,327,328,396,398-402]. This correction method usually requires either repeated imperfect measurements[402] or a separate validation study with some 'gold-standard' measurement technique[403] in order to obtain an idea of the level of variability in the measurement. Often, however, no such gold-standard measurement is available.

7.2 The simulation model

The relationship between the underlying CD4 trend and the measured CD4 count may be expressed as :

Measured CD4 count = Underlying CD4 trend + random error

where this random error may be a result of measurement error or natural biological variation. As the underlying CD4 trend cannot be measured, all estimates of the relative hazard associated with a drop in the CD4 count are based on the measured CD4 count. When studying HIV pathogenesis, it is usually assumed that the relationship between the measured CD4 count and the risk of disease progression is the same as that

between the underlying CD4 trend and disease progression. Whether this is an appropriate assumption to make, however, is rarely questioned.

In 1991 Taylor[302] suggested the use of a simulated model of CD4 decline, based on the relationship between the measured CD4 count and the underlying CD4 trend described above, for the study of HIV infection. Underpinning the model is the assumption that the underlying CD4 trend, or something strongly related to it, is fundamental to HIV disease progression and that the prognosis of a patient is fully determined by this trend. By making use of a similar model and by simulating CD4 data for individuals, the relationship between the relative hazards associated with the underlying CD4 trend and the measured CD4 count can be studied.

The aim of the simulation studies reported in this chapter is to quantify the effect of variability and missing CD4 values on the relative hazard estimate from the proportional hazards model. Using a method similar to Taylor[302] I have randomly generated underlying CD4 trend data for samples of 500 individuals, based on the typical patterns of decline seen in the cohort. Making the assumption that the underlying CD4 trend is fundamental to the development of AIDS, a date of AIDS diagnosis is selected for each individual purely on the basis of their underlying CD4 trend. In this situation, therefore, only long-term changes in the CD4 count are assumed to result in disease progression; short-term variation around the underlying trend is assumed to have no effect on clinical outcome. The validity of this assumption will be discussed later in the chapter.

7.3 Simulated CD4 paths - the model parameters

The pattern of CD4 decline over HIV infection was described in Chapter 5 and was found to decline in a quadratic manner over time. A linear rate of decline for the square root of the CD4 count has therefore been assumed for the underlying CD4 trend. In the cohort data there remained some dependence of the within-individual variation on time after taking a square root transformation. However, in order to provide a simple computational model I have assumed that the use of the square root transformation successfully stabilises the variation and have not attempted to incorporate changes in the variability around the underlying CD4 trend over time. The model chosen, therefore, is an easily interpretable model. More complex models could be fitted if desired although it is believed that the results in this section are largely independent of such refinements to the underlying model.

A simple linear multi-level model for the measured $\sqrt{CD4}_{ij}$ (the square root of the *j*th CD4 count for the *i*th patient) will therefore be given by:

$$\sqrt{CD4}_{ij} = (intercept + v_i) + (slope + w_i) \times time_{ij} + \varepsilon_{ij}$$

+----------underlying trend----------+ +-random-+ variation

where *intercept* is the overall mean $\sqrt{CD4}$ at seroconversion, v_i describes how each individual's $\sqrt{CD4}$ count at seroconversion deviates from the overall mean. *slope* is the overall mean decline in $\sqrt{CD4}$ per year and w_i expresses how each individual's rate of decline deviates from the overall mean decline. ε_{ij} is the within-individual random component of the model which will be controlled to assess the effects of variability.

The parameters used for the simulations for this simple model were estimated using data from the cohort. The following estimates of the parameters were obtained :

$intercept = 30.05$ $\quad v_i \sim N(0 , 7.22^2)$

$slope = -1.88$ $\quad w_i \sim N(0 , 1.44^2)$

$\varepsilon_{ij} \sim N(0 , 3.3^2)$

Hence, $\sqrt{CD4}$ counts are estimated to be 30.05 at seroconversion on average (903 cells/mm^3) and were estimated to decline at the rate of 1.88 per year. However, as described in Chapter 5, there is wide inter-patient variation around these values and the CD4 counts at seroconversion for individual patients are estimated to fall between 15.6 and 44.5 (243 and 1979 cells/mm^3) and slopes will range between a one unit per year increase and 4.76 units per year decrease in approximately 95% of patients. It is assumed, for simplicity, that the estimates of the intercept and slope are independent for each patient ie. there are no correlations between the parameters. Estimates of the intercept and slope were randomly generated from the above distributions for each individual. The underlying CD4 count was then calculated for each individual at monthly intervals.

The variance of the within-individual variation, ε_{ij}, was estimated to be 10.90 ($=3.3^2$). By increasing or decreasing this estimate, the amount of variation around the underlying CD4 count at each measurement can be increased or decreased. For the following analyses, this variance will be multiplied by a value, k, which will take one of eight different values (0, 1/8, 1/4, 1/2, 1, 2, 4, 8). When k=0 no variation is added; as k

increases the amount of variation increases. When k=1 the variation is assumed to be similar to that seen in the cohort.

The CD4 count at diagnosis was chosen to reflect information from published papers and from the cohort on the distribution of CD4 counts at AIDS. The range in which the CD4 count at the time of AIDS diagnosis lies was first selected with the following probability :

Range (cells/mm^3)	0-50	51-100	101-150	151-200	201-300
Probability	0.35	0.3	0.15	0.1	0.1

After selecting a CD4 range, the patient's CD4 count at diagnosis was then generated by assuming that the CD4 count at AIDS is distributed uniformly over the chosen range. The mean CD4 count at diagnosis is therefore estimated to be approximately 93 cells/mm^3 (standard deviation 71 cells/mm^3). The effect of changes in this distribution on the results will be discussed in section 7.6.

The CD4 count is included in the proportional hazards model as a time-updated covariate. For this simulated data it was found that the best fit of the model was obtained when untransformed values were used and hence the relative hazards quoted refer to a 100 cell/mm^3 drop in the CD4 count. Each simulated individual has been followed for up to 14 years before being censored at that date if their estimated date of AIDS diagnosis had not been reached. This time was chosen in order to assess the effect of variability over a similar time scale to that seen in the Royal Free Hospital Cohort. Each data set of 500 patients is randomly generated 200 times for each analysis, and for each data set the relative hazard associated with the measured CD4 count is estimated. The figures quoted are the median and 90% range of the 200 relative hazard estimates.

In the absence of any variability in the CD4 count (when k=0) and when CD4 counts are measured every month, the median relative hazard estimate is 4.58 (90% range 4.02 to 5.28). This is therefore the 'gold-standard' relative hazard for this model.

7.4 Effect of missing values on the relative hazard estimate

In order to assess the effect of missing values on the relative hazard estimate, different missing value scenarios have been considered. Missing values may either occur totally at random or, more realistically, may depend on either the underlying CD4 trend or the

measured CD4 count. For example, as the underlying CD4 trend becomes lower the patient is more likely to become ill and to present for medical care. Alternatively, if the measured CD4 count is low, the patient may be advised to return sooner for review even if clinically well. The probability that the CD4 count is missing may also be related to the time from seroconversion.

The left-hand side of Table 7.1 shows the median relative hazard estimate and 90% range of estimates for the relative hazard estimate when different proportions of CD4 measurements are missing at random and when no variability is added. There is a gradual drop in the relative hazard estimate from the 'gold standard' as the proportion of missing values increases. However, it is only when the proportion missing reaches 11 out of 12 (corresponding to one measured CD4 count roughly every year) that the relative hazard is considerably reduced.

Table 7.1 : The effect of different patterns of missing values on the relative hazard estimate. CD4 counts are measured every month and are missing at random.

Proportion of CD4 counts missing	k=0		k=1	
	Median estimate	90% range of estimates	Median estimate	90% range of estimates
None	4.58	4.02 - 5.28	2.83	2.57 - 3.11
1 out of 12	4.51	4.02 - 5.21	2.83	2.58 - 3.09
3 out of 12	4.47	3.98 - 5.16	2.83	2.59 - 3.14
6 out of 12	4.35	3.92 - 4.92	2.78	2.55 - 3.03
9 out of 12	4.02	3.57 - 4.62	2.63	2.42 - 2.86
11 out of 12	2.85	2.59 - 3.17	2.14	1.99 - 2.31

As it is unlikely that random missingness is appropriate for HIV infection, the analysis was repeated using three additional missing value scenarios. Table 7.2 shows the median relative hazard estimates and 90% ranges for scenarios where the proportion of missing values has been assumed to be related to (i) the underlying CD4 trend (>500 cells/mm^3: 75% missing, $\leq$ 500 and > 200 cells/mm^3 : 50% missing, $\leq$ 200 cells/mm^3 : 25% missing), (ii) the measured CD4 count (same cut-offs and proportions as above) and (iii) the time from seroconversion of the measurement (up to 4 years after seroconversion : 75% missing, 4-6 years after seroconversion : 50% missing, 7+ years after seroconversion : 25% missing).

Table 7.2 : The effect of different patterns of missing values on the relative hazard estimate. The probability of the count being missing depends on (i) the underlying CD4 trend, (ii) the measured CD4 count and (iii) the time from seroconversion.

	k=0		k=1	
	Median estimate	90% range of estimates	Median estimate	90% range of estimates
No missing values	4.58	4.02 - 5.28	2.83	2.57 - 3.11
Missing depends on underlying CD4 trend	4.43	3.92 - 5.06	2.81	2.56 - 3.09
Missing depends on measured CD4 count	4.41	3.98 - 5.13	2.73	2.50 - 3.06
Missing depends on time from seroconversion	4.26	3.82 - 4.90	2.75	2.52 - 3.02

When the CD4 count is measured perfectly the results in the left-hand side of Table 7.2 suggest that, as expected, there is little difference between the relative hazard estimates when the proportion of missing values depends on either the underlying CD4 trend or measured CD4 count (when k=0 the underlying CD4 count equals the measured CD4 count). Both relative hazard estimates are reduced slightly from that when no values are missing. When the proportion of values missing depends on the time from seroconversion the relative hazard is further reduced.

7.5 Effect of changing random component of measured CD4 count on relative hazard estimate

In order to assess the effect of variability on the relative hazard estimate, k was allowed to take each of the eight values specified in Section 7.3. The proportion of missing values was assumed to be related to the underlying CD4 trend as described in the previous section. Table 7.3 shows the results from this analysis.

As the amount of variability increases the relative hazard estimate falls from a value of 4.43 towards one. When k=8 the variability around the underlying CD4 trend becomes so large that the relative hazard estimate is very close to one, although the lower limit of the 90% range remains above one. This suggests that whilst some prognostic value would be attributed to the CD4 count, it is unlikely to be seen as an important marker.

Table 7.3 : The effect of changing the random variability in the CD4 count on the relative hazard estimate. CD4 counts are measured monthly and the proportion missing depends on the underlying CD4 trend

Variance is multiplied by:	Median estimate of relative hazard	90% range of estimates
0	4.43	3.92 - 5.06
1/8	4.34	3.84 - 4.92
1/4	4.23	3.74 - 4.81
1/2	3.74	3.37 - 4.26
1	2.81	2.56 - 3.09
2	1.75	1.64 - 1.88
4	1.19	1.15 - 1.23
8	1.03	1.02 - 1.04

The analysis in section 7.4 was repeated with k set to one (ie. in the presence of a realistic amount of variability). The results are shown in the right-hand sides of Table 7.1 and 7.2. When no values are missing, the relative hazard estimate associated with a one unit drop in the measured CD4 count (when a realistic amount of variability is added) is 2.83 (Table 7.1). Again, the relative hazard is only reduced noticeably when the proportion of missing values reaches 11 out of 12. When the proportion of missing values depends on the underlying CD4 trend (Table 7.2), the relative hazard estimate is also only slightly reduced. However, when the proportion of missing values depends on either the measured count or the time from seroconversion, the estimate is more severely affected.

7.6 Effect of variability on the estimation of relative hazards associated with other covariates

The effect of variability in the CD4 count on the relative hazard estimate is well documented and has been confirmed in this thesis. However, of interest is the effect that this variability has on the relative hazard estimates of other covariates included in the proportional hazards model. For example, if the relative hazard for the CD4 count is underestimated because of this variability, then the importance of other covariates in the model after adjustment may be overstated.

In order to assess these effects I have chosen to consider two covariates. Firstly, I will consider a fixed covariate at the time of seroconversion. I will call this a 'co-factor' effect although the covariate could represent any fixed covariate which has an effect on the CD4 count but no other effect on AIDS-free survival (e.g. a treatment). Secondly, I will consider the effect of a second time-dependent covariate which has no independent effect on AIDS-free survival, but which may be correlated with the CD4 count. Again, I will call this a 'laboratory marker', but it may represent any other time-updated covariate which is related to the CD4 count in some way.

For each of these analyses, CD4 counts are assumed to be measured monthly and the probability that they are missing is related to the underlying CD4 trend as described earlier. Results for k=1/4, 1 and 4 are included as these are sufficient to illustrate the pattern of the results.

7.6.1 A co-factor which improves the immune status of patients

I will firstly consider a co-factor which has the effect of raising the underlying CD4 count at baseline, in this case at seroconversion, but which doesn't have any effect on the subsequent rate of CD4 decline. I will then consider a co-factor which slows down the rate of CD4 decline but which doesn't change the absolute level at seroconversion. In both cases any effect of the co-factor on AIDS-free survival acts solely through the CD4 count. Once the CD4 count is adjusted for in the proportional hazards model, therefore, no residual effect of the co-factor should remain and its relative hazard should be approximately equal to one.

For the first scenario, the co-factor is assumed to raise the underlying CD4 trend at seroconversion by a mean of six units on the square root scale. Individual responses to the co-factor are assumed to vary around this normally with a standard deviation of three units. Hence, for a patient initially with a CD4 count of 500 cells/mm^3, the co-factor raises the underlying CD4 count at seroconversion to 804 cells/mm^3 on average whereas for a patient with only 200 cells/mm^3 at seroconversion the therapy raises the CD4 count to only 406 cells/mm^3 on average. The size of this effect is chosen so that the unadjusted co-factor relative hazard is large and that the effects of adjustment on the relative hazard can easily be seen.

Unadjusted for the CD4 count (top half of Table 7.4) this co-factor appears to lead to a 31% reduction in the hazard of AIDS. After adjusting for the CD4 count, either with mild (k=1/4) or moderate (k=1) variability, an increase in the relative hazard estimate is seen. The 90% range of the relative hazard estimates includes one in each case. However, of

interest is the finding that the median relative hazard estimate is now greater than one, suggesting a detrimental effect of the co-factor after adjustment for the CD4 count. When heavy variability (k=4) is added to the CD4 count, the results remain heavily biased with both the adjusted relative hazard estimate and the upper limit of the 90% range remaining below one.

Table 7.4 : The effect of variability in the measurement of the CD4 count on the relative hazard estimate of a co-factor which improves the immune status of patients by (i) raising the CD4 count at seroconversion and (ii) slowing the rate of CD4 decline. CD4 counts are measured monthly and the proportion missing depends on the underlying CD4 trend

Effect of co-factor		Variance is multiplied by :	Median estimate of relative hazard	90% range of hazards
Raise CD4 count at seroconversion	Unadjusted co-factor effect	-	0.69	0.54 - 0.86
	Adjusted co-factor effect	1/4	1.20	0.98 - 1.51
		1	1.11	0.96 - 1.33
		4	0.81	0.68 - 0.99
Slow rate of CD4 decline	Unadjusted co-factor effect	-	0.68	0.53 - 0.84
	Adjusted co-factor effect	1/4	0.92	0.75 - 1.11
		1	0.87	0.72 - 1.01
		4	0.73	0.61 - 0.88

For the second scenario, the co-factor is assumed to slow the rate of $\sqrt{CD4}$ decline by, on average, 0.5 units per year. Individual responses vary around this normally with standard deviation 0.25 units. Unadjusted for the CD4 count, this co-factor again appears to reduce the hazard of AIDS considerably. After adjustment for the CD4 count, with either light or moderate variability added, the relative hazard estimates still overstate the residual co-factor effect. However the 90% ranges include one suggesting that in many cases the correct conclusion regarding the effect of the co-factor would be reached. After adjustment for the CD4 count with heavy variability, however, the relative hazard estimate still suggests a large residual co-factor effect and the upper limit of the 90% range remains below one.

7.6.2 A second laboratory marker

Very often the prognostic values of other laboratory markers, which may be correlated with the CD4 count, are of interest. These markers are often measured at the same timepoints as the CD4 count. For this analysis I will assume that a second laboratory marker is measured perfectly and values will be missing whenever the CD4 count is missing. The second marker in itself is assumed to have no independent effect on AIDS-free survival. However, any correlation between this marker and the underlying CD4 trend will result in apparent prognostic value for the development of AIDS in analyses where the CD4 count is not adjusted for. After adjustment for the CD4 count, however, no residual effect on AIDS-free survival should remain.

The top section of Table 7.5 shows the results for a laboratory marker which is uncorrelated with the CD4 count. Adjusting for the CD4 count would not be expected to have any great effect on the estimate of its relative hazard. Unadjusted for the CD4 count this laboratory marker has no prognostic value for the development of AIDS. After adjustment there are no consistent patterns to the relative hazard estimate and any differences are likely to be due to random variation rather than any other effect. The range of estimates is wide, however, both before and after adjusting for the CD4 count, which is again most likely a function of the variability introduced when generating 200 different data sets for each analysis. I will address this issue in the Discussion.

The middle section of Table 7.5 shows the results for the situation where the second laboratory marker is moderately correlated with the CD4 count (correlation coefficient of between 0.3 and 0.4 at each time point). For this and the following analysis, the marker was generated by adding a random amount to the underlying CD4 trend; the larger this amount the smaller the correlation between the two markers. In a univariate analysis, it appears that a one unit drop in the second laboratory marker is associated with an 8% increase in the hazard of developing AIDS. Adjustment for the measured CD4 count, either when light or moderate variability is added, brings the relative hazard estimate down towards one. However, with heavy variability (k=4) the relative hazard estimate and the lower limit of the 90% range both remain above one, suggesting that there is a residual marker effect.

When the two laboratory markers are more highly correlated (correlation coefficients of 0.7 to 0.8), the results are more heavily biased (bottom section of Table 7.5). Unadjusted for the CD4 count, a one unit drop in the laboratory marker appears to be associated with a 42% increase in the hazard of developing AIDS.

When the CD4 count is measured with very little variability, the adjusted relative hazard correctly falls towards one. After adjustment for the CD4 count with either moderate or heavy variability, however, the relative hazards and lower limits of the 90% range remain above one, suggesting that there is at least an 7-32% increase in the hazard of AIDS per unit increase in the marker, even after adjustment for the CD4 count.

Table 7.5 : The effect of variability in the measurement of the CD4 count on the relative hazard estimate of a second laboratory marker which is (i) uncorrelated with the CD4 count, (ii) moderately correlated with the CD4 count and (iii) highly correlated with the CD4 count, but which does not in itself have any independent effect on AIDS-free survival. The CD4 count and the second laboratory marker are measured monthly and the proportion of both markers which are missing depends on the underlying CD4 trend

Correlation with CD4 count		Variation is multiplied by :	Median estimate of relative hazard	90% range for estimates
None	Unadjusted	-	1.05	0.37 - 3.09
	Adjusted for CD4 count	1/4	1.02	0.43 - 2.65
		1	0.94	0.39 - 2.58
		4	1.00	0.42 - 2.54
Moderate	Unadjusted	-	1.08	1.06 - 1.10
	Adjusted for CD4 count	1/4	1.00	0.99 - 1.02
		1	1.02	1.00 - 1.03
		4	1.06	1.05 - 1.08
High	Unadjusted	-	1.42	1.37 - 1.48
	Adjusted for CD4 count	1/4	1.01	0.97 - 1.06
		1	1.11	1.07 - 1.18
		4	1.37	1.32 - 1.43

7.7 Robustness of the results to model assumptions

Many assumptions about the underlying CD4 trend have been made when performing these simulations. These assumptions have been chosen to reflect current knowledge of the pattern of decline of the CD4 count throughout HIV infection, and have been influenced by findings from this cohort. Further, a number of assumptions have been

made to ensure computational simplicity and interpretability. As it appears that the pattern of HIV infection may be changing over time, it is of interest to assess whether the results from these simulations depends on some of these assumptions.

Tables 7.6, 7.7 and 7.8 show results from sensitivity analyses for the assumptions about the parameters of the fitted model. Table 7.6 shows the results of changing the distribution of the CD4 count at seroconversion. Both the mean and standard deviation of the CD4 count are in turn decreased and increased to illustrate situations where the underlying $\sqrt{CD4}$ count at seroconversion is, on average, lower or higher than that seen in our cohort, or where the underlying $\sqrt{CD4}$ count at seroconversion is more or less variable between individuals.

Table 7.6 : Robustness of methods to changes in the CD4 count at the time of seroconversion. CD4 counts are measured every month and the proportion missing depends on the underlying CD4 trend

Change in assumption		Variance is multiplied by :	Median estimate of relative hazard	90% range of estimates
Mean $\sqrt{CD4}$ at seroconversion	25.05	1/4	4.28	3.78 - 4.92
		1	2.76	2.47 - 3.06
		4	1.16	1.13 - 1.20
	30.05	1/4	4.23	3.74 - 4.81
		1	2.81	2.56 - 3.09
		4	1.19	1.15 - 1.23
	35.05	1/4	4.09	3.60 - 4.64
		1	2.80	2.55 - 3.05
		4	1.21	1.17 - 1.26
standard deviation of $\sqrt{CD4}$ at seroconversion	5	1/4	4.29	3.89 - 4.90
		1	2.81	2.56 - 3.08
		4	1.18	1.15 - 1.22
	7.22	1/4	4.23	3.74 - 4.81
		1	2.81	2.56 - 3.09
		4	1.19	1.15 - 1.23
	10	1/4	4.04	3.52 - 4.64
		1	2.70	2.49 - 3.05
		4	1.20	1.17 - 1.25

Table 7.7 shows the results of analyses for the underlying rate of √CD4 decline over time. Again, both the mean and standard deviation of the rate of √CD4 decline have been changed.

Table 7.7 : Robustness of methods to changes in the rate of CD4 decline. CD4 counts are measured every month and the proportion missing depends on the underlying CD4 trend

Change in assumption		Variance is multiplied by :	Median estimate of relative hazard	90% range of estimates
Mean rate of √CD4 decline	-1.58	1/4	4.44	3.94 - 4.98
		1	2.87	2.64 - 3.23
		4	1.19	1.15 - 1.24
	-1.88	1/4	4.23	3.74 - 4.81
		1	2.81	2.56 - 3.09
		4	1.19	1.15 - 1.23
	-2.18	1/4	4.32	3.90 - 4.87
		1	2.78	2.54 - 3.04
		4	1.17	1.14 - 1.21
standard deviation of √CD4 decline	1	1/4	4.25	3.78 - 4.81
		1	2.70	2.49 - 2.98
		4	1.15	1.12 - 1.19
	1.44	1/4	4.23	3.74 - 4.81
		1	2.81	2.56 - 3.09
		4	1.19	1.15 - 1.23
	2	1/4	4.37	3.99 - 5.01
		1	2.91	2.69 - 3.16
		4	1.20	1.17 - 1.25

Table 7.8 shows the results when the distribution of the CD4 count at AIDS diagnosis is changed. Three different distributions have been considered. The first situation forces the overall CD4 count at AIDS diagnosis to be lower, with a 0.5 probability of AIDS developing in the range 0-50 cells/mm^3, 0.4 probability whilst the CD4 count is in the range 51-100 cells/mm^3 and a 0.1 probability whilst the CD4 count is in the range 101-150 cells/mm^3 (mean count at AIDS 55 cells/mm^3, standard deviation 33 cells/mm^3). AIDS is assumed not to develop at higher counts.

The second situation assumes a higher CD4 count at the time of AIDS on average, with the probabilities of AIDS developing in the five CD4 ranges of 0.1, 0.3, 0.4, 0.15 and 0.05 respectively (mean 114 cells/mm^3, standard deviation 53 cells/mm^3). Finally, the situation where the probability of developing AIDS in each range is 0.2 is considered (mean 130 cells/mm^3, standard deviation 78 cells/mm^3).

Table 7.8 : Robustness of methods to changes in the CD4 count at the time of AIDS diagnosis. CD4 counts are measured every month and the proportion missing depends on the underlying CD4 trend

Change in assumption	Probabilities	Variance is multiplied by :	Median estimate of relative hazard	90% range of estimates
CD4 counts at AIDS	0.5, 0.4, 0.1, 0, 0	1/4	12.11	10.12 - 14.67
		1	4.65	4.13 - 5.34
		4	1.22	1.18 - 1.28
	0.1, 0.3, 0.4, 0.15, 0.05	1/4	5.35	4.57 - 6.18
		1	2.97	2.71 - 3.24
		4	1.19	1.15 - 1.23
	0.2, 0.2, 0.2, 0.2, 0.2	1/4	3.18	2.94 - 3.42
		1	2.36	2.21 - 2.52
		4	1.17	1.13 - 1.20

The choice of the CD4 count at seroconversion and the subsequent rate of decline throughout infection have little effect on the relative hazard estimate. However, the choice of the distribution for the CD4 count at AIDS diagnosis does have a large impact on the relative hazard estimate, with values being higher when the distribution of CD4 counts at AIDS is lower and more concentrated. In all cases, whilst the relative hazard may be higher or lower as a result of the different assumptions regarding the relationship between the CD4 count and the development of AIDS, the effect of increasing variability on these relative hazard estimates is unchanged and the addition of a higher level of variability leads to a reduced relative hazard estimate in each case.

7.8 Discussion

Despite the introduction of new laboratory methods and quality control schemes to standardise methods, the CD4 count remains an imperfectly measured marker. The problem of measurement variability in the CD4 count is of particular concern because it is the gold-standard marker against which all others are compared. In this chapter I have shown that as the variability in the CD4 count increases, the relative hazard estimate for the CD4 count drops quite considerably. The effect of missing values on this estimate are small in comparison and do not really become apparent until a large proportion of values are missing. In the presence of variability in the CD4 count, erroneous conclusions may be drawn about the value of other covariates in the model, whether they are measured with error or not.

Much work has already been carried out documenting the variability in the CD4 count[306,317,326]. Indeed clinicians now prefer to take confirmatory readings before making clinical decisions. However, the implications of this variability have not yet been transferred to the research setting where single CD4 measurements, or serial measurements over time are frequently used. For example, the CD4 count has been suggested as a possible surrogate marker for the development of AIDS in clinical trials. Prentice's criteria for surrogate endpoints in clinical trials[404] state that the CD4 count should predict the development of AIDS. Further, it should totally explain any treatment's effect on the development of AIDS, ie. after adjustment for the CD4 count there should be no residual treatment effect. Recently, in the AIDS Clinical Trials Group (ACTG) 019 randomised controlled trial of zidovudine versus placebo[405] it was suggested that the CD4 percentage was not a good surrogate marker as it did not explain all of the treatment's effect on the development of AIDS. The results in this chapter illustrate that even if a co-factor, or equivalently a treatment effect, acts solely through the underlying CD4 count, the measured count may not explain all of this effect and qualitative conclusions about the effect of the co-factor may be wrong. Under Prentice's definition of a surrogate marker, therefore, the measured CD4 count will not be a good surrogate marker for the development of AIDS. As almost all laboratory measurements are subject to some variation, either due to measurement imprecision or due to natural biological effects, this casts doubt on whether a laboratory marker could ever meet Prentice's stringent criteria and therefore be used as a surrogate marker for the development of AIDS. It may be necessary to adapt the currently used definition of a surrogate marker in order to allow for the effects of variation.

The implications of the results in this chapter for other laboratory markers are also potentially serious. The results presented do not suggest that these other variables should not be used as prognostic markers. In this case it is the relationship between the measured CD4 count and disease progression which is of primary interest. However, the care should be taken when using the results to search for pathogenic mechanisms by which other markers may have an effect on disease progression. In this situation it is the relationship between the underlying CD4 trend and disease which is of interest and this is not the same as the relationship between the measured CD4 count and disease progression. A correlation of 0.7-0.8 between the CD4 count and a second laboratory marker may seem rather high. However, correlations of this level are not uncommon in HIV infection. In this cohort, both the IgA level and the CD8 count are correlated with the CD4 count. Therefore, these results may be pertinent in these analyses.

The effect of measurement variability on a single covariate is well documented[396,397]. The loss of information which results from measurement errors reduces the precision of parameter estimates as well as the power of statistical tests about these parameters[403]. When more than one variable is included in the model incorrect conclusions may be drawn about all covariates even if many of them are measured perfectly[406,407], with the degree and direction of the bias on other covariates in the model depending on the strength of correlation between these covariates and the CD4 count[402]. Where models include covariates which are highly correlated the contribution of individual covariates is hard to distinguish even in the absence of variability[407,408]. In these cases correction methods are unlikely to give the correct estimates of coefficients in the model.

Many of the proposed correction methods for parameter estimates require knowledge of the conditional distribution of the true values of the covariate given the measured values[399]. In practice, however, this information is rarely available although it may be possible to use repeated measurements at each time point to get some idea of this distribution[406]. The process of estimating this distribution may also, however, influence the precision of the estimates for the parameters of interest[402]. An alternative approach which does not require knowledge about the distribution is to reduce the level of variability as far as possible. Repeated measurements may be taken at each visit and the average of these used in the model in place of the single measurement[409]. However, this may not be cost-effective or feasible. Whilst improvements in laboratory techniques may have a beneficial effect, it is likely that improvements in measurement will only reduce the variation to a certain degree. Hughes[328] suggested that the effect of measurement variability could be reduced either by following a large number of

individuals for a short period of time to ensure high levels of censoring or by selecting a more homogeneous population. However, in cohort studies designed to assess the long-term outcome of patients neither approach is practical.

A number of assumptions have been made when setting up the simulation model. The results appear to be fairly robust to changes in model parameters. However, other more fundamental assumptions should be validated. The underlying assumption in these simulation studies is that the CD4 count, or something closely related to it, is directly related to the development of AIDS. The effect of other factors which may not act through the CD4 count, such as the age of the patient and the development of either p24 antigenaemia or bacterial infections, are not considered to have an effect. However, it is believed that the results from this Chapter will remain valid even if the patients prognosis does not fully depend on the underlying CD4 trend. Simulation models which incorporate other factors could be investigated if desired. More complex variability structures could also be built into the model although it is unlikely that this will have a large effect on the resulting conclusions. The findings in this thesis regarding the prognostic value of some co-factors and laboratory markers for the development of AIDS should also now be questioned in light of the results in this chapter.

A more fundamental assumption made is that the underlying CD4 trend follows some smooth pattern throughout infection, in this case a linear decline in the square root. Deviations from this smooth pattern are assumed to represent random noise which has no effect on disease progression, ie. short-term fluctuations in the CD4 count are unrelated to disease progression and it is only the long-term change over time which is important. It may be the case, however, that some of this short-term variability may, in itself, be important for assessing prognosis. For example, a sudden increase in HIV viraemia may be associated with a short-term drop in the CD4 count. It is tempting to see this drop in CD4 count as random 'noise' and to disregard this as having no effect on disease progression. However, in doing so any direct or indirect effect of the virus on both the CD4 count and disease progression would be overlooked.

When using the real cohort data, the use of six-monthly mean values improved the fit of the model and increased the relative hazard estimate from that obtained using all routine CD4 counts. This suggests that much of the short-term variability is indeed unrelated to disease progression in this cohort. Running averages of the previous three values, however, did not improve the fit of the model. As described in Chapter 5, this is likely to be due to the fact that previous CD4 counts may have, in some cases, been measured a long time before the most recent count. This simulation model could be used to assess

the impact of different smoothing methods on the relative hazard. However, smoothing methods shown to be effective will be very dependent on the model specified, in particular on the choice of the distribution of the random variability around the underlying CD4 trend. Consequently, this is one aspect of variability which may be better studied in real rather than simulated data.

In carrying out the simulations presented in this Chapter, separate data sets of 500 individuals were generated 200 times for each analysis. The rationale behind generating separate data sets was to remove any reliance of the results on any particular data set generated. However, the generation of separate datasets does introduce a further level of random variability. This is particularly noticeable in Table 7.5 where the 90% range of estimates for the marker effect are in some cases very wide. This level of variation could be eliminated by generating only one data set consisting of a much larger number of individuals and then using this one data set for all analyses. However, as the number of individuals in the data set increases, there is a large increase in the time required to calculate the relative hazard estimate from the proportional hazards model. Whilst I plan to consider the effect of eliminating this extra level of random variability in the future, only the first option, of using 200 smaller data sets for each analysis, has been presented in this thesis.

CHAPTER 8 : CONCLUDING REMARKS

8.1 Summary of main findings

In this thesis I have presented the findings from an epidemiologic follow-up of a well-characterised cohort of 111 HIV-positive men with haemophilia registered at the Royal Free Hospital Haemophilia Centre. The men were infected with HIV between 1979 and 1985 after treatment with infected blood products and dates of seroconversion can be estimated for all of the men. As the men are followed closely for the development of HIV disease, they are an ideal group in which to study the progression of HIV disease and to identify factors associated with disease progression.

By 14 years after seroconversion to HIV, 47 of the men have developed AIDS and 45 have died, the majority of deaths (93%) being either directly or indirectly related to HIV infection. The median time to the development of AIDS in this cohort is now 13.1 years after seroconversion, somewhat longer than that reported in other cohorts of HIV-infected individuals. This may partly be due to the long follow-up time in these individuals but may also be a result of careful patient monitoring and the use of prophylaxis and zidovudine once the CD4 count falls to a low level. Prior to AIDS the vast majority of patients (73.9%) have developed some manifestation of their HIV infection, the development of the first HIV-related event occurring an average of only 6.8 years after seroconversion. These conditions may include the development of bacterial infections, skin complaints, thrombocytopenia, oral candida or herpes zoster.

The CD4 lymphocyte count declines in a quadratic manner over time, with patients becoming increasingly likely to develop AIDS or die once their CD4 count has fallen to a very low level. The CD4 count has been confirmed as a good prognostic marker for the development of AIDS, death and minor clinical events in this cohort. The rate of CD4 decline provides some additional prognostic information, suggesting that individuals whose CD4 counts have dropped to low levels rapidly are more likely to develop disease than those whose counts have dropped to the same low levels less rapidly.

As patients included in the cohort are of a wide age range, this cohort offers a good opportunity to study the effect of age on disease progression. Disease progression, especially progression to death, is more rapid in individuals who are older at the time of seroconversion, and this age effect cannot totally be explained by more rapidly declining CD4 counts in these individuals. Patients who are thought to have seroconverted either

prior to 1981 or from 1983 onwards also have faster disease progression. Whilst improved clinical care for HIV-infected patients has undoubtedly resulted in a gradual lengthening of incubation periods over time, increased awareness of AIDS and changes in the AIDS definition may also have artefactually shortened incubation periods in some patients as clinicians diagnose patients with AIDS sooner.

The wide variation in incubation periods amongst patients in the cohort, even amongst those with similar CD4 counts, means that the search for additional markers of disease progression continues. With this in mind, a number of other routinely measured laboratory markers have been studied in this thesis. A drop in the CD8 lymphocyte count at first appears to provide prognostic information for HIV disease progression. However, at late stages of disease, patients with the lowest CD4 counts also tend to have low CD8 counts. Consequently, the CD8 count simply identifies these individuals. After adjusting for the CD4 count, the CD8 count does not, therefore, provide any independent prognostic information. As IgA levels rise throughout infection it appears that they may offer some additional prognostic information. Despite the finding that IgA levels are correlated with the CD4 count, and as such may be expected not to provide any independent information on prognosis, the IgA level does appear to be a useful prognostic marker for the development of clinical disease. The B2M level may also offer some additional prognostic information for the development of clinical disease when patients have relatively high CD4 counts, although it is debatable whether any additional information is provided at lower CD4 counts. The development of p24 antigenaemia, traditionally one of the first indications of HIV infection, heralds a deterioration in the patient's clinical condition, even after considering all other markers of disease progression. In addition, the development of bacterial infections is also a bad sign, with patients progressing more rapidly after the development of such conditions.

In general CD4 counts are measured every three to six months on patients in the cohort. However, their measurement has become more frequent as the value of the CD4 count has become recognised, and also as patients become ill and attend the Centre more frequently, resulting in irregular time intervals between measurements. Despite improvements in laboratory methods, the CD4 count is still an imperfectly measured marker and this may have implications for the value attributed to it as a prognostic marker. A simple model for the measured CD4 count has been presented in this thesis. The use of this model to simulate CD4 data for a large number of individuals has provided a simple way of studying the effects of both missing values and of variability on the relative hazard associated with a drop in the CD4 count. The findings in this thesis

could be applied to any other condition where a laboratory marker changes almost deterministically over infection and where the prognosis of the patients depends fully on this marker. As the CD4 count becomes more variable, the relative hazard estimate drops towards one. In the presence of a very poorly measured CD4 count, the value of the CD4 count may therefore be vastly underestimated and this may lead to incorrect conclusions about the role of this marker in HIV pathogenesis.

8.2 Implications of results and future plans

Before any new and potentially expensive laboratory marker is used for routine patient monitoring, it is important to establish that the information provided by this marker does not simply duplicate information already available from the CD4 count. The markers chosen for analysis in this thesis have already been shown to provide prognostic information in univariate models at fixed time points after seroconversion. However, the amount of additional information that each marker provides when all markers are measured regularly throughout HIV infection has rarely been studied. The results in this thesis suggest that IgA levels, B2M levels and the presence of p24 antigen should be measured routinely in HIV-infected patients. These markers could then be used in combination with the CD4 count, and possibly the rate of CD4 decline, to enable clinicians to make more informed decisions about the prognosis of the patient. For example, the possible long-term adverse effects and the development of resistance to antiretroviral therapies may mean that clinicians wish to delay the initiation of such treatments in patients with low CD4 counts who are clinically well and have relatively low IgA and B2M levels, and who are p24 antigen negative. However, clinicians may wish to consider starting such treatments sooner than usual in individuals who have higher CD4 counts, but who have very high IgA and B2M levels, who may have developed either p24 antigenaemia or a bacterial infection.

With its long incubation period, clinical trials which assess the value of new treatments for HIV infection are often constrained to follow a large group of patients for a long period of time, in order that the number of clinical endpoints (eg. the development of AIDS or death) is sufficiently large for comparisons to be made. The search for suitable surrogate endpoints in clinical trials is, therefore, of key importance. Unfortunately, it appears that the CD4 count is not likely to be useful as a surrogate marker on its own as treatment-induced rises in the CD4 count may not necessarily lead to clinical benefit to the patients. The combined use of the CD4 count, B2M and IgA levels and the development of p24 antigenaemia may improve on the CD4 count alone as a surrogate

endpoint for clinical trials. Further, these markers may also provide a means to restrict clinical trial entry to high-risk patients which may also lead to a reduction in the sample size required for such studies.

With increased follow-up on patients in the cohort it will be possible to further study trends in these prognostic markers throughout infection and to consider the impact of HIV therapies and prophylaxis on these markers. Unfortunately, new developments in laboratory methods mean that some of the markers studied in this thesis have now been superseded by other markers. For example, the development of p24 antigenaemia is now of less clinical importance as it has become possible to directly measure HIV viral load. Other virological markers, such as the development of SI phenotypes, and the number and type of different viral strains, may all be of use when determining patient prognosis. Rather than simply consider the CD4 or CD8 count, studies are now underway to identify whether particular subsets of these lymphocytes provide more prognostic information than others. If these markers are demonstrated to provide additional or better prognostic information than that provided by the CD4 count, then it is likely that the routine measurement of these markers in patients remaining alive in the cohort will commence. Where markers are measurable in stored serum, their value can be assessed in all patients in the cohort. However, in many cases, it will only be possible to consider the effect of these markers in living patients.

All haemophilic patients were co-infected with HCV after treatment with unheated clotting factor concentrates. The impact of coinfection with HCV on HIV disease progression is now of interest. As patients survive for longer with HIV they are increasingly likely to develop additional complications as a result of infection with HCV. So far, nine patients have died during liver failure in the cohort (including four patients who died of AIDS). Further, liver function tests are abnormal in the majority of patients who remain alive and AIDS-free. Hence, there is a real possibility of increased morbidity and mortality from HCV infection in these patients. Further, the effect of co-infection with other viruses, such as Epstein-Barr virus, varicella zoster virus, and human herpesviruses 6 and 7 is as yet unknown. It is planned to measure antibodies to these viruses in patients in the cohort in the near future.

In conclusion, therefore, this thesis has presented a selection of work carried out on this well-defined cohort of men with haemophilia. Many of the results presented may be generalised to other groups of HIV-infected patients and, as follow-up continues, it is hoped that the cohort will continue to provide answers to important clinical questions in the future.

APPENDIX I - LABORATORY METHODS

Antibodies to HIV

Antibodies to HIV were measured by enzyme immunoassay ('Wellcozyme', Wellcome Diagnostics, Dartford, UK). All sera screening positive for HIV antibodies were sent to a reference laboratory for confirmation. All were confirmed as positive, on the basis of the strong concordance between the results of testing sera by gelatin particle agglutination (Fujirebio, Tokyo, Japan as supplied by Mast Diagnostics, Liverpool, UK), antiglobulin immunoassay containing recombinant antigens (Abbott Diagnostics, Maidenhead, UK) and the original screening method.

CD4 and CD8 lymphocyte counts

Absolute CD4 and CD8 lymphocyte counts were calculated from the lymphocyte count and CD4/CD8% values which were measured as follows: Absolute lymphocyte counts were determined by an automated whole blood counter (Ortho 'ELT 800' with differential screen). Between 1982 and 1986 the percentages of CD4 and CD8 lymphocytes were counted in Ficoll-Hypaque separated blood mononuclear cell suspensions using an EPICS V flow cytometer (Coulter Electronics, Luton, UK). Since 1986 a whole blood lysis method has been used, and the percentage of CD4 and CD8 lymphocytes analysed by flow cytometry either using an EPICS V or FACScan (Becton Dickinson, Crawley, UK). A monoclonal CD4 antibody, RFT4, to the CD4 antigen, and a CD8 antibody, RFT8, to the CD8 antigen were used throughout, either singly, or since 1986 in double concentration with a monoclonal CD3 antibody (OKT3 or UCHT1). Flow cytometer quality control was monitored using Q.C. beads, and in the UK National External Quality Assurance Scheme (NEQAS).

IgA

IgA is measured using a commercial reagent from Boehringer Mannheim (Germany) and is measured on a Hitachi 911. Human IgA (Ag) in a sample reacts with anti-IgA antiserum (Ab) in a buffered solution to form Ag/Ab complexes. The complexes cause turbidity of the buffered solution. The turbidity is measured at 340 nm and is proportional to the IgA concentration. Polyethylene glycol is added to accelerate the Ag/Ab reaction.

p24 antigenaemia and antibody levels

The presence of p24 antigen was measured by enzyme immunoassay (Abbott Diagnostics, Maidenhead, UK). p24 antibody levels were measured using a kit from Wellcome Diagnostics (Dartford, UK).

Beta-2 microglobulin

A commercial radial immunodiffusion (RID) method was used to measure B2M retrospectively in stored serum samples (NanoRID, Binding Site Ltd, Birmingham, UK) and monitored by QC controls and the UK NEQAS quality assurance scheme.

CMV

Antibodies to CMV were measured by radioimmunoassay for IgG antibodies to the virus using cell extracts from CMV infected fibroblasts (Behring Diagnostics, Germany)[410,411].

APPENDIX II - STATISTICAL METHODS

1 The Cox Proportional hazards model

1.1 Introduction

The Cox proportional hazards model has been used frequently when analysing the data for this thesis. Altman and de Stavola[412] and Andersen[413] have written very good reviews of the model and of some of the problems encountered when fitting the model. This section will give a short summary of the statistical ideas which form the basis of the model.

Let an individual i, i=1, 2, .., N, be observed from time 0 to a failure or censoring time, T_i, and let D_i be the corresponding censoring indicator for that individual which takes the value 1 if T_i is a failure time and 0 if T_i is a censoring time. It is of interest to study the relationship between the intervals $(0, T_i)$ and a set of covariates, $z_1, \ldots z_q$.

The hazard function, $\lambda_i(t)$, defines the probability that an individual i fails at time t, given that they have survived up to that point in time. The cumulative survival function at time t, $S_i(t)$, often estimated using the methods of Kaplan and Meier[42], expresses the cumulative probability of having survived to that point in time and is related to the cumulative hazard function, $H_i(t)$, by :

$$S_i(t) = \exp(-H_i(t)) \quad (1)$$

$$= \exp\left(-\int_0^t \lambda_i(u)\,du\right) \quad (2)$$

It is possible to make univariate comparisons of the effect of covariates on survival using the log-rank test[43]. However, it is often the independent effects of multiple covariates on survival that is of particular interest.

1.2 The basic model

The proportional hazards model was proposed by David Cox in 1972[414], who suggested that the hazard function could be expressed as

$$\lambda_i(t) = \lambda_0(t) \exp\{\beta_1 z_{i1} + \beta_2 z_{i2} + \ldots\ldots + \beta_q z_{iq}\} \quad (3)$$

or, in vector form

$$\lambda_i(t) = \lambda_0(t) \exp(\beta \mathbf{z}_i) \quad (4)$$

where $\lambda_0(t)$ is some baseline hazard function, $\mathbf{z}_i$ is the vector of the covariate values z_{ij}, j=1,...,q, measured on individual i at baseline (at time zero) and β is the vector of unknown model parameter values, β_j, j=1,....,q, which are to be estimated.

The ratio of the hazards for two individuals i and k with covariate values z_{ij} and z_{kj} respectively, is therefore given by :

$$\lambda_i(t)/\ \lambda_k(t) = \exp\{\beta(\mathbf{z}_i - \mathbf{z}_k)\} \qquad (5)$$

This is known as the relative hazard and, since it is independent of t, the value is constant over time. The hazards for the two individuals are therefore proportional. This value is also independent of the baseline hazard, $\lambda_0(t)$.

When there are no ties among the failure times, the estimation of the parameters β_i, j=1,....,q, is performed by maximising the partial likelihood L(β), given by:

$$L(\beta) = \prod_i \left[\frac{\exp(\beta \mathbf{z}_i)}{\sum_{k \in R_i} \{\exp(\beta \mathbf{z}_k)\}} \right]^{Di} \qquad (6)$$

where R_i is the set of all subjects at risk at each event time, T_i, and D_i is the censoring indicator. Each element in the likelihood is the probability that an individual, i, fails at time T_i , given that only one individual fails at T_i. Where tied event times occur an approximation suggested by Peto is often used[415].

1.3 The time-updated model

The above model is based on covariates measured at baseline. Hence, relative hazard estimates are expressed in terms of each individual's baseline value of the covariates only. However, many covariate values change over follow-up and repeated measurements are often available for analysis. These covariates are known as 'time-updated' or 'time-dependent' covariates. The Cox model can be extended to incorporate such covariates.

If

$$\lambda_i(t) = \lambda_0^{u}(t) \exp(\beta^{u} \mathbf{z}_i(t)) \qquad (7)$$

where $\mathbf{z}_i(t)$ are the updated covariate values for subject i at time t. Estimation of the parameters β^u is carried out again by maximising the log of the partial likelihood, with equation (6) re-expressed as :

$$L(\beta) = \prod_i \left[\frac{\exp(\beta^u z_i(t))}{\sum_{k \in R_i} \{\exp(\beta^u z_k(t))\}} \right]^{Di} \quad (8)$$

Estimates of the baseline hazard function, $\lambda_0{}^u(t)$ can be made if desired[43,416]. However, as these estimates are usually based only on the baseline values of the covariates the estimate of this hazard is of little relevance when considering time-updated covariates.

1.4 Confidence intervals and testing for significance

In large samples the distribution of the parameters β can be approximated by a normal distribution with mean, variances and covariances which can be estimated from the second derivative of the log of $L(\beta)$. Hence confidence intervals and hypothesis tests for β can be performed in the usual way. Given a parameter estimate β_j and standard error for this estimate, a 95% confidence interval can be calculated as

$$\beta_j \pm 1.96 \times \text{s.e.}(\beta_j) \quad (9)$$

and the ratio of β_j to its standard error can be compared to a Normal distribution in order to test whether it is significantly different from zero.

1.5 Proportionality assumption

This model relies on the fact that the ratio of hazards in equation (5) is independent of time. This assumption can be tested, both for fixed and time-updated covariates, by incorporating the interaction between the covariate of interest and the log of time in the model[414]. If the parameter estimate for this interaction term is significantly different from zero then there is evidence that the relative hazards are not proportional over time. Alternatively, the total follow-up time can be split into intervals and the relative hazard estimated separately in each interval. These estimates can be visually inspected to see if the hazard appears to change over the different time intervals[412].

2 MODELS FOR REPEATED MEASUREMENTS

2.1 Introduction

Frequently, multiple measurements of the same marker are obtained from individuals at different time points. It may not be possible to control the circumstances under which measurements are taken and there may be considerable variation among individuals in

both the number and timing of observations. The resulting unbalanced data sets could be modelled using a general multivariate model. However, unless some restrictions are placed on the structure of the covariances between the repeated measurements on the same individual, a large number of parameters will be required and many of these will be poorly estimated.

Models which study this type of data by imposing some restrictions on the covariance structure have been proposed since the late 1970s[417-421]. Such models make no requirement of balance in the data, i.e. the number and timing of measurements can vary between individuals. Further, they usually allow for the explicit modelling and analysis of the covariance structure. Very often the covariance structure may be of interest in its own right. However, even if not, the modelling of a parsimonious error structure leads to more efficient parameter estimates.

Given an individual i, i=1,.....,n, with t_i repeated measurements of each variable j, j=1,....,p, the general formulation for these models can be given by :

$$\mathbf{y}_i = \mathbf{X}_i\beta + e_i \qquad (10)$$

where $\mathbf{y}_i$ is the vector of responses for the individual i, $\mathbf{X}_i$ is a (t_i x p) flexible design matrix, β is a (p x 1) vector of parameters and e_i is independently and identically distributed $N(\boldsymbol{\theta}, \Sigma_i)$. Σ_i describes the covariance structure for the individual i, i=1,.....,n, and can be written as a function of the parameters θ, i.e. $\Sigma_i = \Sigma_i(\theta)$ for i=1,...,n. The general formulation comprises of two parts, therefore, the regression part of the model in which β is estimated, and the model for the covariance structure, $\Sigma_i(\theta)$, in which θ is estimated. The specific form of the covariance structure chosen, and the interpretation of the resulting coefficients differs according to the type of model fitted.

This general structure includes marginal models, transition (or Markov) models and random or mixed effects models as special cases. In the case of marginal models the two stages of the model are modelled separately. The coefficients are interpreted as 'population averages', as if they had been estimated from a cross-sectional study. Transition models attempt to address both the regression part of the model and the covariance structure simultaneously in a common equation. Finally, in random-effects models the probability distribution for the multiple measurements has the same form for each individual, but the parameters of that distribution, β, vary over individuals. It is this class of models which are of most relevance for the data contained in this analysis, as repeated measurements of many laboratory markers are available for each patient in the

cohort and it is important to consider the within-person changes of these markers over time. Both growth curve analyses[422] and repeated measures analyses could be modelled as special forms of a general random effects model.

The first random effects models were proposed by Laird and Ware in 1982[417]. In this paper, the general model was reformulated as :

$$\mathbf{y}_i = \mathbf{X}_i\alpha + \mathbf{Z}_i\beta_i + e_{ij} \qquad (11)$$

where α is a (p x 1) vector of unknown population 'fixed' parameters and $\mathbf{X}_i$ is the (t_i x p) design matrix which links α to $\mathbf{y}_i$. β_i is a (k x 1) vector of unknown individual 'random' effects and $\mathbf{Z}_i$ is the (t_i x k) design matrix which links β_i to $\mathbf{y}_i$. The β_i are distributed as N(**0**, **D**) independently of each other, where **D** is a (k x k) covariance matrix. e_{ij} is distributed as N(**0**,$\mathbf{R}_i$), where $\mathbf{R}_i$ is a (t_i x t_i) covariance matrix with parameters which do not vary between individuals and is independent of the individual covariance matrix, **D**. Very often e_{ij} is given by the simple form of σ^2I, where I is the identity matrix. In other words, after taking account of the parameters α and β_i, measurements on the same individuals at different time points are independent. In this case the model is called a 'conditional-independence' model.

The estimation of the parameters from these models has to be performed iteratively, as both the covariance structure and the population parameters need to be estimated. Either maximum likelihood or restricted maximum likelihood methods were suggested, with the EM algorithm being proposed as a means of estimating these parameters[417]. However, at the time there was no simple computer package which enabled parameters to be estimated easily. As a consequence, the use of random effects models was limited. Goldstein[420] reformulated this random effects model as a two-level model in which level one was taken to be the within-individual level and level 2 was taken to be the between-individual level. He showed that the within-individual covariance, e_{ij}, could be allowed to be a function of individual patient characteristics. More recently, the availability of special software for the estimation of the parameters of the model has meant that the use of multi-level modelling is becoming more widespread. The software has recently been further extended to allow for more than two levels of data, allowing for a far more complex hierarchy of data to be modelled.

APPENDIX III : RECENT PUBLISHED PAPERS ARISING FROM RESEARCH ON ROYAL FREE HOSPITAL HAEMOPHILIA COHORT

Papers included

6. Sabin CA, Phillips AN, Lee CA, Elford J, Timms A, Bofill M, Janossy G 221
Beta-2 microglobulin as a predictor of prognosis in HIV-infected men with haemophila: a proposed strategy for use in clinical care.
Brit J Haematol 1994; **86**: 366-371.

7. Sabin CA, Pasi J, Phillips A, Elford J, Janossy G, Lee CA. 228
CD4+ counts before and after switching to monoclonal high-purity factor VIII concentrate in HIV-infected haemophilic patients.
Thromb Haem 1994; **72**: 214-217.

8. Sabin CA, Phillips AN, Lee CA, Janossy G, Emery V, Griffiths PD. 232
The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion.
Epidemiol Infec 1994; **114**: 361-372.

9. Phillips AN, Sabin CA, Elford J, Bofill M, Janossy G, Lee CA. 244
Use of CD4 lymphocyte count to predict long term survival free of AIDS after HIV infection.
BMJ 1994; **309**: 309-313.

1995

10. Sabin CA, Elford J, Phillips AN, Janossy G, Lee CA. 249
Prophylaxis for *Pneumocystis carinii* pneumonia: its impact on the natural history of HIV infection in men with haemophilia.
Haemophilia 1995; **1**: 37-44.

British Journal of Haematology, 1993, 83, 330–333

RAPID PAPER

The progression of HIV disease in a haemophilic cohort followed for 12 years

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Summary. A cohort of haemophilic patients who seroconverted to HIV-1 between October 1979 and July 1985 has been followed to 1 January 1992. The median age at initial seropositivity was 24 years with a range of 2–77 years. By January 1992, 38/111 (34%) had developed AIDS and 39/111 (35%) had died (four of liver failure including one hepatoma). Using Kaplan-Meier plots, the calculated progression to AIDS at 12 years is 45% (95% CI 31, 58): for age >25 years 63% (95% CI 45, 82), age <25 years 32% (95% CI 15, 48) $P=0{\cdot}0001$: CMV positive 68% (95% CI 48, 87) CMV −ve 20% (95% CI 8, 32) $P=0{\cdot}0009$. The 12-year progression rate to CD4 + 0·2 or AIDS is 66% (95% CI 55, 76). 21/34 (63%) of patients who are p24 antigen positive have developed AIDS compared to 17/77 (22%) who are p24 antigen negative ($=0{\cdot}0001$). 19/34 (56%) and 20/77 (23%) of those p24 positive and negative respectively have died ($P=0{\cdot}007$). Before antiviral and prophylactic treatment for asymptomatic patients there were nine AIDS cases in 3·84 years experience with CD4 + <0·05 (1/0·43 years) and since treatment, 10 AIDS cases in 18·22 years (1/1·8 years). Age, CMV status and p24 remain strongly predictive of disease progression. Treatment appears to reduce the incidence of AIDS.

We have previously described the natural course of HIV infection in a cohort of haemophilic patients infected with HIV-1 (Lee *et al.* 1989, 1990, 1991). The earlier use of zidovudine, the use of prophylaxis against *Pneumocystis carinii* pneumonia and the widespread use of anti-candida therapy appears to have led to a fall in the incidence of AIDS-defining conditions (Khoo *et al.* 1992). Thus the Centres for Disease Control (CDC) has proposed a new AIDS definition which would include people who already meet the 1987 AIDS definition (CDC 1987), as well as all adults and adolescents infected with HIV-1 who have a CD4 lymphocyte (CD4+) count of less than $0{\cdot}2\times10^9$ l (CDC, 1992). In this 12-year follow-up of a haemophilic cohort, we have described the progression of HIV-1 disease to AIDS. In order to describe progression independent of the effect of treatment, we have used as an endpoint the time at which treatment would normally be instigated in the cohort: that is the date on which a patient's CD4 count falls to $0{\cdot}2\times10^9$ l or on development of AIDS whichever event occurs first.

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PATIENTS

The cohort consists of 111 patients who seroconverted to HIV-1 between October 1979 and July 1985. The follow-up is now complete to 1 January 1992. The majority (109) of these patients were infected by unheated factor VIII concentrate, one patient received unheated factor IX concentrate and another with moderate von Willebrand's disease is thought to have had other risk factors. All patients are male and the median age at time of initial seropositivity was 24 years with a range 2–77 years.

METHODS

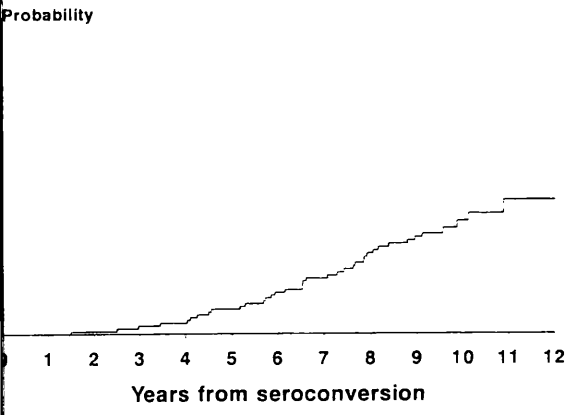
The investigation of T-lymphocyte subsets started in November 1982. Between 1982 and 1985 the subsets were determined on Ficoll–Hypaque separated mononuclear cells. Since then a whole blood method has been introduced (Bofill *et al.* 1992).

TREATMENT

In this cohort of haemophilic patients we have been using zidovudine in the treatment of AIDS and CDC IV disease since

Table I. First AIDS defining event in 38/111 HIV-infected haemophilic patients (1987 CDC definition).

PCP	18
Oesophageal candida	3
Toxoplasma	3
Lymphoma	3
HIV wasting	3
Cryptosporidia	2
Septicaemia	2
CMV infection	1
HIV encephalopathy	1
Aspergillosis	1
Chickenpox pneumonia	1



. Kaplan-Meier estimate of the rate of progression to AIDS. ored at death or December 1991.

ıst 1987. Treatment of asymptomatic patients began in mber 1988 when patients were recruited into the cal Research Council Concorde trial—a double blind bo-controlled trial of zidovudine. Our current protocol ıe treatment of asymptomatic individuals is to start anti- therapy with zidovudine, and prophylaxis with penta- ne or co-trimoxazole and fluconazole at a CD4+ count of 10^9/l.

ULTS

January 1992, 38/111 (34%) have developed AIDS the 1987 CDC definition (CDC, 1987) (Table I). Since troduction of prophylaxis for pneumocystis pneumonia in February 1989, one patient has presented with pcp first AIDS defining events. 38/111 (35%) patients have Hepatic failure was the primary cause of death in four of patients (including one hepatoma). A further three nts died in liver failure and thus the total deaths in liver e were 7/39 (18%). In 8/18 post-mortems the liver logy showed cirrhosis (44%).

e Kaplan-Meier (Kaplan & Meier, 1958) progression rate

Table II. Kaplan-Meier progression rates to AIDS (1987 CDC definition (1)) at 12 years of follow-up.

	Percentage	95% confidence intervals	
AIDS whole cohort	45	31–58	
AIDS age >25 years	63	45–82	$P=0{\cdot}0001$
AIDS age <25 years	32	15–48	
Relative risk (>25 years)	3·99	2·01–7·90	
AIDS CMV positive	68	48–87	$P=0{\cdot}0009$
AIDS CMV negative	20	8–32	
Relative risk (CMV positive)	3·3	1·57–7·01	
Relative risk (adjusted for age)	2·62	1·23–5·58	

to AIDS at 12 years is 45% (95% CI 31%, 58%) (Fig 1). Table II shows that progression to AIDS is speeded with increasing age at seroconversion and CMV seropositivity, as defined by the presence of IgG antibody to CMV. 21/34 (62%) patients who have been p24-positive on at least one occasion have developed AIDS compared with 17/77 (22%) who are p24-negative ($P=0{\cdot}0001$). 19/34 (56%) and 20/77 (26%) of those who are p24-positive and negative, respectively, have died ($P=0{\cdot}007$).

The Kaplan-Meier progression of time from seroconversion to reach either a CD4 count of 0·2 or AIDS was 66% (95% CI 55%, 76%).

Before the use of antiviral and prophylactic treatment for non-AIDS patients (November 1988) there were nine AIDS cases in 3·84 years of experience with a CD4+ $<0{\cdot}05\times10^9$/l (1/0·43 years); after the introduction of treatment for asymptomatic individuals 10 AIDS cases have occurred in 18·22 years of experience with a CD4+ $<0{\cdot}05\times10^9$/l (1/1·8 years). $P<0{\cdot}00001$.

DISCUSSION

It is clear from this 12-year follow-up that HIV-1 disease has a long and variable latent period—two-thirds of our cohort remain AIDS-free. Although over half of AIDS defining events were pcp, the occurrence of only one case of pcp since February 1989 reflects the success of prophylactic treatment (Simmonds *et al.* 1989). More recently because of the reported higher relapse rate of pentamidine compared to co-trimoxazole in secondary prophylaxis (Carr *et al.* 1992) we have been using co-trimoxazole where tolerated. Kaposi sarcoma does not appear to occur in this patient group.

It is significant that 18% of deaths were associated with liver disease and that at post-mortem 44% of liver histologies showed cirrhosis. It is likely that the predominant cause of this liver disease is chronic hepatitis C virus (HCV) disease. It has been shown that transfusion of unsterilized pooled plasma products to previously untreated patients (PUPS) invariably caused non A non B hepatitis or HCV hepatitis (Fletcher *et al.* 1983; Kernoff *et al.* 1985). Thus any individual who acquired HIV from unheated clotting factor concentrate must also have been infected with HCV. There is increasing

evidence that haemophilic patients with concomitant HIV and HCV infection are likely to be HCV viraemic as shown by per (Fedder *et al.* 1991) and that HIV may accelerate liver failure in HCV infected individuals (Eyster *et al.* 1992).

Age remains a powerful factor influencing progression. This was first demonstreated in a haemophilic cohort by Eyster *et al* (1987) and has been since confirmed by others (Darby *et al.* 1989; Goedert *et al.* 1989). The rate of decline of CD4+ count is similar in different age groups after the age of 10 years and it seems that there is a greater risk of developing AIDS among older people at a given low CD4+ count (Phillips *et al.* 1991).

This 12-year progression continues to show that patients positive for antibodies to cytomegalovirus progress to AIDS more quickly and that this effect is independent of age. Although this has been a consistent finding in our cohort (Webster *et al.* 1989, 1992) others have found in haemophilic patients that the apparent association of CMV and AIDS is confounded by age (Rabkin *et al.* 1992).

Detection of p24 antigen has been shown by others to be associated with poor prognosis (de Wolf *et al.* 1987). This has been a consistent finding in this cohort and we have demonstrated that the association between p24 antigenaemia and the rate of progression of AIDS can be largely explained by a more rapid decline in CD4 count among patients with p24 antigenaemia than those without (Phillips *et al.* 1991).

We have shown previously that prior to the use of therapy for asymptomatic patients, AIDS occurs on average at a CD4+ count $0{\cdot}05 \times 10^9$/l (Lee *et al.* 1989). In this 12-year follow-up, therapy has influenced the natural history of HIV disease: whereas previously one AIDS case occurred in 0·43 patient years with a CD4+ count $< 0{\cdot}05 \times 10^9$/l, now the length of time has extended to 1·8 years. This reflects the many patients presently existing with late-stage HIV-1 disease, manifested by a CD4+ count $< 0{\cdot}05 \times 10^9$/l, but AIDS-free.

The new AIDS definition proposed by CDC would include all individuals with a CD4+ count $< 0{\cdot}2$ (MMWR, 1992). This would recognize HIV infection as a chronic disease by using a marker of immune deficiency to move the AIDS diagnostic label earlier in the disease natural history (Chang *et al.* 1992). Patients would be diagnosed as having AIDS earlier: in this cohort the 12-year progression rate to the new definition, 66%, compares to 40% to the 1987 AIDS definition. Although the psychological and social effect of this early AIDS definition might not necessarily be desirable, the new definition could become of major economic significance in countries, including the U.K., where health care funding for a patient with HIV-1 infection is triggered by the diagnosis of AIDS.

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The incidence of HIV-related disease in a cohort of haemophilic men: natural history and changes since the introduction of pre-AIDS treatment

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Summary One hundred and eleven men with haemophilia at the Royal Free Hospital Haemophilia Centre, London, became infected with HIV between October 1979 and July 1985. This paper describes the incidence of HIV-related disease in this cohort in the absence of pre-AIDS treatment and assesses the effect of antiretroviral and prophylactic therapies on this. In particular the relationship between the CD4 count and the development of HIV-related disease is investigated. Before the introduction of pre-AIDS treatment in November 1988, 60 patients (54%) had developed some type of HIV-related pre-AIDS condition or AIDS, a cumulative incidence of 77% (95% Confidence Interval 63% to 91%) by nine years after seroconversion. The probability of developing such conditions was associated with falling CD4 lymphocyte counts and an estimated 51% of patients (95% Confidence Interval 38% to 63%) would be expected to develop some manifestation of HIV-related disease before their CD4 count has fallen to $0{\cdot}2 \times 10^9$/l. Consequently, only conditions which develop later in HIV infection (AIDS, oral candida, herpes zoster) are likely to be influenced by treatment. Since November 1988, there has been a reduction in these conditions, although the reduction in the rate of new AIDS cases was small ($P = 0{\cdot}16$). The risk of developing oral candida has been reduced at low CD4 counts from 12 cases in 76 years of experience below 0.2×10^9/l, prior to November 1988, to two cases in 81 years after November 1988, ($P = 0{\cdot}005$). Similarly, since that date no herpes zoster infections were observed in our cohort.

Keywords: haemophilia, CD4 counts, HIV, AIDS, oral candida, herpes zoster

People infected with HIV are likely to experience a number of less serious HIV-related symptoms and conditions before developing AIDS. Patients may suffer from skin problems, mild bacterial infections, diarrhoea, fever, weight loss

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and night sweats, amongst other complaints (Lang *et al.* 1987; Giesecke *et al.* 1988; Farizo *et al.* 1992). Whilst most of the conditions tend not to be severe, they are often recurrent and are frequently resistant to normal treatment (Tindall *et al.* 1988).

Many studies have considered the prevalence of these conditions, and progression rates to AIDS amongst patients suffering from them. Commonly studied symptoms include lymphadenopathy syndrome (Kaplan *et al.* 1987a; Kaslow *et al.* 1987), herpes zoster (Friedman-Kien *et al.* 1986; Melbye *et al.* 1987; Alessi, Cusini & Zerboni 1988), oral candidiasis (Klein *et al.* 1984; Greenspan & Greenspan 1988), hairy leukoplakia (Greenspan *et al.* 1987; Matis *et al.* 1987), and skin problems (e.g., seborrhoeic dermatitis) (Berger *et al.* 1988), some of which have been found to be predictive of a more rapid rate of progression to AIDS. In the past, papers which have assessed the prevalence of certain clinical conditions have often been based on follow-up of patients over a relatively short period of time (e.g., six months), or have estimated the prevalence rates at one particular point in time. Few studies have been carried out which have followed patients throughout infection, or have reported on the incidence and timing of these conditions at all stages of HIV infection. In particular, the link between these conditions and the CD4 lymphocyte count has only recently been considered (Lifson *et al.* 1991; Coates *et al.* 1992).

These conditions are distressing to patients, many of whom see them as a sign that they are progressing more rapidly to the stage at which they will develop AIDS. Since the end of 1988 a number of new treatment and prophylactic regimes have been introduced and are now generally available to patients with low CD4 counts who have yet to develop AIDS. These have included primary prophylaxis for some opportunistic infections (e.g. pentamidine or cotrimoxazole for *pneumocystis carinii* pneumonia (PCP) and fluconazole for candida) and general antiretroviral treatments for HIV infection, such as zidovudine and dideoxyinosine (ddI). Due to limited follow-up of patients receiving these therapies and to the inherent potential for biases associated with assessment of the treatment effect in observational studies (Gail & Mark 1992), little work has been carried out on the effect of such therapies on HIV-related conditions other than AIDS and overall survival. In a recent paper (Lee *et al.* 1991) we have assessed the effect of treatment on the development of AIDS. This paper describes the incidence of HIV-related conditions in a cohort of haemophilic men, and investigates the relationship between declining CD4 count and the development of these conditions. Finally, we estimate the effect that new treatments have had on HIV-related pre-AIDS conditions.

Methods

PATIENTS

The cohort has been described elsewhere (Lee *et al.* 1989, 1991; Phillips *et al.* 1991). In brief. 111 of the haemophiliacs based at the Royal Free Hospital

Haemophilia Centre became infected with HIV between 1979 and 1985, the point at which heat-treated clotting factor concentrate was introduced. These haemophilic patients have blood regularly stored, and as a result of this it is possible to estimate patients' dates of seroconversion (Lee *et al.* 1991). The patients are reviewed for clinical and laboratory assessment at least every six months at which time CD4 counts are measured. These counts have been measured since December 1982 up to the end of December 1991, the cut-off point for the analysis. A median of 16 CD4 counts have been carried out on each patient (range 1–40 counts).

From August 1987, zidovudine became available in the UK and to date 51 patients have been treated with the drug. This includes ten patients receiving active therapy in the MRC/INSERM Concorde trial of zidovudine *vs.* placebo in asymptomatic HIV-infected patients. Secondary prophylaxis with pentamidine for PCP began in March 1988 and primary prophylaxis with pentamidine for this infection began in February 1989. To the end of 1991, 26 patients have received primary PCP prophylaxis and 9 patients have received secondary PCP prophylaxis. Currently, all patients with CD4 counts of less than 0.2×10^9/l are given PCP prophylaxis and zidovudine. Since April 1988, patients with a CD4 count $< 0.2 \times 10^9$/l, have received fluconazole 150 mg weekly as secondary prophylaxis against candida (26 patients), and primary prophylaxis for this condition began in 1990 (13 patients). Such prophylaxis may also play a role in preventing seborrhoeic dermatitis where there is a fungal aetiology and other fungal infections such as cryptococcal infection. Prophylaxis for herpes virus infection is not currently prescribed in the cohort.

LABORATORY METHODS

Absolute CD4 counts were calculated from the lymphocyte count and CD4% values. These were measured as follows: Absolute lymphocyte count was determined by an automated whole blood counter (Ortho 'ELT 800' with differential screen). Between 1982 and 1986, the percentages of CD4 lymphocytes were counted in Ficoll-hypaque separated blood mononuclear cell suspensions using an EPICS V flow cytometer (Coulter Electronics). Since 1986, a whole blood lysis method has been used, and percentage of CD4 lymphocytes analysed by flow cytometry, using either an EPICS V or FACScan (Becton Dickinson) (Bofill *et al.* 1992). A monoclonal CD4 antibody, RFT4, to the p55 CD4 antigen was used throughout, either singly or, since 1986, in double concentration with a monoclonal CD3 antibody (OKT3 or VCHT1). Flow cytometer quality control was monitored using QC beads, and in the UK NEQAS external quality assurance scheme.

DATA COLLECTION

The HIV-related conditions considered in this paper have been grouped into five categories: mild bacterial infections (most commonly sinusitis, tonsillitis and

otitis), skin problems (serborrhoeic dermatitis and folliculitis), oral candida, herpes zoster (single and multidermal) and thrombocytopenia (platelet count $< 50 \times 10^9/l$). These symptoms and conditions were only included in the analysis if they were judged to have occurred as a result of HIV infection. In patients who developed AIDS, conditions were only included at their first occurrence, and if they had occurred before the diagnosis of AIDS. The diagnosis of AIDS was made according to the Centers for Disease Control classification (1987 revision).

STATISTICAL METHODS

The Lifetest procedure in SAS (SAS User's Guide 1985) was used to generate Kaplan-Meier plots of the probability of developing any condition by time from seroconversion, and according to the level of the patient's CD4 count (Phillips *et al.* 1992). The patient's first HIV-related event was defined as the date of either one of the listed conditions, or AIDS, according to which event occurred first. In both cases, patient follow-up was right-censored on death, and, in order to show disease progression independently of treatment, at the end of November 1988, if they had not experienced any condition or developed AIDS by that time. To assess whether the risk of developing pre-AIDS conditions, or AIDS, changed after the introduction of treatment, we considered the total patient years free of conditions after the CD4 count had fallen below a number of cut-off levels, and the number of times these conditions had been recorded below each CD4 cut-off level. The dates at which a patients CD4 count had first fallen below these levels was estimated by linear interpolation of the dates of CD4 measurements preceding and immediately after the patients count had fallen. The differences in rates before and after the introduction of treatment in November 1988 were then assessed for significance using standard procedures (Daly, Bourke & McGilvray 1991). When considering the incidence of any HIV-related event, the patient-years observation is censored at the date of the first event.

Results

INCIDENCE OF PRE-AIDS HIV-RELATED CONDITIONS AND AIDS BEFORE THE INTRODUCTION OF TREATMENT

To November 1988, 25 (23%) members of the cohort had developed AIDS and 16 members had died (14%); 9 deaths were from AIDS. Fifty-six patients (50%) had experienced at least one of the conditions studied, skin problems and oral candida being the most common complaints (Table 1). A further patient was diagnosed as suffering from persistent weight loss, without having developed one of the listed conditions. Most of those who experienced such conditions had developed only one type prior to development of AIDS. However, as this is likely to be related to the length of follow-up of the study, the number of patients suffering from more than one type of condition would be expected to increase with further follow-up.

Table 1. The incidence of early pre-AIDS HIV-related conditions prior to the introduction of therapy in November 1988

Condition	No. of patients	% of cohort
Skin complaints	22	19.8
Oral candida	18	16.2
Bacterial infections	16	14.4
Herpes zoster	11	9.9
Thrombocytopenia	7	6.3
AIDS	25	22·5
Any condition (including AIDS)	60	54.1

CUMULATIVE INCIDENCE OF CONDITIONS BY THE TIME FROM SEROCONVERSION AND BY THE CD4 COUNT

Figure 1 shows a Kaplan-Meier plot of the time from seroconversion to the occurrence of patients' first HIV-related event (pre-AIDS condition or AIDS). Of patients alive nine years after seroconversion, the probability of having experi-

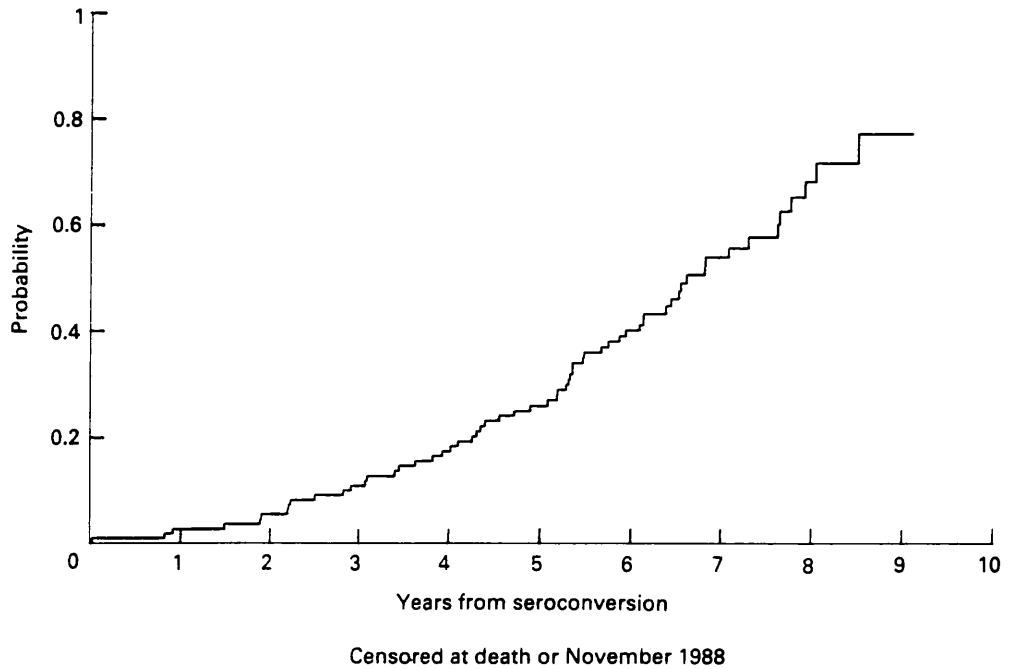

Figure 1. Kaplan-Meier plot showing the proportion of AIDS-free patients experiencing an HIV-related condition or AIDS in the absence of pre-AIDS treatment, according to the number of years from seroconversion.

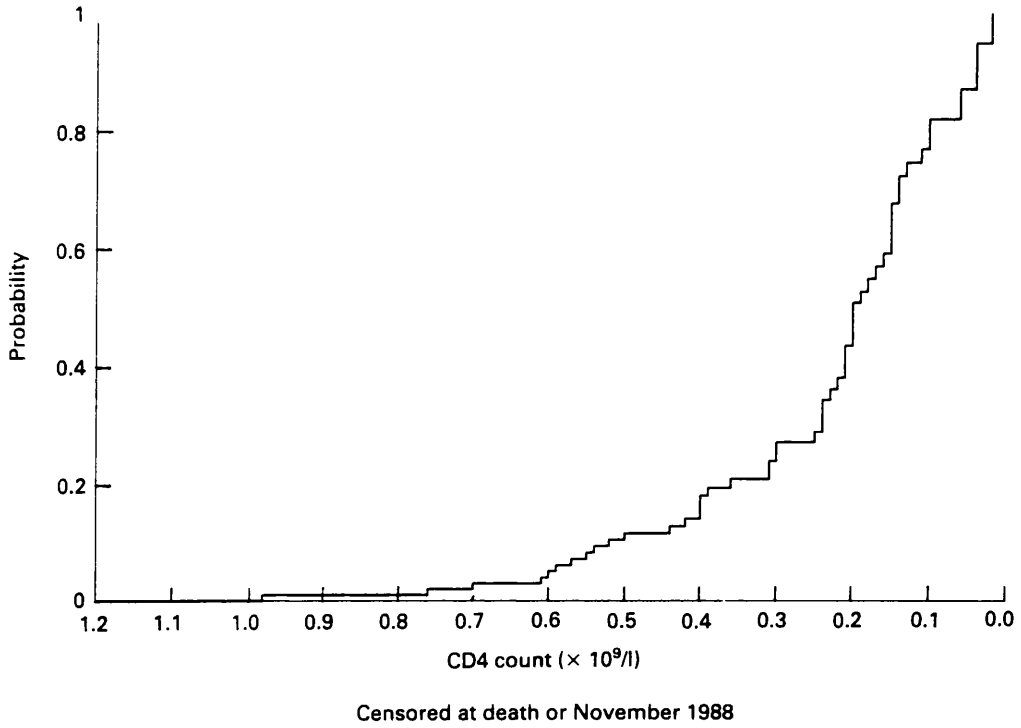

Figure 2. Kaplan-Meier plot showing the proportion of AIDS-free patients experiencing an HIV-related condition or AIDS in the absence of pre-AIDS treatment before the CD4 count has declined to a certain level.

enced an HIV-related event in the absence of pre-AIDS treatment was very high (77%, 95% CI (63%–91%)).

In a similar way to that above, a Kaplan-Meier plot of the time to the occurrence of the first HIV-related event was drawn, according to the patients' CD4 counts (Figure 2). The plot shows the probability of experiencing an HIV-related event, before the patient's CD4 count has fallen below any given level. Although there is only a 12% chance (95% Confidence Interval 5% to 18%) of experiencing an HIV-related event before the CD4 count has fallen below 0.5×10^9/l, this probability rises to 51% (95% Confidence Interval 38% to 63%) by the time the CD4 count has fallen to 0.2×10^9/l, and rises rapidly thereafter.

THE EFFECT OF TREATMENT AFTER NOVEMBER 1988

Table 2 shows the incidence rates of the first HIV-related condition (including AIDS) before the introduction of treatment in November 1988, after the patients' CD4 counts have fallen below certain cut-off levels. The increase in the incidence rate as the CD4 count declines can clearly be seen, with a much larger rate at CD4 counts below 0.1×10^9 l. Prior to November 1988, a total of 25 first HIV-related

Table 2. The incidence of the first pre-AIDS HIV-related event (including AIDS) before 30th November 1988

CD4 count	No.	Patient-years	Rate
> 0.5	10	131.2	0.08
$> 0.4, \leq 0.5$	5	70.1	0.07
$> 0.3, \leq 0.4$	7	43.5	0.16
$> 0.2, \leq 0.3$	13	49.9	0.26
$> 0.1, \leq 0.2$	16	41.3	0.39
$> 0.05, \leq 0.1$	5	3.5	1.41
≤ 0.05	4	1.3	3.02
Total	60	340.7	0.18

conditions were seen in 46.1 patient-years of follow-up below a CD4 count of 0.2×10^9/l (follow-up censored after first HIV-related event). After the introduction of treatment in November 1988, six conditions were seen in 15.9 patient-years of follow-up, indicating a marginal reduction in rates which is not statistically significant ($P = 0.43$).

At CD4 counts less than 0.2×10^9/l, there has been a significant reduction in the rates of both oral candida and herpes zoster after the introduction of treatment in November 1988 (Table 3). There has been a reduction in the rate of new AIDS cases since that date, although this reduction is not significant ($P = 0.16$).

Discussion

This study shows that pre-AIDS HIV-related conditions are a major problem in HIV-infection. Without treatment, an estimated 77% of patients will experience at least one HIV-related event by nine years after seroconversion, and hence

Table 3. The incidence of individual pre-AIDS HIV-related condition types first occurring at a CD4 count $\leq 0.2 \times 10^9$/l, before and after November 1988

	Before November 1988		After November 1988		
Condition	No.	Patient-years	No.	Patient-years	Relative risk
Skin complaints	10	68.34	5	71.96	0.47
Oral candida	12	75.98	2	81.00	0.16**
Bacterial infections	6	72.98	3	72.54	0.50
Herpes zoster	3	79.46	0	100.35	0.00*
Thrombocytopenia	3	83.31	2	97.06	0.57
AIDS	20	71.11	13	76.30	0.61

*Significant at 5% level. **Significant at 1% level.

the implications for patient management are great. Over half of patients infected with HIV would be expected to develop some manifestation of their disease before their CD4 count has fallen below 0.2×10^9/l and under most current treatment policies (i.e., the introduction of treatment at CD4 counts below 0.2×10^9/l), the introduction of pre-AIDS treatment would not be expected to affect this probability. It is therefore not surprising that the first HIV-related event (including AIDS) has not been significantly reduced after the introduction of treatment in November 1988. The conditions likely to be affected by the introduction of treatment are those which tend to occur at lower CD4 counts and later on in infection than those which are not affected. These include three conditions: (i) new AIDS diagnoses, (ii) oral candida, and (iii) herpes zoster. In all three conditions we have observed a reduced incidence since November 1988.

In the last year no new cases of oral candida have been seen amongst members of the cohort, an effect which may well be due to the introduction of fluconazole. It is also hoped that prophylaxis has reduced the number of patients developing oesophageal candida, although, as this has never been a common AIDS-defining condition in the cohort (one case prior to and two cases after the introduction of primary prophylaxis in April 1988), any increase in AIDS-free survival seen after November 1988 cannot be as a result of this.

There is a decrease in the incidence of AIDS after November 1988, at low CD4 counts, although this does not reach statistical significance due to the relatively short follow-up on patients after this date. The effect of PCP prophylaxis and zidovudine has been to delay an AIDS diagnosis in some patients, and, in the case of zidovudine, to raise the patient's CD4 count temporarily (Volberding *et al.* 1990). It has also been suggested that zidovudine, whilst affecting the absolute number of CD4 lymphocyte cells in peripheral blood, may improve CD4 lymphocyte performance (Miedema *et al.* 1992). The introduction of zidovudine may therefore be expected to reduce the incidence of HIV-related disease at each CD4 level in two ways. If the risk of developing AIDS at certain CD4 levels remains constant, then a rise in CD4 counts would obviously result in a lower incidence. In addition, if the CD4 cells remaining are functioning better, then the incidence of HIV-related disease would be expected to be reduced at equivalent CD4 levels. It is likely, therefore, that any increase in AIDS-free survival is attributable, at least in part, to the introduction of these therapies. Further studies assessing the relative importance of absolute numbers and functional performance of CD4 cells on HIV-related disease are essential for the targeting of new forms of antiretroviral therapies.

Our study is a prospectively followed cohort of patients in whom pre-AIDS therapy is instigated either at low CD4 counts or when the patients become symptomatic. As such the direct assessment of individual treatment effects is subject to many biases inherent in the design. For example, a direct assessment of the effect of zidovudine may show that patients treated had poorer survival than those not treated. This would simply reflect the fact that many patients receiving the drug were symptomatic and/or with low CD4 counts and therefore would have a shorter expected survival than patients with higher CD4 counts who

remained asymptomatic. Further, in order to receive zidovudine, patients had to have survived at least until the introduction of the drug in 1988, resulting in difficulty in interpreting the results from standard survival methods. At the time of starting zidovudine, many patients would also have started on other pre-AIDS therapies, and so the effect of zidovudine would be confounded with the effects of these. By using November 1988 as a proxy measure for the introduction of any pre-AIDS therapies we are simply measuring the combined effect of any therapy. Of course, the use of a proxy measure does not rule out the effect of other external factors which may also affect patient prognosis after 1988, for example, earlier AIDS diagnosis, better patient care or other changes in disease progression.

Estimates of the prevalence of various conditions from other studies vary greatly, mainly due to the differences in study designs. The cumulative incidence of conditions (excluding AIDS) ranged from 50%, 5–6 years after seroconversion (Giesecke *et al.* 1988) to 74% over a six month period of infection (Kaslow *et al.* 1987). These authors include lymphadenopathy amongst the conditions studied. Whilst persistent generalized lymphadenopathy syndrome affects a large proportion of HIV-infected individuals early on in infection, it is non-specific to HIV infection (Greenberg *et al.* 1992) and is not thought to be prognostic of AIDS, and was therefore not included in our symptom list.

The prevalence of individual conditions has also been studied by a number of authors. Reported prevalence rates of seborrhoeic dermatitis vary from 10% to 40% (Matis *et al.* 1987; Sindrup *et al.* 1987; Berger *et al.* 1988). Oral candida has been shown to occur in around 3–6% of HIV positive patients (Lang *et al.* 1987; Matis *et al.* 1987; Alessi *et al.* 1988), and the incidence rates of herpes zoster are also found to vary between 2 and 10% in these studies. Clearly there is a wide difference in estimates of incidence rates and this is mainly due to the different times from seroconversion when subjects are studied in these papers. The associations between CD4 counts and clinical conditions have not been studied in great depth by many authors, although Kaslow *et al.* (1987) have shown that oral candida is associated with CD4 counts below $0.25 \times 10^9/l$, and that thrombocytopenia is weakly associated with the CD4 count. Further, Kaplan *et al.* (1987b) have shown that of 118 men with a CD4 count below $0.1 \times 10^9/l$, only 15 had no cutaneous disorders.

It is uncertain how directly our results can be generalized to other groups of HIV-infected individuals (e.g., homosexual males, intravenous drug users, heterosexual males or females etc.). Some AIDS-defining conditions are more common in other groups and certain manifestations of the disease are known to be different, such as the occurrence of Kaposi's Sarcoma in homosexual men—a condition which was not seen in our cohort. Contrary to some other studies, we have diagnosed hairy leukoplakia only rarely, with none of these diagnoses being the primary one. In common with intravenous drug users, patients with haemophilia have the added problem of hepatitis C virus (HCV) related disease (Lee & Kernoff 1990). Deaths and morbidity due to HCV have not been defined as HIV-related in this study and have therefore not had a great effect on estimates of progression rates to AIDS or to the development of HIV-related conditions.

There is a need for resources to be made available for the large numbers of HIV-infected individuals who will develop HIV-related conditions in the future. The development of conditions may indicate that a patient is becoming increasingly immunocompromised. Their incidence is a particularly important sign if the CD4 count is not being regularly monitored. As hoped, the combined effect of treatment appears to have reduced the rates of oral candida and AIDS in patients with low CD4 counts. However, over half of infected patients are likely to develop HIV-related disease before their CD4 count has fallen below $0.2 \times 10^9/l$ and, as pre-AIDS treatment is rarely introduced in those with higher CD4 counts, this is unlikely to be affected by the introduction of treatment.

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CD8 lymphocyte counts and serum immunoglobulin A levels early in HIV infection as predictors of CD4 lymphocyte depletion during 8 years of follow-up

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Objective: To assess the ability of the CD8 lymphocyte count and immunoglobulin (Ig) A level, measured at the early stage of HIV infection when the CD4 lymphocyte count remains relatively high, to predict the future rate of CD4 lymphocyte loss and hence the risk of AIDS.

Design: Cohort of recently infected haemophiliacs with relatively high CD4 lymphocyte counts followed for up to 8.5 years from baseline measurement of CD8 lymphocyte counts and IgA levels.

Setting: A regional haemophilia centre based in a major teaching hospital.

Patients: Eighty-four of 111 patients with haemophilia who seroconverted to HIV between 1979 and 1985 in whom CD8 lymphocyte counts and IgA levels were measured soon after seroconversion (mean, 2.7 years; maximum, 5 years) while CD4 lymphocyte counts remained relatively high (median, 600 × 10^6/l; minimum, 300 × 10^6/l).

Outcome measures: Development of severe immunodeficiency defined by a CD4 lymphocyte count falling below 50 × 10^6/l, and AIDS.

Results: Individuals with high CD8 counts ($P < 0.008$) and high IgA levels ($P < 0.003$) at baseline experienced a more rapid rate of CD4 lymphocyte loss than those with low baseline levels. A score was derived to combine the predictive ability of CD8 count and IgA level. Estimated proportions with CD4 counts below 50 × 10^6/l after 8 years of follow-up were 100, 30 and 14% for those with high, intermediate and low baseline scores, respectively. The CD8/IgA score showed similar ability to predict the future occurrence of AIDS ($P < 0.0001$; log-rank test).

Conclusion: Immune activation seen in HIV infection, as reflected by raised CD8 counts and IgA levels, appears to be linked to the process of CD4 lymphocyte depletion. Measurement of these markers in the years following infection, when CD4 lymphocyte counts remain high, provides a first indication of a patient's long-term prognosis.

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Keywords: HIV infection, CD4 lymphocyte count, serum immunoglobulin A, CD8 lymphocyte count, immune activation, AIDS.

Introduction

The gradual loss of CD4 lymphocytes in HIV infection that typically occurs over several years is of fundamental importance in the pathogenic process [1–5]. The risk of serious disease is low before cell numbers in peripheral blood become severely depleted [1–6]. Indeed, differences in the rate of CD4 lymphocyte loss appear to explain the large variations in the interval after infection before the development of AIDS [7].

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Since the course of immunological decline tends to be slow, individuals typically have only moderately depressed CD4 lymphocyte counts within the first few years after HIV infection. At this early stage of HIV infection, little can be inferred about the time that will elapse before AIDS occurs; this is largely dependent on the rate of CD4 lymphocyte loss in the following years. Thus, it would be of considerable interest if markers could be identified at this early stage (when counts remain relatively high) that would indicate the likely future rate of CD4 lymphocyte loss.

Here we used data from a cohort of haemophiliacs followed for up to 12 years from serconversion, whose CD4 lymphocyte counts were monitored regularly to evaluate the predictive ability of two immune markers, the CD8 lymphocyte count and serum immunoglobulin (Ig) A levels. In HIV infection, high CD8 lymphocyte counts are associated with the accumulation of primed CD8 T lymphocytes that include cytotoxic effector cells [8,9], while raised IgA levels reflect B-cell activation [10]. Both CD8 counts and IgA levels were measured soon after seroconversion (mean, 2.7 years; maximum, 5 years) when patients' CD4 lymphocyte counts were only moderately diminished (median, 600 × 10^6/l). These individuals have been subsequently followed for up to 8 years to ascertain whether and when the CD4 count declined below 50 × 10^6/l. The time to development of AIDS was also studied.

Patients and methods

The cohort of 111 haemophiliacs and the methods for estimating our patients' dates of seroconversion have been described in detail [7,11,12]. All patients seroconverted between October 1979 and July 1985, and have been under the care of the Haemophilia Centre, Royal Free Hospital, London, UK. The median age at seroconversion was 24 years (range, 2–77 years). For this analysis, we considered follow-up information on patients until 1 January 1992. Since 1982, a series of CD4 and CD8 lymphocyte counts has been recorded for each patient by standard methods [7,13]. The median number of counts per patient was 16 (range, 1–40 counts).

Total serum IgA concentration was measured for 106 patients. The patients' baseline IgA value was the first serum concentration recorded after lymphocyte subset measurement had begun. When lymphocyte counts were not taken on the same day as the baseline IgA measurement, the CD4 and CD8 lymphocyte counts were estimated by linear interpolation of the two counts made immediately before and after the baseline IgA measure. Patients for whom baseline IgA was measured more than 5 years after seroconversion were not included in the analysis.

Treatment

Patients with AIDS were prescribed zidovudine from August 1987. None had received pre-AIDS treatment prior to November 1988. Since that time, 26 patients have been entered into the MRC/INSERM Concorde trial (zidovudine versus placebo in asymptomatic individuals), which is no longer blinded. Patients have also been prescribed open zidovudine and anti-*Pneumocystis carinii* pneumonia (PCP), and anti-*Candida* prophylaxis with pentamidine or trimethoprim-sulphamethoxazole and fluconazole, respectively.

Statistical methods

The Cox proportional hazards model [14] was used to determine the association between the baseline CD8 count and IgA concentration, and the rate of development of severe CD4 lymphocyte depletion, defined as at least one count below 50 × 10^6/l. The estimates of log relative hazard from the model were used as a basis for combining the two activation markers into a baseline CD8/IgA score. For each patient the CD8/IgA score is merely the log relative hazard for the CD8 count (0.00131) multiplied by their CD8 count plus the log relative hazard for IgA (0.56) multiplied by their IgA level. Thus, for a patient with a CD8 count of 900 × 10^6/l and an IgA level of 2.0 g/l we would have: CD8/IgA score = (0.00131 × 900) + (0.56 × 2.0) = 2.3.

Patients were grouped into three categories on the basis of their baseline CD8/IgA score. Kaplan–Meier estimates were used to estimate the percentage of patients with a CD4 count < 50 × 10^6/l in each category. The log-rank test was used to test for differences between groups.

When a CD4 count of 50 × 10^6/l was the outcome measure in the Cox proportional hazards model and the Kaplan–Meier estimates, patient follow-up was censored 6 months after the final CD4 count, if this timepoint was reached before death or 1 January 1993 (16 patients). The occurrence of AIDS was ignored. Although there has been a policy of more regular CD4 counting when patients have reached a count of 200 × 10^6/l, the development of AIDS did not result in more frequent CD4 count measurements.

Results

The current analysis was restricted to the 84 patients of the total 111 haemophiliacs for whom baseline CD4 counts were > 300 × 10^6/l, and the serum IgA concentrations were measured within a period of 5 years from seroconversion. The mean length of time from seroconversion of the baseline marker measurements was 2.7 years. For these 84 patients the median and range of serum IgA levels were determined together with the corresponding CD8 and CD4 counts at baseline (Table 1). During the follow-up period, CD4 counts of 22 (26%) of the patients declined below

Table 1. Three categories of CD8/IgA score and the association with the development of severe immunodeficiency and AIDS over 8 years' follow-up.

	CD8/IgA score			
	Low (< 1.5)	Intermediate (1.5–2.99)	High (≥ 3.0)	All
No. patients	24	49	11	84
Baseline median (range)				
CD4 count	505 (300–1180)	695 (310–1920)	650 (450–1000)	600 (300–1920)
IgA level (g/l)	1.2 (0.6–2.2)	2.0 (0.3–4.1)	3.1 (1.4–6.9)	1.8 (0.3–6.9)
CD8 count	420 (10–770)	910 (200–1670)	1300 (400–1920)	590 (10–1920)
No. with CD4 count < 50 during follow-up (%)	3 (13)	10 (20)	9 (82)	22 (62)
CD4 count below 50 by 8 years* (%)	14 (0–30)	34 (14–54)	100 (66–100)	47 (28–66)
No. with AIDS during follow-up (%)	3 (13)	12 (24)	9 (82)	24 (29)
AIDS after 8 years* (%)	14 (0–29)	30 (13–47)	100 (56–100)	41 (24–58)

*Kaplan–Meier estimate with 95% confidence interval. CD8/IgA score: (CD8 count × 0.00131) + (IgA × 0.56).

50 × 10^6/l. The Kaplan–Meier estimate for the proportion of patients whose CD4 count reached this level after 8 years is 47% (95% confidence interval, 28–66).

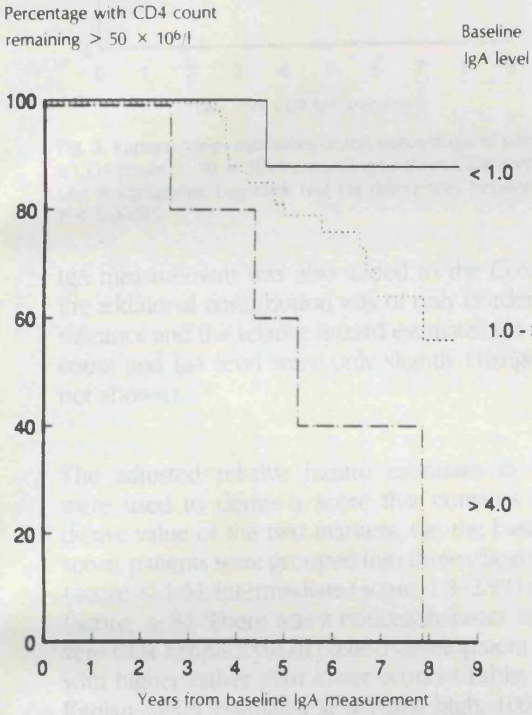

Fig. 1. Kaplan–Meier estimates of the percentage of patients with a CD4 count < 50 × 10^6/l according to three immunoglobulin concentration categories. Log-rank test for difference between groups, $P < 0.03$.

The ability of the baseline CD8 counts and IgA levels to predict the subsequent risk of rapid CD4 lymphocyte decline is shown in Figs 1 and 2. Those with higher CD8 counts and IgA levels experienced a more rapid rate of development of severe immunodeficiency. The unadjusted and mutually adjusted relative hazard estimates obtained from fitting the Cox proportional hazards model (Table 2) were statistically significant for both markers. The effect of neither marker is materially changed by adjustment for the other, suggesting that

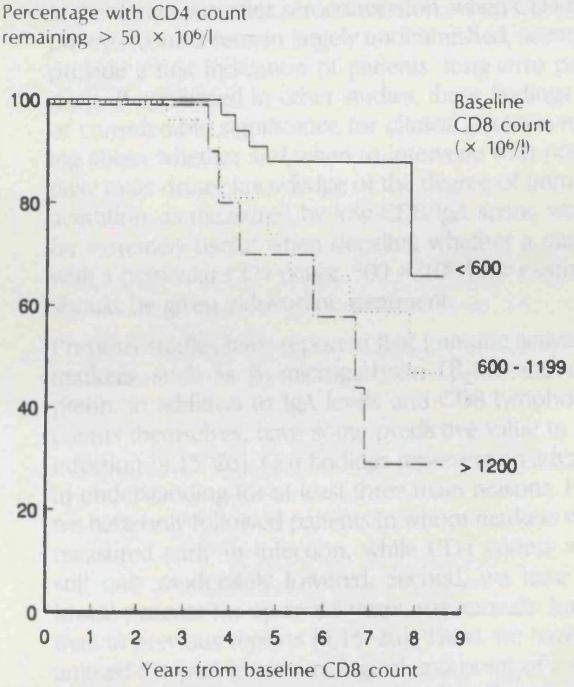

Fig. 2. Kaplan–Meier estimates of the percentage of patients with a CD4 count < 50 × 10^6/l according to three CD8 count categories. Log-rank test for differences between groups, $P < 0.03$.

Table 2. Relative hazard estimates indicating the predictive value of baseline immunoglobulin (Ig) A and CD8 counts for the development of severe CD4 lymphocyte depletion.

	Relative hazard	95% CI	*P*
Unadjusted			
IgA*	1.67	1.17–2.37	0.005
CD8 count†	1.12	1.02–1.24	0.016
Mutually adjusted			
IgA*	1.75	1.22–2.52	0.003
CD8 count†	1.14	1.04–1.25	0.008

*Per g/l; †per 100 × 10^6/l. CI, confidence interval.

each marker provides information useful for prediction over and above that provided by the other. When the CD4 count at the time of baseline CD8 count and

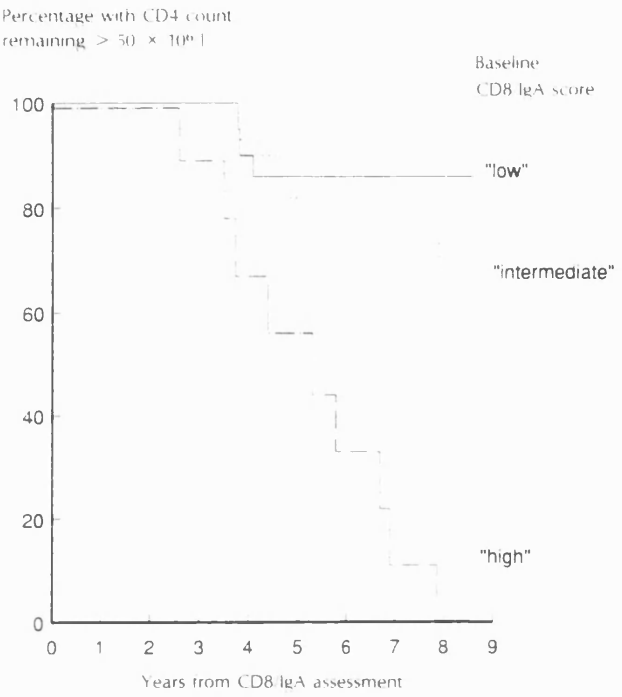

Fig. 3. Kaplan–Meier estimates of the percentage of patients with a CD4 count $< 50 \times 10^6$/l according to three CD8 immunoglobulin A categories. Log-rank test for differences between groups, $P < 0.00005$.

IgA measurement was also added to the Cox model, the additional contribution was of only borderline significance and the relative hazard estimates for the CD8 count and IgA level were only slightly changed (data not shown).

The adjusted relative hazard estimates in Table 2 were used to derive a score that contains the predictive value of the two markers. On the basis of this score, patients were grouped into three categories: low (score < 1.5), intermediate (score, 1.5–2.99) and high (score, ≥ 3). There was a noticeably faster rate of severe CD4 lymphocyte depletion development in those with higher rather than lower scores (Table 1, Fig. 3; Kaplan–Meier estimates at 8 years: high, 100%; intermediate, 34%; low, 14%; log-rank test, $P < 0.0001$). This was despite the fact that baseline CD4 counts were, on average, slightly higher in those patients with higher scores (medians: high, 650×10^6 l; intermediates, 695×10^6/l; low, 505×10^6/l).

All the analyses performed were also performed using counts of 100, and 200×10^6/l as end-points, instead of 50×10^6/l as presented above. The results were only slightly changed. In order to ascertain whether treatment might have influenced the results, analyses in which patient follow-up was censored at November 1988, the date at which pre-AIDS treatment began, were also performed. Again, the results were little changed.

Although CD4 lymphocyte loss was the principal focus of this analysis, we also considered the rate of AIDS development in the three CD8/IgA score groups. This rate was more rapid in those with higher scores (Table 1; Kaplan–Meier estimates at 8 years: high, 100%; intermediate, 30%; low, 14%; log-rank test, $P = 0.0001$).

Discussion

These findings indicate that immune activation seen in HIV infection, as reflected by raised CD8 counts and IgA levels, appears to be linked to the process of CD4 lymphocyte depletion. Measurement of these markers in the years soon after seroconversion, when CD4 lymphocyte counts remain largely undiminished, seems to provide a first indication of patients' long-term prognosis. If confirmed in other studies, these findings are of considerable significance for clinical decision-making about whether and when to intervene with potentially toxic drugs; knowledge of the degree of immune activation, as measured by low CD8/IgA score, would be extremely useful when deciding whether a patient with a particular CD4 count, 500×10^6/l for example, should be given zidovudine treatment.

Previous studies have reported that immune activation markers, such as β_2-microglobulin (β_2M) and neopterin, in addition to IgA levels and CD8 lymphocyte counts themselves, have some predictive value in HIV infection [4,15–26]. Our findings represent an advance in understanding for at least three main reasons. First, we have only followed patients in whom markers were measured early in infection, while CD4 counts were still only moderately lowered. Second, we have followed patients for up to 8.5 years; substantially longer than in previous reports [4,15–26]. Third, we have examined the purely immunological end-point of a CD4 count $< 50 \times 10^6$/l as well as the clinical end-point, AIDS. This has enabled us to illustrate not only that the CD8/IgA score predicts the rate of AIDS development but also, at least on one level, why it does so, i.e., because it predicts CD4 lymphocyte loss. In contrast, previous studies on the predictive value of serum β_2M and neopterin have suggested that the effects act 'independently' of the CD4 lymphocyte count [15–17,21]. In addition, although they are often grouped together as 'activation markers' in HIV infection, it is important to note that the measurement of IgA levels and CD8 lymphocyte counts cannot in any way be considered equivalent to measurement of β_2M or neopterin. Correlations between these are of the order of less than 0.2 [16].

Among those studies that have linked immune activation markers with future CD4 lymphocyte count decline, Hofmann *et al.* [27] and Melmed *et al.* [28] have shown that raised β_2M and neopterin levels are related to more rapid CD4 lymphocyte loss over 2.5 years, while Muñoz *et al.* [29] found that increased

IgA levels and CD8 counts, among other factors, were related to more rapid CD4 count decline over the following 6-month period. Salmon *et al.* also reported that IgA level predicted the rate of CD4 lymphocyte decrease over 18 months' follow-up [30]. These findings are consistent with our own, but our results further suggest that the association persists over a far greater length of time — for at least 8 years — during which CD4 cells are observed to decline from levels in the normal range for uninfected individuals [13] to a severely depleted state. Findings of inverse correlations between CD4 counts and both the levels of serologic activation markers and activated CD8 subsets in cross-sectional studies of HIV-infected individuals also support our finding [31]. As in other studies [4,25,26], raised levels of IgG and IgM seen in HIV infection have been shown to be less strongly predictive of prognosis than the IgA level (data not shown).

Our finding of more rapid CD4 cells loss in individuals with higher CD8 lymphocyte counts appears to be in conflict with observations that CD8 lymphocytes include HIV-specific cytotoxic T cells that control HIV replication [8,9,32]. Nevertheless, CD8 lymphocytes include phenotypically and functionally diverse subsets. It appears that the CD8 subsets associated with overt immune activation in HIV infection, including the CD38+ CD45RO+ subset, are particularly increased in number [31,33,34], revealing severe functional defects with failure to proliferate [35] and susceptibility to cell death and apoptosis [34,36]. Thus, the further dissection and quantitation of these populations may increase the prognostic significance of our observations. Similarly, the non-specific activation of Ig synthesis at the time of a severe deficiency of antibody responses may be further refined, for example, with the inclusion of other parameters such as IgD synthesis and pokeweed mitogen tests [37].

Although an association between early immune activity and more rapid loss of CD4 lymphocytes has been identified, further investigations are required before concluding that the immune system itself is responsible for the cell loss where the virus acts as a trigger for the destruction process [38–42]. For example, the raised CD8 and IgA levels may merely reflect enhanced HIV-specific immune responses in individuals with a higher viral load which, due to the direct cytopathic effect of HIV, leads to more rapid CD4 cell depletion. In addition, the fact that activated cells are more readily infected with HIV [43] could also contribute to immune activation leading to more rapid CD4 cell loss. Regardless of these uncertainties, the strength of the relationship presented in this paper suggests that HIV-induced immune activation (as reflected by raised IgA and CD8 lymphocyte levels) should be carefully studied by those seeking to understand how HIV decimates the CD4 lymphocyte population, since the phenomenon is unlikely to be far removed from the central cause or causes of CD4 lymphocyte loss.

In summary, the measurement of the CD8 count and IgA level in HIV infection provides an early indication of the subsequent rate of CD4 cell loss. These markers may thus be of considerable importance, both for clinical decisions over patient management, and for continuing efforts to understand the pathogenic pathways in HIV infection.

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Acquired Immunodeficiency Syndrome (AIDS) Risk in Recent and Long-standing Human Immunodeficiency Virus Type 1 (HIV-1)-infected Patients with Similar CD4 Lymphocyte Counts

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The loss of CD4 lymphocytes is known to be an important component of human immunodeficiency virus type 1 (HIV-1) pathogenesis. It remains unclear, however, whether the importance of the CD4 lymphocyte count is such that individuals who have been infected for widely different lengths of time, but for whom the CD4 lymphocyte count is the same, have the same risk of developing acquired immunodeficiency syndrome (AIDS). The authors directly addressed this question for 111 HIV-1-infected hemophiliacs who had been followed for up to 12 years from seroconversion and for whom a median of 16 CD4 lymphocyte counts had been made. Thirty-eight patients had developed AIDS by January 1, 1992. As of August 1, 1985, the time from seroconversion to AIDS ranged from 3 weeks to almost 6 years. The risk of AIDS increased with time from seroconversion (relative hazard = 1.25 per year; $p = 0.09$). CD4 lymphocyte count was strongly related to time from seroconversion and also to the risk of AIDS (relative hazard = 3.25 per 100/mm^3; $p < 0.00005$). In a bivariate Cox regression model, the relative hazard for time from seroconversion fell markedly towards unity (1.05; $p = 0.7$), while that for CD4 lymphocyte count remained unchanged. This suggests that time from seroconversion is a relevant factor in HIV-1 infection only insofar as it provides information as to the probable degree of loss of CD4 lymphocytes. If the number of these cells is known, time from seroconversion seems to be of little additional value in assessing the risk of AIDS. This finding has implications for the clinical assessment of individual patient prognosis, for the design of epidemiologic studies of HIV-1 infection, and for our understanding of how HIV-1 causes disease. *Am J Epidemiol* 1993;138:870–8.

acquired immunodeficiency syndrome; antigens, CD4; cell count; cross-sectional studies; HIV-1; lymphocytes

After a variable period of time—usually measured in years—infection with human immunodeficiency virus type 1 (HIV-1) leads to the development of severe immunodeficiency and, hence, various opportunistic infections and malignancies, the presence of any one of which leads to a diagnosis of acquired immunodeficiency syndrome (AIDS). Previous analyses from a cohort of HIV-1-infected men with hemophilia from

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Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1.

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the Royal Free Hospital in London, England (1), along with reports from several other studies (2–10), have suggested that the immunodeficiency is chiefly characterized by severe depletion of the number of circulating CD4 lymphocytes. This implies that individuals infected with HIV-1 many years ago have a similar risk of AIDS as those infected more recently, so long as their CD4 lymphocyte counts are the same. To our knowledge, however, this important question has not been directly addressed, principally because of the paucity of cohort studies in which dates of seroconversion have been reasonably well characterized and CD4 lymphocytes have been measured regularly.

In this paper, we present data from further analyses of the cohort of HIV-1-infected hemophiliacs, which has now been followed for up to 12 years and in which a median of 16 CD4 lymphocyte counts have been made per patient. We estimate the prognostic importance of the time from seroconversion to AIDS and assess the extent to which this is modified after controlling for CD4 lymphocyte count.

MATERIALS AND METHODS

Patients

The cohort, which has been described previously (1, 6, 11), consists of 111 HIV-1-infected men with hemophilia under the care of doctors at the Haemophilia Centre and Haemostasis Unit, Royal Free Hospital, London. All but two patients received unheated factor VIII concentrates: one was treated with unheated factor IX concentrate and the other with cryoprecipitate. The concentrates received by the patients have been described in more detail elsewhere (6).

Estimation of the dates of seroconversion

For 63 patients, the dates of their last negative and first positive anti-HIV-1 antibody tests were available. The date of HIV-1 seroconversion was estimated as the midpoint between these two dates (median difference between the dates, 11 months; 10th percentile, 41 days; 90th percentile, 21.5 months; range, 15 days–24 months). The earliest date of seroconversion was estimated as October 1979 (difference between dates of last negative and first positive tests = 7 days) and the last as July 1985 (difference between dates of last negative and first positive tests = 10.1 months). These dates approximately agree with data from other sources as to the period within which contaminated blood products were inadvertently administered (12). Within this range, patients' estimated dates of seroconversion followed an approximately uniform distribution (6). For 36 of the 48 patients for whom no anti-HIV-1 negative test result was available, the date of seroconversion was estimated as the midpoint between October 1979 (the presumed first possible date of infection) and the date of the first anti-HIV-1 test (median difference, 44 months; 10th percentile, 5.5 months; 90th percentile, 64.3 months; range, 1.5–67 months). This approach minimizes the maximum error that can be made in estimating the seroconversion date. For the remaining 12 patients, the first positive anti-HIV-1 test result appeared after the presumed last possible date of infection (July 1985), and the date of seroconversion was estimated as the midpoint between October 1979 and July 1985: September 1982 (difference = 68 months).

The analysis was also carried out after restricting the cohort to only those for whom the date of seroconversion was known within, at most, 6 months (i.e., the period of possible infection times was shorter than 12 months). The 49 individuals falling into this category comprised 39 subjects with a last negative test result appearing less than 12 months before the first positive test and 10 subjects without a negative test result but for whom the first positive test occurred within 12 months (i.e., before October 1980) of what was considered the earliest possible seroconversion date, October 1979.

Follow-up

We considered patient information collected up to January 1, 1992. The median length of follow-up from seroconversion to this date was 9.3 years. Eight patients were lost to clinical follow-up after moving to different hemophilia centers (see "Statistical methods" for censoring strategy). However, information on the clinical status of these patients is sought annually. A series of CD4 lymphocyte counts was recorded for each patient (1). The median number of counts was 16 (range, 1–40). The median age at seroconversion was 24 years (range, 2–77).

HIV-1-related treatment

Zidovudine has been used for the treatment of AIDS and Centers for Disease Control group IV disease since August 1987. The current protocol for the treatment of asymptomatic individuals is to start antiretroviral therapy with zidovudine and prophylaxis with pentamidine or cotrimoxazole and fluconazole at a CD4 lymphocyte count of 200/mm^3. Treatment of asymptomatic patients began in November 1988 when 25 (23 percent) patients were recruited into the Medical Research Council Concorde Trial, which compares zidovudine with a placebo. Forty-four (40 percent) patients have been treated with open zidovudine, 40 (36 percent) with anti-*Pneumocystis carinii* pneumonia prophylaxis, and 36 (32 percent) with prophylactic fluconazole.

Statistical methods

The Cox proportional hazards model with time-dependent covariates was fitted using the PHREG program in SAS (SAS Institute, Inc., Cary, North Carolina). Time zero was taken as August 1, 1985. A fixed date was chosen in order to mimic the enrollment of a cohort with heterogeneous durations of infection, so that such a variable could be incorporated into a regression model. August 1, 1985, was selected as the fixed date because it was the date immediately after the last patient had seroconverted. Sensitivity analyses of the effect of choosing other possible dates were performed. CD4 lymphocyte count was fitted as a time-dependent covariate; i.e., patients' counts were continually updated throughout the follow-up period. It should be noted that for a time-dependent variable that changes in a deterministic manner over time, such as time from seroconversion to AIDS, the relative hazard estimate is the same regardless of whether the variable is fitted as a fixed variable or as a time-dependent variable (13). The proportional hazards assumption for the CD4 lymphocyte count was investigated by fitting the interaction of the CD4 count with survival time. Follow-up of patients was censored at non-AIDS death, the date of last follow-up (January 1, 1992), or 6 months after the last available CD4 lymphocyte count if any of these events occurred before the development of AIDS.

The median CD4 lymphocyte count was plotted for various time points based on all patients for whom counts were available before and after each time point. The count at a given time point for each patient was estimated by linear interpolation from the counts immediately before and after.

RESULTS

Thirty-eight of the 111 subjects had developed AIDS by January 1, 1992. We assessed the association between time from seroconversion and the risk of developing AIDS. Table 1 shows the number of AIDS cases, the number of person-years of experience, and the average rate of developing AIDS per person-year of observation. Not surprisingly, the rate is small close to the time of seroconversion and rises rapidly in the ensuing years. There is no observed increase in the rate after 8 years of follow-up, but the numbers in the 9- to 12-year column are too small to derive reliable conclusions concerning the shape of the relation. Nonetheless, the observed pattern is consistent with that reported from cohorts of homosexual men infected with HIV-1 (14–16).

A Cox proportional hazards model was also fitted, with time from seroconversion

TABLE 1. Numbers of acquired immunodeficiency syndrome (AIDS) cases, patient-years of experience, and rate of developing AIDS per patient-year among 111 HIV-1*-positive men with hemophilia, according to the number of years since seroconversion: Royal Free Hospital Haemophilia Centre, London, England, 1979–1991

Years since seroconversion	No. of AIDS cases	Patient-years of experience	Rate per patient-year
<3	3	327.1	0.009
3–5	12	303.4	0.040
6–8	18	228.6	0.079
9–12	5	67.1	0.075
Total	38	926.2	0.041

* HIV-1, human immunodeficiency virus type 1.

fitted as a continuous variable and August 1, 1985, as time zero (see "Statistical methods"). On this date, time from seroconversion ranged from 3.5 weeks to 5 years and 9 months. The hazard rate increased by an average of 25 percent for every additional year after seroconversion (table 2). This association was only of borderline significance, which may be due to the limited size of the cohort. The implicit assumption in this analysis, of an exponential relation between time from seroconversion and the risk of developing AIDS, could be questioned. Results shown in table 1 suggest that, after an initial sharp rise, the hazard increases rather slowly. Thus, models incorporating various transformations of time from seroconversion were also fitted. As would be expected from the shape of the relation in table 1, an exponential transformation, but not a logarithmic or square root transformation, improved the fit of the model. (The Wald chi-squared statistic for the test of whether the log relative hazard estimate was significantly different from zero was 4.57, as compared with 2.87 using the untransformed variable; in all cases, Wald test statistics were very similar to the corresponding values from the likelihood ratio test.) Results from the use of this transformation of time from seroconversion are given in table 3. Note that the relative hazard for the exponential function of time from seroconversion (table 3) cannot be meaningfully compared with that for the untransformed variable (table 2).

The univariate association between CD4 lymphocyte count and the risk of developing AIDS was found to be extremely strong and highly significant ($p < 0.00005$). There was an estimated 3.25-fold increase in risk with every 100/mm^3 decrease in CD4 lymphocyte count (tables 2 and 3). Since studies have found the average rate of loss of CD4 lymphocytes to be between 50 and 100 cells/mm^3 per year (1, 10), the relative hazard for CD4 lymphocyte count can be directly compared with that for time from seroconversion

TABLE 2. Estimated relative hazard of developing acquired immunodeficiency syndrome among 111 HIV-1*-positive men with hemophilia, according to time from seroconversion and CD4 lymphocyte count, fitting them as time-dependent variables in a Cox proportional hazards model: Royal Free Hospital Haemophilia Centre, London, England, 1979–1991

	Time from seroconversion (relative hazard per year)			CD4 lymphocyte count (relative hazard per 100/mm^3 decrease)		
	Relative hazard estimate	95% confidence interval	p	Relative hazard estimate	95% confidence interval	p
Unadjusted	1.25	0.96–1.62	0.09	3.25	2.29–4.63	0.00005
Adjusted†	1.05	0.81–1.34	0.7	3.25	2.30–4.61	0.00005

* HIV-1, human immunodeficiency virus type 1.
† Time from seroconversion was adjusted for CD4 lymphocyte count and vice versa.

TABLE 3. Estimated relative hazard of developing acquired immunodeficiency syndrome among 111 HIV-1*-positive men with hemophilia, according to time from seroconversion (fitted as the exponential of time from seroconversion) and CD4 lymphocyte count, fitting them as time-dependent variables in a Cox proportional hazards model: Royal Free Hospital Haemophilia Centre, London, England, 1979–1991

	Time from seroconversion (relative hazard per 1,000 expyears†)			CD4 lymphocyte count (relative hazard per 100/mm³ decrease)		
	Relative hazard estimate	95% confidence interval	p	Relative hazard estimate	95% confidence interval	p
Unadjusted	4.01	1.12–14.40	0.03	3.25	2.29–4.63	<0.00005
Adjusted‡	1.58	0.41–6.13	0.5	3.29	2.31–4.68	<0.00005

* HIV-1, human immunodeficiency virus type 1.
† Time from seroconversion was adjusted for CD4 lymphocyte count and vice versa.

in table 2. Clearly, from these univariate analyses, the former appears to have the much stronger association. Once again, transformations of the CD4 lymphocyte count were considered. It was found that the square root or logarithmic transformation of the count gave a slightly better fit than the untransformed variable. However, for the purposes of simplicity, the untransformed results are presented throughout this paper, since the conclusion remained unchanged.

The relation between CD4 lymphocyte count and time from seroconversion is shown in figure 1. The tendency for a gradual fall to take place in the count during HIV-1 infection can be clearly seen. When time from seroconversion and CD4 lymphocyte count were considered together in a

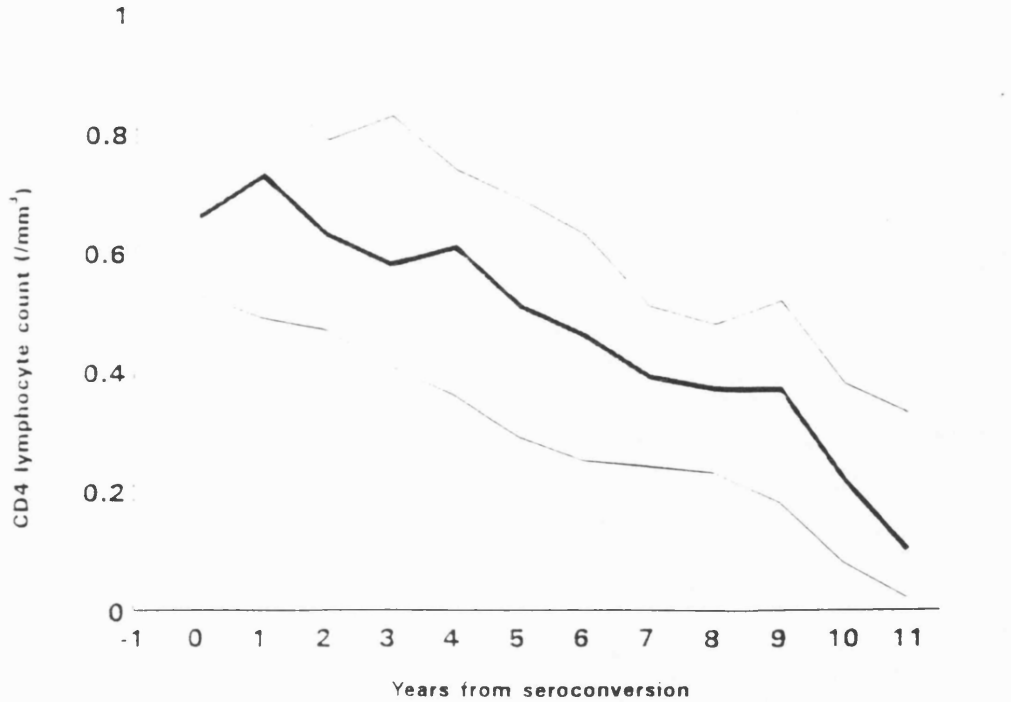

FIGURE 1. Median CD4 lymphocyte count (dark line) and its interquartile range (lighter lines) among 111 HIV-1-seropositive men with hemophilia, according to the number of years since seroconversion: Royal Free Hospital Haemophilia Centre, London, England, 1979–1991. Values at exact years from seroconversion were calculated for each patient by linear interpolation between the counts immediately before and after each date. (HIV-1, human immunodeficiency virus type 1)

Cox multiple regression model, the relative hazard for time from seroconversion fell dramatically towards unity (tables 2 and 3). In sharp contrast, the relative hazard for CD4 lymphocyte count was unchanged. The results shown in table 3 convey the same message as those in table 2. We included them to show that the findings do not result merely from inadequate modeling of the effect of time from seroconversion.

To illustrate the findings simply and without any reliance on statistical models, table 4 extends the results from table 1 and gives the AIDS rate per patient-year of follow-up according to whether the CD4 count had fallen to 200/mm^3 or below, as well as by the number of years since seroconversion. When the AIDS rate was considered separately for patient experience before and after reaching a CD4 count of 200/mm^3, the increasing trend with time from seroconversion disappeared. In patients with CD4 lymphocyte counts that had fallen to 200/mm^3 or below, the AIDS rate was similar in those who had seroconverted within the previous 6 years (11 AIDS cases/49.2 patient-years experience; rate = 0.224 per year) and those who had been seropositive for 6–12 years (21 AIDS cases/92.3 patient-years experience; rate = 0.228 per year). Similarly, there was no increasing trend in AIDS rate with years from seroconversion at higher counts. It is clear from table 4 that the only reason for the overall increase in the AIDS rate over time (see the column on the far right) is the fact that there is proportionately more patient experience at lower CD4 counts with increasing years from seroconversion. In marked contrast, there is a strong effect of CD4 count at each time from seroconversion (table 4).

Other analyses

To assess whether pre-AIDS treatment could have influenced the findings, we reanalyzed the data taking the last date of follow-up to be November 1988, the point at which pre-AIDS treatment started. The results are shown in table 5. Again, the findings were similar. We also repeated the analysis presented in table 2, restricting the data to those 49 subjects for whom the date of seroconversion was known within, at most, 6 months. Again, the results (table 6) were only slightly different. The relative hazard for the CD4 lymphocyte count was still highly significant, even in this smaller number of subjects.

The analyses presented are not sensitive to the choice of time zero (i.e., August 1, 1985), as might be expected with a model in which covariate values are updated during follow-up. When other dates were used (January 1986, January 1987, and January 1988), the results were similar. Models in

TABLE 4. Numbers of acquired immunodeficiency syndrome (AIDS) cases, patient-years of experience, and rate of developing AIDS per patient-year among 111 HIV-1*-positive men with hemophilia, according to the number of years since seroconversion and whether the CD4 lymphocyte count had declined to ≤200/mm^3: Royal Free Hospital Haemophilia Centre, London, England, 1979–1991

Years since seroconversion	CD4 count had not declined to 200/mm^3 or below			CD4 count had declined to 200/mm^3 or below			Total†		
	No. of AIDS cases	Patient-years of experience	Rate per patient-year	No. of AIDS cases	Patient-years of experience	Rate per patient-year	No. of AIDS cases	Patient-years of experience	Rate per patient-year
<3	1	322.0	0.003	2	3.9	0.513	3	326.9	0.009
3–5	1	248.2	0.004	9	45.3	0.199	10	293.5	0.034
6–8	2	145.0	0.014	16	65.6	0.244	18	210.6	0.085
9–12	0	34.0	0.000	5	26.7	0.187	5	60.7	0.082
Total	4	750.2	0.005	32	141.5	0.226	36	891.7	0.040

* HIV-1, human immunodeficiency virus type 1

† Total numbers of AIDS cases and patient-years of experience are slightly lower than the numbers in table 1 because of censoring of patient experience 6 months after the final CD4 count in individuals whose count had yet to reach 200/mm^3

tial cofactors for progression of HIV-1 infection to AIDS when such designs are employed. These biases relate to the inability to control for the unknown time from infection. However, if, as these results imply, knowledge of individuals' dates of infection adds nothing to the ability to predict the development of AIDS over and above that afforded by the CD4 lymphocyte count, this suggests that biases can be largely removed by appropriate statistical standardization for the CD4 lymphocyte count when assessing the effect of potential cofactors.

It should be noted that although the relative hazard for the number of years from seroconversion fell dramatically towards 1 after adjustment for CD4 lymphocyte count, the upper limit of the 95 percent confidence interval was 1.34. Thus, we have not eliminated the possibility that there is some effect of time from seroconversion after adjustment for CD4 lymphocyte count. However, even if the relative hazard is approximately 1.34, it is still an order of magnitude smaller than that for the CD4 lymphocyte count (relative hazard = 3.25), and its residual confounding effect in any prevalent cohort study is likely to be weak.

Similarly, it is important to note that the relative degree of imprecision in measurement of the covariates in a multiple regression model can heavily influence the resulting coefficient estimates (21–23). In this case, both the date of seroconversion and the CD4 lymphocyte count were subject to some error, and it is unclear exactly how this factor influenced the results obtained. However, the results were changed little even when the analysis was restricted to those with the most precisely characterized dates of seroconversion (table 6): thus, again, it is unlikely that we have significantly underestimated the effect of time from seroconversion on the rate of progression to AIDS.

Regarding HIV-1 pathogenesis, these results suggest that the loss of CD4 lymphocytes is of central importance in HIV-1 infection. This is consistent with an earlier report from this study (1) which indicated that differences in the time at which HIV-1-infected individuals progress to AIDS can largely be explained by differences in the rate of loss of CD4 lymphocytes. In contrast to that report, however, we have here made no assumptions concerning either the pattern of loss of CD4 lymphocytes or the count at which AIDS develops.

Future work in this area must address the causes of the depletion of CD4 lymphocytes. Thus, follow-up studies of patients with high CD4 lymphocyte counts are required to ascertain the predictors of the rate of CD4 lymphocyte loss. Studies of patients who are already severely immunosuppressed are much less likely to reveal the mechanisms by which the immune-deficient state has been reached, since the causes and consequences of immunodeficiency are likely to interact.

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The Use of Backcalculation to Estimate the Prevalence of Severe Immunodeficiency Induced by the Human Immunodeficiency Virus in England and Wales

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SUMMARY

As patients infected with the human immunodeficiency virus (HIV) become more severely immunosuppressed, they are at increased risk of developing one of a number of opportunistic infections or lymphomas, which define them as suffering from the acquired immune deficiency syndrome (AIDS). Estimates of the number of individuals who could benefit from antiretroviral treatment and prophylactic therapies against the main opportunistic infections are not readily available, as many individuals infected with HIV are asymptomatic and unaware of their infection. Using the method of backcalculation, we suggest that there are currently at least 2161 individuals with severe immunodeficiency but not AIDS in England and Wales. We demonstrate that this figure is sensitive to the choice of model for the severe immunodeficiency period and to the assumed effect of pre-AIDS therapy. Using a definition of severe immunodeficiency based on a single CD4 count rather than two consecutive counts leads to substantially increased estimates of the prevalence of severe immunodeficiency.

Keywords: ACQUIRED IMMUNE DEFICIENCY SYNDROME; BACKCALCULATION; EPIDEMIOLOGY; HUMAN IMMUNODEFICIENCY VIRUS; IMMUNODEFICIENCY

1. INTRODUCTION

CD4 lymphocytes, which are central to the body's immune system, are the main target of the human immunodeficiency virus (HIV), and, as the number of these cells decreases, the infected individual becomes increasingly susceptible to various opportunistic infections and lymphomas (Eyster *et al.*, 1987; Fahey *et al.*, 1990; Phillips *et al.*, 1991a). Although this decline in CD4 cells occurs at differing rates in different individuals, those with very low CD4 counts (less than $0.2 \times 10^9 l^{-1}$) have been shown to be at an increased risk of developing acquired immune deficiency syndrome (AIDS). (Masur *et al.*, 1989; Eyster *et al.*, 1989; MacDonell *et al.*, 1990; Aledort *et al.*, 1992). Despite this, many patients remain asymptomatic for a long time throughout infection, even for a while after reaching this level of immunodeficiency (Masur *et al.*, 1989; Sabin *et al.*, 1993), and a large number probably remain unaware that they are infected with the virus.

Currently, many treatment centres have adopted policies which involve the introduction of antiretroviral treatment and prophylactic therapies for the main AIDS defining opportunistic infections when a patient's CD4 count drops to $0.2 \times 10^9 l^{-1}$

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(Kovacs and Masur, 1988; Chave *et al.*, 1989; Centers for Disease Control, 1992a). It is believed that, although overall survival may not be increased dramatically, the effect of these therapies may be to lengthen the time to an AIDS diagnosis, and hence a greater proportion of time during HIV infection is now AIDS free (Volberding *et al.*, 1990; Hamilton *et al.*, 1992). However, there is little available information about the numbers of individuals with low CD4 counts who remain untreated when it would be of likely benefit. The new Centers for Disease Control (CDC) AIDS surveillance definition adopted in the USA (Centers for Disease Control, 1992b) includes all HIV-infected individuals with a CD4 count less than $0.2 \times 10^9 l^{-1}$. If it were possible to estimate the numbers of individuals with CD4 counts below $0.2 \times 10^9 l^{-1}$, it would go at least part of the way to assessing the effect of this new definition on surveillance figures. Additionally, recent research suggests that patients infected with HIV may also be at a higher risk of transmitting the virus once their CD4 count drops to a low level (European Study Group on Heterosexual Transmission of HIV, 1992). Models which aim to study the epidemic through transmission dynamics could benefit from an estimate of the number of individuals with very low CD4 counts.

The aim of the research was to use the method of backcalculation (Brookmeyer and Gail, 1986, 1988; Day *et al.*, 1989; Rosenberg and Gail, 1991) to estimate the current prevalence of severe immunodeficiency in England and Wales, as defined by the date of the first of the first two consecutive CD4 counts below $0.2 \times 10^9 l^{-1}$. In this paper, we assess the sensitivity of the method to changes in the reported AIDS figures and to different possible treatment scenarios. Further, we consider the effect of variability in the measurement of CD4 counts (Hoover *et al.*, 1992; Mientjes *et al.*, 1992; Stein *et al.*, 1992) by comparing the above definition of severe immunodeficiency with a definition based on a single CD4 count only.

2. METHODS AND DATA

2.1. *Method of Backcalculation*

The method of backcalculation has been well described (Brookmeyer and Gail, 1988; Day *et al.*, 1989). The incidence of severe immunodeficiency and the incidence of AIDS are linked by the equation

$$a(t) = \int_0^t m(s) f(t-s)\, ds \tag{1}$$

where $a(t)$ is the incidence of AIDS at time t, $m(s)$ is the incidence of patients initially reaching a CD4 count of $0.2 \times 10^9 l^{-1}$ at time s and $f(\ )$ is the severe immunodeficiency period distribution, i.e. the distribution of the number of years from reaching a CD4 count of $0.2 \times 10^9 l^{-1}$ to developing AIDS. If any two of these functions are known, or can be estimated, then the third can be inferred.

Now, suppose that calendar time is divided into intervals $(i-1, i)$, $i = 1, 2, 3, \ldots, T$. Further, using a discrete approximation and making the assumption that all AIDS cases occurred at the start of the time interval, the above equation becomes

$$a_i = \sum_{j=1}^{i} m_j f_{i-j} \qquad i = 1, 2, \ldots, T \tag{2}$$

where a_i and m_i represent the number of new AIDS cases and new cases of severe immunodeficiency in the time interval $(i-1, i)$, $i=1, 2, \ldots, T$ respectively and

$$f_{i-j} = \begin{cases} F(i-j+1) - F(i-j) & i > j, \\ F(1) & i = j, \\ 0 & \text{otherwise} \end{cases}$$

with

$$F(t) = \int_0^t f(u)\,du.$$

In matrix form this becomes

$$\mathbf{a} = \mathbf{Fm} \tag{3}$$

where $\mathbf{a}$ is a $T \times 1$ column matrix of known AIDS figures from each time interval, $\mathbf{m}$ is the unknown $T \times 1$ column matrix of the number of HIV-infected individuals with a CD4 count first dropping to $0.2 \times 10^9 l^{-1}$ or less in each time interval and $\mathbf{F}$ is a $T \times T$ triangular matrix of the form

$$f_{ij} = \begin{cases} f_{i-j} & i \geqslant j, \\ 0 & \text{otherwise.} \end{cases}$$

Then

$$\mathbf{m} = \mathbf{F}^{-1}\mathbf{a} \tag{4}$$

which can be evaluated easily in any package which allows for basic matrix algebra. Any changes in the severe immunodeficiency period over time are assumed to be caused by a number of other factors, such as the introduction of treatment. These factors can therefore be modelled and a different probability distribution estimated for each combination of factor levels.

The usual method of backcalculation assumes that the number of new individuals with severe immunodeficiency in each time period has some functional form (e.g. quadratic exponential). When backcalculating to HIV infection, this can be chosen so that the estimates are always positive and conform to the expected shape of the epidemic. As CD4 count monitoring has only recently come into vogue, information on the shape of such a distribution for the incidence of severe immunodeficiency is not available, and the reasons for the choice of any particular model form would be largely dependent on the researcher. To obtain a very simple method which does not rely on too many assumptions, the estimates have not been constrained in this way. However, without such a fixed form, it is quite possible for estimates of the incidence of severe immunodeficiency to jump wildly between time intervals or even to become negative. To overcome this partly, therefore, we have subtracted the number of newly reported AIDS cases from the incidence of severe immunodeficiency in each time interval. The resulting figures have been accumulated over time. The cumulative figures shown, therefore, represent the prevalence of severe immunodeficiency but not AIDS. These estimates will be rather more stable than the incidence figures, although further smoothing could be applied to the results if desired.

2.2. *Acquired Immune Deficiency Syndrome Figures*

The Communicable Disease Surveillance Centre (CDSC) have provided the reported AIDS figures for England and Wales. Half-yearly diagnoses up to the end of 1991, and reported by the end of June 1992, have been used. The backcalculation has been performed on the total figures for England and Wales, and also for homosexual men only.

AIDS figures reported to the CDSC are subject to a number of known errors. Several papers have been published which attempt to model the delay between diagnosis of AIDS and reporting of the AIDS case (Heisterkamp *et al.*, 1989; Brookmeyer and Damiano, 1989). To be consistent with the methods used by the 1992 HIV/AIDS Predictions Working Group, an adjustment for reporting delay suggested by Rosenberg (1990) has been used. Under-reporting of AIDS cases has been assumed to be constant over time and, again in line with the Predictions Working Group, has been taken to be 20%, i.e. 20% of AIDS cases are never reported to the CDSC for some reason. Within risk groups this figure is likely to vary: under-reporting may be lower in groups known to be at a high risk of infection. Finally, there will be a number of individuals who develop AIDS without ever having been diagnosed as severely immunodeficient. Published estimates of the number of HIV-infected patients developing AIDS without their CD4 count ever being measured below $0.2 \times 10^9 l^{-1}$ range from upwards of 15% (Farizo *et al.*, 1992; Schwartlander *et al.*, 1992). When using a definition based on two consecutive counts, this proportion would be expected to be higher. Owing to differences in the frequency of certain AIDS defining conditions, homosexual men may be expected to develop AIDS at higher CD4 levels (Masur *et al.*, 1989; Schwartlander *et al.*, 1992).

A summary of the assumptions made for under-reporting, AIDS at counts above $0.2 \times 10^9 l^{-1}$ and the age distributions is shown in Table 1. The final adjusted AIDS figures, both for England and Wales as a whole and for homosexual men, are shown in Fig. 1.

2.3. *Modelling Severe Immunodeficiency Period Distribution*

The severe immunodeficiency period was modelled by using procedure LIFEREG in SAS (SAS Institute, 1989). Three underlying parametric models were compared:

TABLE 1

Assumptions made for backcalculation both for England and Wales as a whole and for homosexual men only

	All AIDS cases (%)	*AIDS cases in homosexual men only (%)*
Proportion of AIDS cases never reported to the CDSC	20	20
Proportion of AIDS cases at CD4 counts greater than $0.2 \times 10^9 l^{-1}$		
1-count definition	17	18
2-count definition	20	22
Age at development of AIDS		
<35 years	44	41
≥ 35 years	56	59

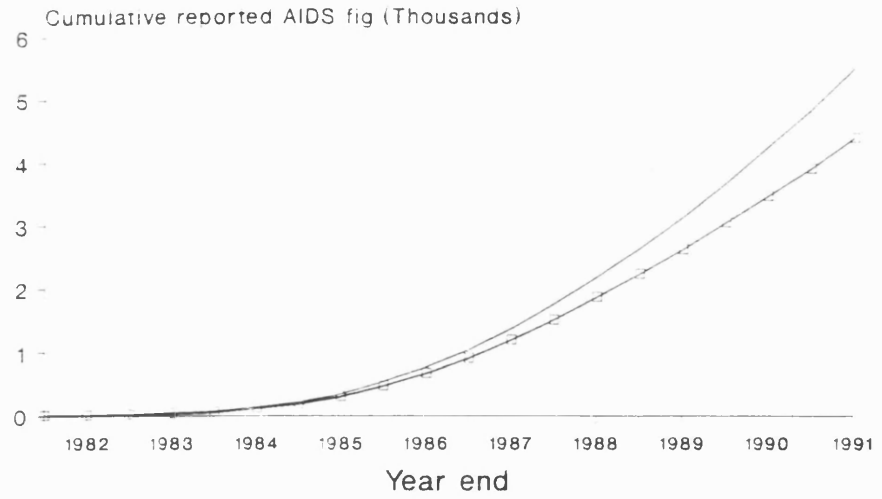

Fig. 1. Cumulative reported AIDS figures for England and Wales, adjusted for reporting delay and under-reporting: ——, total; □, homosexual men

Weibull, log-logistic and log-normal. Two definitions of severe immunodeficiency were compared: the date of the first CD4 count less than $0.2 \times 10^9 l^{-1}$ and the date of the first of the first two consecutive CD4 counts less than $0.2 \times 10^9 l^{-1}$. The latter definition is affected much less by measurement variability in CD4 counts and has been accepted as the standard definition of severe immunodeficiency by the Predictions Working Group.

2.4. *Severe Immunodeficiency Period*

To estimate the severe immunodeficiency period distribution, data were taken from a cohort of 111 patients with haemophilia at the Royal Free Hospital Haemophilia Centre, London, who became infected with HIV over the period when infected blood products were inadvertently administered to patients (1979–85). The patients are all men and are followed regularly for both clinical and laboratory review. To date, a median of 16 CD4 counts has been measured on each patient (range 1–40 counts). Currently, all patients are offered treatment once their CD4 counts drop below $0.2 \times 10^9 l^{-1}$. The patients have been described in more detail elsewhere (Lee *et al.*, 1989, 1991; Phillips *et al.*, 1991a).

To the end of 1991, 45 patients' CD4 counts had fallen below $0.2 \times 10^9 l^{-1}$ on two consecutive occasions, of whom 26 had gone on to develop AIDS. Two further patients' CD4 counts had dropped on two consecutive occasions, both after a diagnosis of AIDS, and eight patients are known to have developed AIDS without their CD4 counts falling below $0.2 \times 10^9 l^{-1}$ on two consecutive occasions. To the end of 1991, 58 of the patients' CD4 counts had dropped below $0.2 \times 10^9 l^{-1}$ on at least one occasion. Of these, 33 are known to have developed AIDS.

2.5. *Modelling Effect of Treatment*

From late 1988, the antiretroviral drug zidovudine became available for HIV-infected individuals without AIDS, and since 1989 prophylaxis has been available for *pneumocystis carinii* pneumonia (PCP) (Kovacs and Masur, 1988; Pedersen *et al.*, 1990) and candidiasis (Chave *et al.*, 1989), both common AIDS defining conditions. Treatment within the cohort, therefore, is necessarily limited to those patients who had survived until late 1988. Currently, few results from large-scale trials assessing the effects of various therapies on both AIDS-free survival and CD4 counts have been published (Volberding *et al.*, 1990; Aboulker and Swart, 1991, 1993). Further, treatment take-up in the HIV-infected population is difficult to assess without accurate measures of the number of HIV-infected individuals. In this paper, therefore, the results of several different treatment scenarios are shown.

To investigate the effect of the introduction of treatment, a 'no-treatment' model has been generated by censoring all survival times in 1988, or at death. Assuming that therapy has some effect on AIDS-free survival, this model will underestimate the numbers of individuals with severe immunodeficiency, and so its use is restricted to comparing the choice of parametric models.

One method of modelling the treatment effect has been described by De Angelis *et al.* (1993). The basic matrix equation described earlier is extended to incorporate a 'treatment effect' matrix and becomes

$$\mathbf{a} = \mathbf{TFm} \tag{5}$$

where $\mathbf{T}$ is a $T \times T$ block matrix

$$\begin{pmatrix} \mathbf{I} & \mathbf{0} \\ \mathbf{0} & \mathbf{S} \end{pmatrix}.$$

$\mathbf{I}$ is the $(k-1) \times (k-1)$ identity matrix, where k represents the time of introduction of therapy, and $\mathbf{S}$ represents the overall effect of treatment, including the treatment take-up in the population and the extension to the severe immunodeficiency period as a result of treatment. The treatment scenarios were chosen to match those used by the Predictions Working Group when using backcalculation to estimate the prevalence of HIV infection (Day *et al.*, 1993). We assume that pre-AIDS treatment began in 1988 and that the proportions of infected individuals receiving treatment was either 5% in 1988, 15% in 1989 and 25% in 1990 onwards (a 'low' treatment take-up scenario) or 10% in 1988, 30% in 1989 and 50% in 1990 onwards (a 'high' treatment take-up scenario). We have compared three different extensions to the severe immunodeficiency period. Extensions to the severe immunodeficiency period have been generated from

(a) a uniform distribution with a mean extension of 1 year (referred to as a uni(1) extension),
(b) an exponential distribution with a mean extension of 2 years (exponential(2) extension) and
(c) an exponential distribution with a mean extension of 4 years (exponential(4) extension).

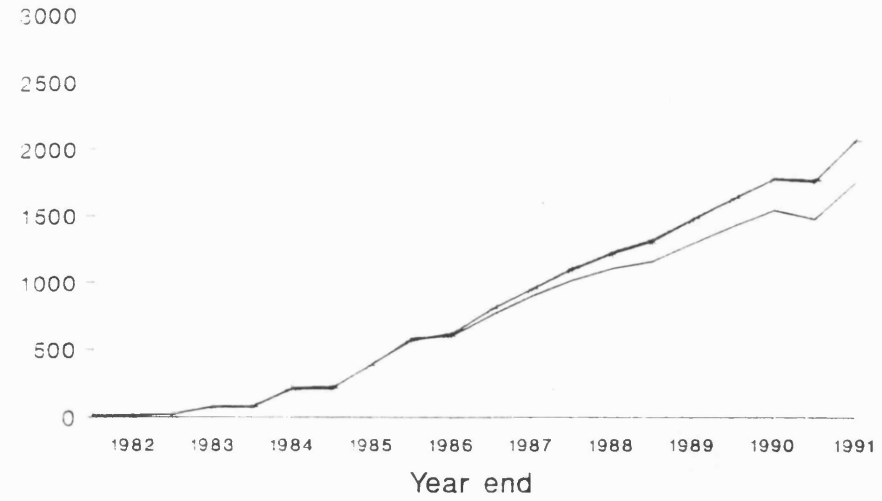

Fig. 2. Cumulative estimates of the numbers with severe immunodeficiency but not AIDS in England and Wales, based on a no-treatment model censored in 1988—comparison of Weibull (———), log-normal (|) and log-logistic (×) models for the severe immunodeficiency period

3. RESULTS

3.1. *Total Figures for England and Wales*

3.1.1. *'No-treatment' model: choice of distribution*

Three different distributions were fitted to the logarithm of the severe immunodeficiency periods, censored in 1988 or at death: Weibull (median 1.19 years), log-logistic (median 1.10 years) and log-normal (median 1.10 years) models. Fig. 2 shows the estimates of the prevalence of severe immunodeficiency as defined by the first of the first two consecutive CD4 counts less than $0.2 \times 10^9 l^{-1}$, but without AIDS, in England and Wales, using each of these three models. There are only small differences between the three models, although both the log-normal and the log-logistic models give slightly higher estimates (prevalence of 2075 and 2087 by December 1991 respectively) than the estimates from the Weibull model (1767).

Because the Weibull model has frequently been used in the past for modelling AIDS incubation periods, the following results will be based on the Weibull model. However, if using the log-normal and log-logistic models, the results can be expected to be slightly higher than those from the Weibull model.

3.1.2. *Effect of treatment: treatment take-up*

The results of using either a high or a low treatment take-up are shown in Fig. 3. The introduction of treatment has been assumed to lead to an extension to the severe immunodeficiency period generated from a uni(1) distribution. When compared with the no-treatment model, the effect of the low treatment take-up has been to increase the estimates by 394 to 2161. The high treatment take-up model increases the estimates by a similar amount to 2537.

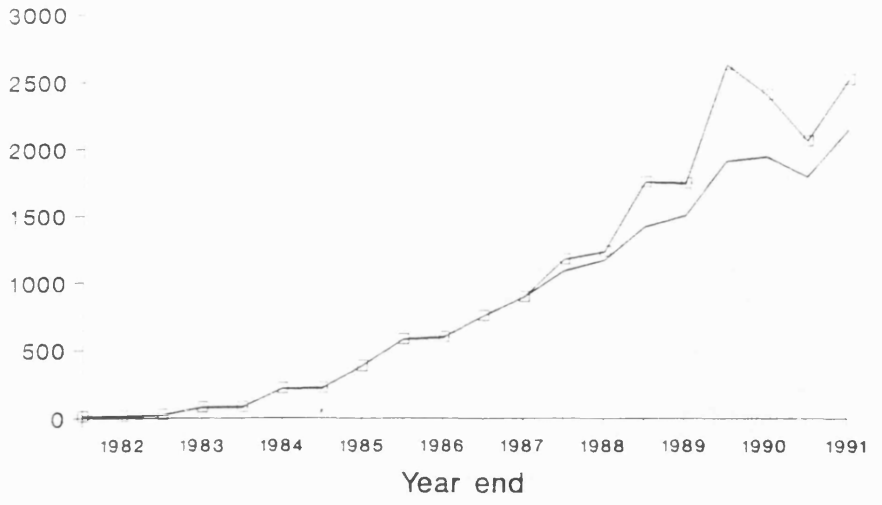

Fig. 3. Cumulative estimates of the numbers with severe immunodeficiency but not AIDS in England and Wales, based on a Weibull model with a uni(1) extension to the severe immunodeficiency period—comparison of low (———) *versus* high (□) treatment take-up in the population

3.1.3. *Effect of treatment: extension to severe immunodeficiency period*

Three different extensions to the severe immunodeficiency period were compared. A Weibull model has been used, and a low treatment take-up has been assumed. The results are shown in Fig. 4.

The longer the average extension of the severe immunodeficiency period due to treatment is, the higher the resulting estimates are. Compared with the extension from the uni(1) distribution (2161), the exponential(2) extension leads to an increase of 186 in the results (2347), and with the exponential(4) extension estimates are increased by another 334 to 2681 individuals with severe immunodeficiency but not AIDS.

3.1.4. *Effect of age*

It has been shown that there is more rapid HIV disease progression in older HIV-infected individuals than in those who are younger (Eyster *et al.*, 1987; Goedert *et al.*, 1989; Blaxhult *et al.*, 1990; Phillips *et al.*, 1991b). The patient's age at development of severe immunodeficiency was added to the model as a covariate. AIDS cases were then stratified by age at diagnosis (below 35 years, 44%; 35 years and above, 56%), representing the overall proportions of AIDS cases in the two age groups (Communicable Disease Surveillance Centre, 1991). Adjustments were made to allow for differences in age at development of severe immunodeficiency and at diagnosis of AIDS. Again, a Weibull model was used including a low treatment take-up effect with uni(1) extension.

The median severe immunodeficiency periods for those under 35 years and those over 35 years of age at diagnosis of AIDS were 1.36 years and 1.04 years respectively. Despite this difference in medians, the addition of an age effect into the model barely affects the estimates, giving a cumulative total of 2148 by the end of

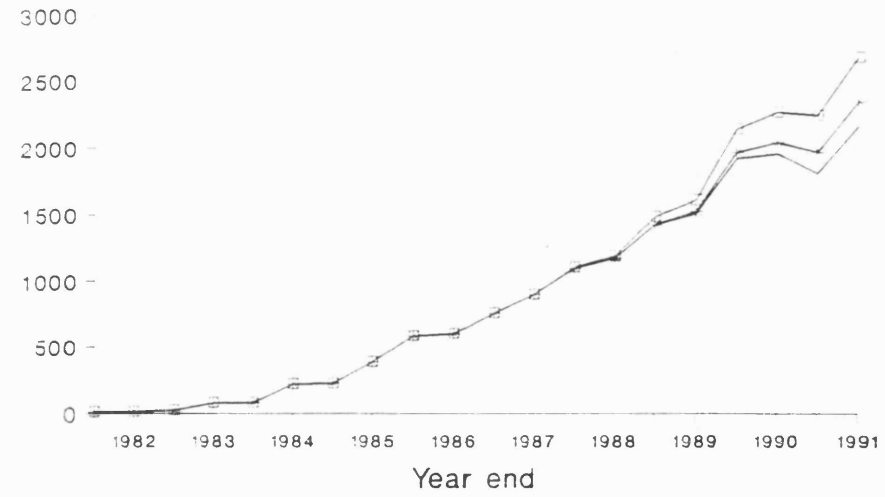

Fig. 4. Cumulative estimates of the numbers with severe immunodeficiency but not AIDS in England and Wales based on a Weibull model with low treatment take-up—comparison of different extensions to the severe immunodeficiency period due to the introduction of treatment in 1988: ———, uni(1) extension; *, exponential(2) extension; □, exponential(4) extension

December 1991. As the estimates are so similar to those from the model without an age effect, no graph has been included to illustrate the estimates.

3.1.5. *Definition of severe immunodeficiency*

The choice of a definition based on either two consecutive or a single CD4 count less than $0.2 \times 10^9 l^{-1}$ has a large effect on the estimate of the prevalence of severe immunodeficiency but not AIDS. When using a definition based on only a single CD4 count, the date at which a person is described as immunodeficient occurs, in general, much earlier than when a definition of two counts is used. When fitting a no-treatment Weibull model to these times, the median severe immunodeficiency period was 2.39 years (compared with 1.19 years for two CD4 counts).

By the end of 1991 a total of 5216 individuals would be classified as severely immunodeficient on the basis of a single-count definition, compared with 2161 under a two-count definition (Fig. 5).

3.1.6. *Sensitivity to most recent acquired immune deficiency syndrome figures*

Fig. 6 shows the effect of increasing and decreasing the most recent reported number of AIDS cases for 1992 by 25 or 50 cases. The model is very sensitive to the choice of this number—each change of 25 leads to a change of 125 in the prevalence of severe immunodeficiency but not AIDS. These most recent figures are unfortunately the figures about which we have most uncertainty due to delays in reporting of AIDS cases to the CDSC.

3.1.7. *Homosexual men*

A summary of the above results for homosexual men appears in Table 2. With a Weibull model, low treatment take-up and a uni(1) extension to the severe

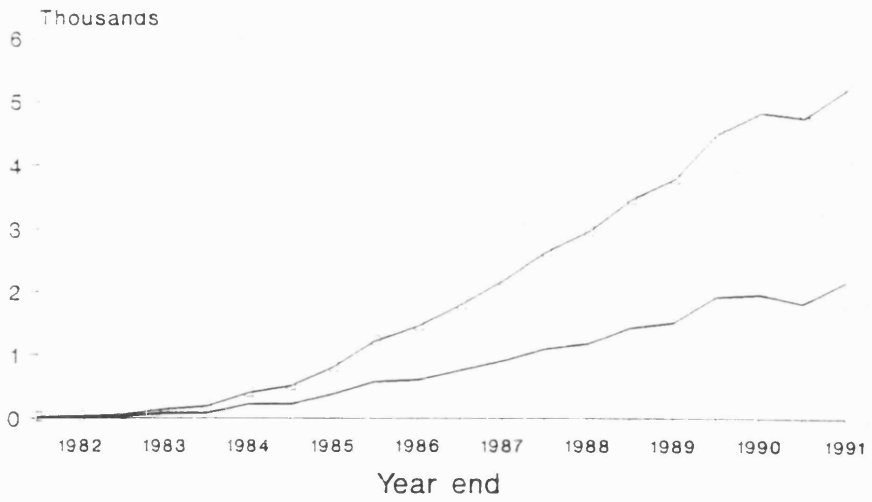

Fig. 5. Cumulative estimates of the numbers with severe immunodeficiency but not AIDS in England and Wales based on a Weibull model with low treatment take-up and uni(1) extension—comparison of severe immunodeficiency definitions based on a single (□) or two consecutive (———) CD4 counts

immunodeficiency period, an estimated 1536 homosexual men would be classified as severely immunodeficient but would not have developed AIDS by the end of 1991. The effects of the choice of the various assumptions are similar to those for England and Wales as a whole, the estimates being affected by the treatment take-up in the population, by the length of the extension to the severe immunodeficiency period and by the choice of definition of severe immunodeficiency.

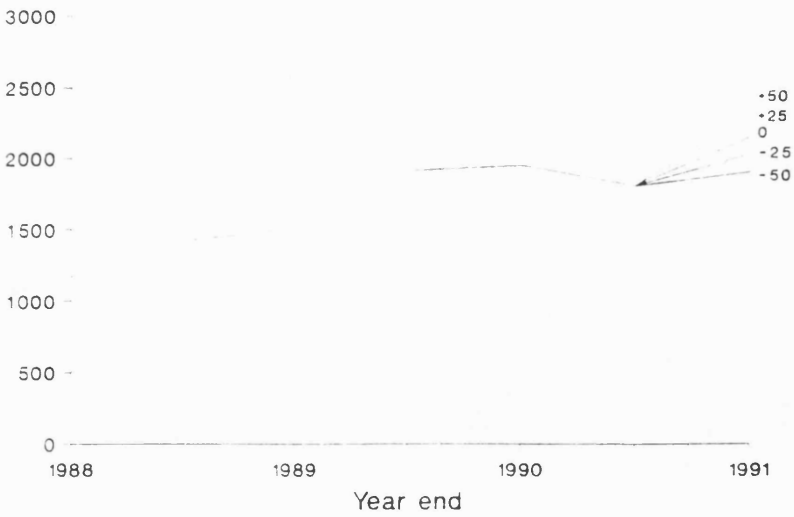

Fig. 6. Cumulative estimates of the numbers with severe immunodeficiency but not AIDS in England and Wales based on a Weibull model with low treatment take-up and uni(1) extension—sensitivity to the most recently reported AIDS figures

TABLE 2
Cumulative estimates of the number of homosexual men with severe immunodeficiency but not AIDS by December 31st, 1991†

AIDS cases occurring after development of severe immunodeficiency (adjusted for reporting delay and under-reporting)	4305
No-treatment model	
Weibull	1259
Log-logistic	1497
Log-normal	1491
Treatment take-up	
Low	1536
High	1796
Extension to immunodeficiency period	
Uniform, mean 1 year	1536
Exponential, mean 2 years	1674
Exponential, mean 4 years	1919
Age effect	1515
Definition based on 1 CD4 count only	3822

†Unless where stated, all results are based on a Weibull model with low treatment take-up giving a uniform extension to the immunodeficiency period with mean 1 year.

4. DISCUSSION

We have estimated that there are at least 2161 individuals in England and Wales who, if their CD4 counts had been continually monitored, would have had two consecutive CD4 counts below $0.2 \times 10^9 l^{-1}$ and who do not currently have AIDS. To put this figure into perspective, there are currently approximately 2120 individuals living with AIDS who have been reported to the CDSC (Day, 1993). For each person alive with AIDS, therefore, there is another infected with HIV who, if their CD4 counts had been continually monitored, would have had two consecutive CD4 counts below $0.2 \times 10^9 l^{-1}$ and who are therefore likely to develop AIDS in the near future.

This estimate assumes use of treatment which has the combined effect of extending the severe immunodeficiency period by 1 year on average, and which is received by 5%, 15% and 25% of individuals developing AIDS in 1988, 1989 and 1990 onwards respectively. We have shown that estimates of the prevalence of severe immunodeficiency are sensitive to the choice of the severe immunodeficiency period distribution and, because of its effect on the duration of severe immunodeficiency, the introduction of pre-AIDS therapy. Estimates are also sensitive to the choice of the most recent AIDS figures, emphasizing the need for continual effort in monitoring reporting delays and under-reporting levels. This sensitivity has also been shown by others (Wilson *et al.*, 1992). Increased age does not affect the results in a large way: most of the effect due to age will occur *before* the CD4 count has fallen to a low level. Other factors which will also affect the results are the levels of under-reporting, diagnosis of AIDS before a diagnosis of severe immunodeficiency and the bias due to severely immunosuppressed patients who die before developing

AIDS. In this paper we have selected a figure for these which, from other sources, is thought to be the best estimate (discussed later). Because of the simplicity of the methods used, a change to the AIDS figures as a result of increasing or decreasing these levels simply results in the same proportionate change in the estimates of severe immunodeficiency. To remain consistent, the assumptions made, and varied, in this paper, are largely those of the Predictions Working Group. We have not illustrated the effect of changes to these levels although in most cases it is quite simple to estimate the likely effect.

Other studies have suggested that the median duration of severe immunodeficiency (from a single CD4 count definition) ranges from just under 2 years to around 2.5 years (Van Griensven *et al.*, 1991; Chene *et al.*, 1992). In the absence of treatment these estimates are similar to ours. Including a treatment effect, however, inevitably leads to a longer severe immunodeficiency period.

A definition based on two consecutive rather than a single CD4 count attempts to reduce the effect of variability in the CD4 count measurement, and the results therefore do not include the large number of individuals who have a single CD4 count below $0.2 \times 10^9 l^{-1}$ before returning to higher levels (Hoover *et al.*, 1992). Under this definition the effect of the introduction of therapy on the prevalence estimates may be much larger than under a single-count definition, dependent on the policy for introducing therapy. In the UK, it is usual to introduce therapy after a CD4 count of $0.2 \times 10^9 l^{-1}$ (Centers for Disease Control, 1992a). However, whether a confirmatory CD4 count is sought before starting therapy depends on the individual centre. Differences between the two definitions will be greater under a policy which introduces treatment after only a single low CD4 count than under a policy in which treatment is withheld until the patient has had two consecutive counts measured below this level.

Very little is known about either the proportion of patients in the population receiving therapy, the effect of combined therapy on the risk of developing AIDS or the length of benefit of any therapy. Recent results from trials of zidovudine suggest that any benefit of the drug is possibly short lived and may not be improved by earlier usage of the drug (Aboulker and Swart, 1993). Prophylaxis for PCP and candidiasis have been successful in reducing the incidence of these AIDS defining conditions (Chave *et al.*, 1989; Pedersen *et al.*, 1990) but have not prevented them altogether. We have illustrated some scenarios suggested by the Predictions Working Group. The low treatment take-up suggested seems plausible and consistent with the fact that in many case AIDS is the first indication of HIV infection. Extensions chosen vary both in terms of their mean length of effect and the choice of probability distribution used to generate them. In general, any effect which extends the severe immunodeficiency period will necessarily increase the resulting prevalence estimates.

Available research suggests that the proportion of patients with severe immunodeficiency who die before a diagnosis of AIDS is at most 2–5% (Eskild *et al.*, 1992; Farizo *et al.*, 1992). Although we have not adjusted for this, it is unlikely to be a major source of bias. The proportions of patients developing AIDS before the development of severe immunodeficiency has been assumed to be 20% (two CD4 count definition). This, again, is consistent with other reports (Farizo *et al.*, 1992; Schwartlander *et al.*, 1992). Finally, under-reporting has been fixed at 20% (Evans and McCormick, 1994). These last two effects essentially cancel each other out, and

the residual effect of one over the other is likely to be small. Any changes in these rates over time would also be expected to act in opposing directions, thus reducing any overall effect.

The effect of a change in the surveillance definition of AIDS in 1987 (Centers for Disease Control, 1987), which included two new AIDS defining conditions and allowed for presumptive diagnoses of certain opportunistic infections, is expected to have been short lived and will not bias the results greatly. The most recent definition change adopted by the Centers for Disease Control in the USA (Centers for Disease Control, 1992b), which includes all HIV-infected individuals with a CD4 count below $0.2 \times 10^9 l^{-1}$ as AIDS cases, will inevitably lead to increased underreporting, as the definition depends on a laboratory test of CD4 counts in potentially asymptomatic people. The inherent biases in future surveillance figures have already been well described (Hoover *et al.*, 1992). There is potential for our figures to be used to assess the effect of this new definition, and to generate comparative AIDS figures for the years before 1993.

Although our estimate is subject to substantial uncertainty, it is possibly the best estimate that is available currently. Using a similar definition of two consecutive CD4 counts less than $0.2 \times 10^9 l^{-1}$, current estimates suggest that there are more than 1000 HIV-infected individuals who have severe immunodeficiency, without AIDS, but are currently receiving care (HIV/AIDS Predictions Working Group, 1992). This number excludes many asymptomatic patients with low CD4 counts and those who are unaware of their HIV status. We may infer, therefore, that of those patients with severe immunodeficiency, but not AIDS, just under a half are currently receiving care. This is consistent with the fact that only 50% of male homosexual and injecting drug user HIV cases, and 25% of heterosexual HIV cases, were diagnosed more than 4 months before a diagnosis of AIDS (HIV/AIDS Predictions Working Group, 1992). Recent estimates for the USA suggest that by the end of 1992 up to 170000 individuals developed severe immunodeficiency but not AIDS, a ratio of between 1.0 and 1.2 to the estimated number of live AIDS cases (Centers for Disease Control, 1992c). The method used is slightly different from our own (Brookmeyer, 1991) and the definition of severe immunodeficiency that has been used is based on a single CD4 count. These results are, however, somewhat lower than ours.

We have not attempted to put any bounds of uncertainty around our estimates. For simplicity, we have suggested a range of possible values by allowing the assumptions to change slightly. Bootstrapping methods could be used to obtain confidence intervals for the estimates (Efron, 1982), and attempts to find bounds of uncertainty for these estimates should certainly be investigated further. Under certain assumptions around 2161 individuals are likely to have severe immunodeficiency but not AIDS under a two CD4 count definition. This is our preferred estimate as the assumptions made are reasonable and the estimate is consistent with other sources of information. However, we have illustrated that if these assumptions are changed slightly the estimated prevalence of severe immunodeficiency can rise quite dramatically. For example, a more positive benefit to AIDS-free survival due to treatment could lead to an increase of 500 in the prevalence estimates. A further methodological point which should be commented on is the decision to assume that all AIDS cases occurred at the start of each time interval. The backcalculation was repeated with quarterly AIDS figures. This would be expected to reduce any bias due

to this assumption, as AIDS cases would be misplaced by at most 3 months, rather than 6 months when using half-yearly data. However, when this analysis was performed the results were essentially unchanged (data not shown). These results were, however, rather less stable than the half-yearly estimates, as they were based on much smaller numbers.

As the cohort is made up of male patients with haemophilia, uncertainty will always lie in whether the estimate of the severe immunodeficiency period is representative of other risk groups. Although certain AIDS defining conditions are more common in different risk groups, little evidence is available to show that progression rates, especially from a low CD4 count to AIDS, vary greatly between the groups. Homosexual men are more likely to develop Kaposi's sarcoma than other risk groups are, and so might be expected to progress more rapidly (Masur *et al.*, 1989; Schwartlander *et al.*, 1992). However, there is a suggestion that other co-infections in injecting drug users and exposure to intermediate purity clotting factor concentrate in patients with haemophilia also speed progression in these groups. Little information is available on HIV disease progression in females. However, as the number of HIV-infected females in England and Wales is small, any differences in progression rates are likely to have little effect on the overall estimates of severe immunodeficiency. Although it would be of interest to apply the method to risk groups, other than homosexual men, in the population, the method used is less robust with smaller numbers of AIDS cases, and the estimates would be affected much more by small perturbations in the data.

There are currently at least 2161 individuals estimated to have severe immunodeficiency (as defined by two CD4 counts below $0.2 \times 10^9 l^{-1}$) but not AIDS in England and Wales. It is thought that less than a half of these patients are currently receiving care. Although there is still work to be done on refining the methods used, this estimate is consistent with those from the other, limited, information sources. These are the patients who are most likely to develop AIDS in the near future and, hence, should be identified and targeted for prophylaxis and other treatment.

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Beta-2 microglobulin as a predictor of prognosis in HIV-infected men with haemophilia: a proposed strategy for use in clinical care

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Summary. Whilst the prognostic value of serum beta-2 microglobulin (s-β_2m) is well documented, the lack of a simple strategy for its use means that it is rarely ever measured in clinical practice. The prognosis associated with s-β_2m at two different points in HIV infection, as defined by the CD4 count, was studied in a cohort of 111 men with haemophilia registered at the Royal Free Hospital School of Medicine, London. At CD4 counts of 0·5 and 0·2 × 10^9/l, a raised s-β_2m level was significantly associated with an increased risk of developing AIDS (P=0·002 and 0·022 respectively, adjusted for the patient's age). Kaplan-Meier progression rates to AIDS by 4·5 years after a CD4 count of 0·5 × 10^9/l were 57% (95% CI 32–82%) in those with s-β_2m levels of 3 mg/l or more, but 20% (95% CI 4–36%) in those with s-β_2m levels of less than 3 mg/l. By 3·5 years after a CD4 count of 0·2 × 10^9/l, Kaplan-Meier progression rates to AIDS were 75% (95% CI 52–98%) in those with s-β_2m levels of 3·8 mg/l or more, and 47% (95% CI 29–66%) in those with s-β_2m levels of less than 3·8 mg/l. In the absence of acute viral infections, a raised s-β_2m indicates those who will tend to progress to AIDS more rapidly than those with lower s-β_2m levels and the same CD4 count. S-β_2m levels in general are likely to be higher in haemophilia patients than in other, non-haemophilic risk groups. Whilst care should be taken, therefore, when applying our chosen cut-off values to non-haemophilic patients, our findings support the introduction of prophylaxis and antiviral therapies at a higher CD4 count in those with raised s-β_2m levels relative to other patients in the same risk group whilst delaying treatment in those with lower CD4 counts, but relatively normal s-β_2m levels.

Keywords: beta-2 microglobulin, CD4, haemophilia, HIV.

CD4 lymphocyte counts have been shown to be one of the best prognostic markers for the development of AIDS in individuals with HIV infection. As the CD4 count declines, the infected individual becomes at a greater risk of developing HIV-related conditions and AIDS. However, although very valuable, the CD4 count is not a perfect indicator of prognosis, and many individuals can survive for long periods of time with low CD4 counts without developing overt signs of immunodeficiency. A number of serological markers have therefore been suggested to complement CD4 counts (Moss *et al.* 1988; Eyster *et al.* 1989; Fuchs *et al.* 1989; Fahey *et al.* 1990; Fernandez-Cruz *et al.* 1990; Polis & Masur, 1990) including serum beta-2 microglobulin (s-β_2m) (Moss *et al.* 1988; Fahey *et al.* 1990; Fernandez-Cruz *et al.* 1990; Schwartlander *et al.* 1993).

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S-β_2m is a small 12k peptide which is normally associated with membrane bound HLA-A,B,C (class I) molecules. Once lymphoid cells are activated, however, s-β_2m is shed and consequently, during infections, elevated levels of s-β_2m are measured (Grey *et al.* 1973). The turnover of activated CD8 lymphocytes is particularly high in the acute phase of viral infections caused by cytomegalovirus (CMV) and Epstein-Barr virus (EBV) when s-β_2m levels are also highly elevated (Cooper *et al.* 1984). In HIV-1 infection, s-β_2m values rise early after seroconversion (Zolla-Pazner *et al.* 1984; El-Sadr *et al.* 1987; Hofmann *et al.* 1990) and then stabilize at various levels, probably reflecting the high turnover of lymphocytes in HIV disease. S-β_2m levels remain predictive of progression

to AIDS, largely independently of CD4 counts (Bhalla *et al.* 1985; Moss *et al.* 1988; Fahey *et al.* 1990; Hofmann *et al.* 1990; Chevret *et al.* 1992; Schwartlander *et al.* 1993). Furthermore, the rise in s-β_2m levels predicts future CD4 decline (Harrison & Skidmore, 1990) and has been shown to correlate with the drop in CD4 counts (Prince *et al.* 1990; Domingo *et al.* 1992; Lifson *et al.* 1992).

Although a number of publications have indicated that the measurement of s-β_2m levels is useful in clinical assessment (Anderson *et al.* 1990; Fahey *et al.* 1990; Hofmann *et al.* 1990), these analyses have either dealt with patients at all stages of HIV disease or have simply stratified patients according to their CD4 counts and s-β_2m levels at entry to the study. Patients with widely differing CD4 counts and, hence, expected survival times are therefore grouped together, confounding the issue and making it difficult for a clinician to interpret the effect of a raised s-β_2m level. The difficulty associated with developing a simple strategy for assessing patient prognosis on the basis of both the s-β_2m level and the CD4 count has meant that, although it is widely recognized that s-β_2m provides prognostic information additional to that given by the CD4 count, this information is seldom used in clinical practice.

In the U.K. it is common practice to start prophylactic and antiretroviral therapy once the CD4 count falls below $0{\cdot}2 \times 10^9$/l (Centers for Disease Control, 1992). However, in the U.S.A. treatment is often started at much higher CD4 counts ($0{\cdot}5 \times 10^9$/l and below) (State-of-the-Art Conference on Azidothymidine Therapy for Early HIV Infection, 1990). These two CD4 counts therefore not only represent different stages of HIV infection but are also critical points for clinical decision making. In this paper we aim to assess the prognosis associated with s-β_2m level in a cohort of HIV-seropositive men with haemophilia, at two points in their infection defined by the CD4 count: firstly when the count falls to below $0{\cdot}5 \times 10^9$/l and, secondly, when the count falls to below $0{\cdot}2 \times 10^9$/l. This provides a practical strategy by which clinicians who are currently regularly monitoring patients' CD4 counts can make use of the additional prognostic information which has generally been shown to be provided by s-β_2m.

METHODS

Patients. 111 men registered at the Royal Free Hospital Haemophilia Centre became infected with HIV between 1979 and 1985. The patients were aged between 2 and 77 years (median 24 years) at the time of their first positive HIV test, and are followed up for clinical and laboratory review approximately every 3–6 months (Lee *et al.* 1989, 1991; Phillips *et al.* 1991). CD4 lymphocyte counts have been routinely measured at these visits since December 1982. As serum is regularly stored on these patients, and since all haemophilia patients with HIV are known to have been infected between 1979 and 1985, it has been possible to estimate seroconversion dates for all 111 men (Lee *et al.* 1989, 1991; Phillips *et al.* 1991).

Since August 1987, 51 patients have been treated with zidovudine, including 10 patients who received active drug in the MRC/INSERM Concorde trial of zidovudine versus placebo in asymptomatic HIV-infected patients. Prophylaxis with pentamidine for PCP began in February 1988 and more recently septrin has been used. To the end of 1992, 36 patients have received PCP prophylaxis (26 primary, nine secondary). Currently, all patients with CD4 counts of less than $0{\cdot}2 \times 10^9$/l are given PCP prophylaxis and zidovudine, and since April 1988, 36 patients with a CD4 count $< 0{\cdot}2 \times 10^9$/l have received fluconazole 150 mg weekly as prophylaxis against candida (13 primary, 26 secondary).

Laboratory methods. Absolute CD4 counts were calculated from the lymphocyte count and CD4 % values which were measured as follows: Absolute lymphocyte count was determined by an automated whole blood counter (Ortho 'ELT 800' with differential screen). Between 1982 and 1986 the percentages of CD4 lymphocytes were counted in Ficoll-Hypaque separated blood mononuclear cell suspensions using an EPICS V flow cytometer (Coulter Electronics). Since 1986 a whole blood lysis method has been used, and percentage of CD4 lymphocytes analysed by flow cytometry, either using an EPICS V or FACScan (Becton Dickinson) (Bofill *et al.* 1992). A monoclonal CD4 antibody, RFT4, to the p55 CD4 antigen was used throughout, either singly or, since 1986, in double concentration with a monoclonal CD3 antibody (OKT3 or UCHT1). Flow cytometer quality control was monitored using Q.C. beads, and in the U.K. NEQAS external quality assurance scheme.

For the purposes of this study, s-β_2m was tested retrospectively on stored frozen sera (Mancini *et al.* 1965). A commercial radial immunodiffusion (RID) method was used on all samples (NanoRID, Binding Site Ltd) and monitored by QC controls and the U.K. NEQAS quality assurance scheme.

Statistical methods. Two baseline dates were estimated for each of the patients: the dates on which each patient's CD4 count had fallen below $0{\cdot}5 \times 10^9$/l and $0{\cdot}2 \times 10^9$/l respectively. These dates were estimated by linear interpolation between the dates of the last CD4 measurement above and the first measurement below these two levels. Patients with a first-recorded CD4 count below that of interest were excluded from the analysis. S-β_2m measurements were included in the analysis if a stored serum sample was available within 6 months of the baseline date. As these measurements were unlikely to be normally distributed, Spearman rank correlations were used to assess the linear relationship between the patient's age at these baseline dates, and the s-β_2m level. As both s-β_2m levels are likely to be higher and AIDS-free survival times shorter in older patients, Cox proportional hazards models were fitted to the survival times with both the s-β_2m measurement and patient's age at baseline as fixed covariates (Cox & Oakes, 1984). Thus the effect of s-β_2m could be assessed independently of the patient's age at baseline. The assumption of proportional hazards was checked by plotting an estimate of the log (− log (survival function)) against time from baseline for each group. The Lifetest procedure in SAS (SAS Institute Inc., 1989) was used to generate Kaplan-Meier plots of the probability of developing AIDS yearly from these baseline dates. Patients were stratified into two groups according to their s-β_2m level at each baseline, the upper 33rd percentile of the s-β_2m level

being chosen as the cut-off point so as to ensure a similar number of AIDS cases in each group. Differences between the survival curves were assessed for significance using the log-rank test (Cox & Oakes, 1984). Finally, as zidovudine has been shown to reduce the level of s-β_2m, at least temporarily (Pedreira *et al.* 1992), all analyses have been repeated, with patient follow-up censored at the end of November 1988, if the patient had not developed AIDS by then.

RESULTS

Forty-seven members of the cohort had a s-β_2m measurement within 6 months of their CD4 count falling below $0{\cdot}5 \times 10^9$/l (Table I). At this point in their infection, the patients' age was correlated with their s-β_2m measurement (Spearman's $r=0{\cdot}35$, $P=0{\cdot}02$). The corresponding s-β_2m measurements for patients when their CD4 count had fallen to $0{\cdot}2 \times 10^9$/l are also shown in Table I. 46 patients had s-β_2m measurements within 6 months of their CD4 count falling below $0{\cdot}2 \times 10^9$/l and, again, the patients' ages were highly correlated with the s-β_2m measurements ($r=0{\cdot}47$, $P=0{\cdot}001$).

In order to assess the relationship between AIDS-free survival, s-β_2m and the patients' age at each baseline, Cox proportional hazards models were used to model survival times, adjusting for s-β_2m levels and age, both separately and in a bivariate model. For each CD4 measurement, the proportional hazards assumption for s-β_2m level was assessed for validity by plotting an estimate of the log (−log (survival function)) against time in each group. As the two plotted lines were approximately parallel in each case, the proportional hazards assumption was felt to be reasonable for each CD4 level. The relative risks associated with a unit increase in s-β_2m and a 5-year increase in age are shown in Table II. At each CD4 level the univariate effect of s-β_2m is highly significant ($P=0{\cdot}003$ and $P=0{\cdot}017$ at CD4 counts of 0·5 and $0{\cdot}2 \times 10^9$/l, respectively). When considering progression from a fixed CD4 count to AIDS, the patient's age has little effect on survival times. Hence, in both cases, when age is added into the model, the effect of s-β_2m remains unchanged ($P=0{\cdot}0007$ and $P=0{\cdot}022$ at CD4 counts of 0·5 and $0{\cdot}2 \times 10^9$/l, respectively).

The upper 33rd percentile of the s-β_2m measurement at a CD4 count of $0{\cdot}5 \times 10^9$/l was 3 mg/l. Patients were stratified according to whether their s-β_2m measurement was above or below this value. There is a substantial difference in progression rates to AIDS after a CD4 count of $0{\cdot}5 \times 10^9$/l, with those patients with the highest s-β_2m levels (3 mg/l or above) progressing at a much faster rate than those with lower measurements (Fig 1). Whilst 57% of patients with s-β_2m measurements of 3 mg/l or more would be expected to progress to AIDS in just under 4·5 years (95% CI 32–82%), only 20% of those with less than 3 mg/l would be expected to have progressed to AIDS over the same period of time (95% CI 4–36%).

At a CD4 count of $0{\cdot}2 \times 10^9$/l, the upper 33rd percentile of the s-β_2m measurements was 3·8 mg/l. Again patients were stratified according to whether their s-β_2m measurement was above or below this value (Fig 2). At a CD4 count of $0{\cdot}2 \times 10^9$/l, patients with s-β_2m levels of 3·8 mg/l or more have a faster progression rate to AIDS than those with lower measurements. By 3·5 years after their CD4 count falling to $0{\cdot}2 \times 10^9$/l, 75% of those with s-β_2m values of 3·8 mg/l or more would have progressed to AIDS (95% CI 52–98%). Over the same period fewer patients (47%; 95% CI 29–66%) with s-β_2m measurements of less than 3·8 mg/l would have progressed to AIDS.

Finally, the effects of introducing antiviral treatment and prophylaxis for opportunistic infections in November 1988 were considered by censoring the data at this point in time. Although no longer statistically significant, the size of the effects was similar and the main conclusions essentially unchanged.

Table I. S-β_2m measurements (mg/l) and patients' age at baseline.

	Baseline CD4 measurement	
	$0{\cdot}5 \times 10^9$/l	$0{\cdot}2 \times 10^9$/l
No. of patients	47	46
S-β_2m		
⩾3·5	6 (12·8%)	23 (50·0%)
<3·5, ⩾3	11 (23·4%)	11 (23·9%)
<3, ⩾2·5	17 (36·2%)	5 (10·9%)
<2·5	13 (27·7%)	7 (15·2%)
Median	2·7	3·5
Range	1·5–6·5	1·8–6·5
Age (years)		
Median	28·0	30·8
Range	0·4–66·4	16·8–74·4
Spearman correlation coefficient (age versus s-β_2m)	0·35	0·47

Table II. Relative hazards (and approximate 95% confidence intervals) associated with a unit rise in s-β_2m and a 5-year increase in age at baseline.

	$0{\cdot}5 \times 10^9$/l to AIDS		$0{\cdot}2 \times 10^9$/l to AIDS	
	Relative hazard	CI	Relative hazard	CI
Univariate				
s-β_2m	1·68	1·20–2·36	1·42	1·07–1·90
Age	1·03	0·86–1·22	1·05	0·93–1·18
Bivariate				
s-β_2m	2·35	1·43–3·87	1·61	1·07–2·43
Age	0·83	0·68–1·01	0·93	0·80–1·09

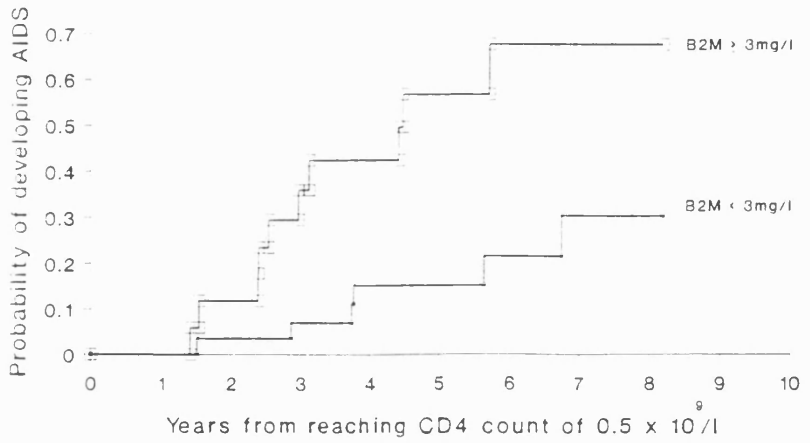

Fig 1. Kaplan-Meier plot of progression from a CD4 count of $0{\cdot}5 \times 10^9$/l to AIDS, stratified by s-β_2m level.

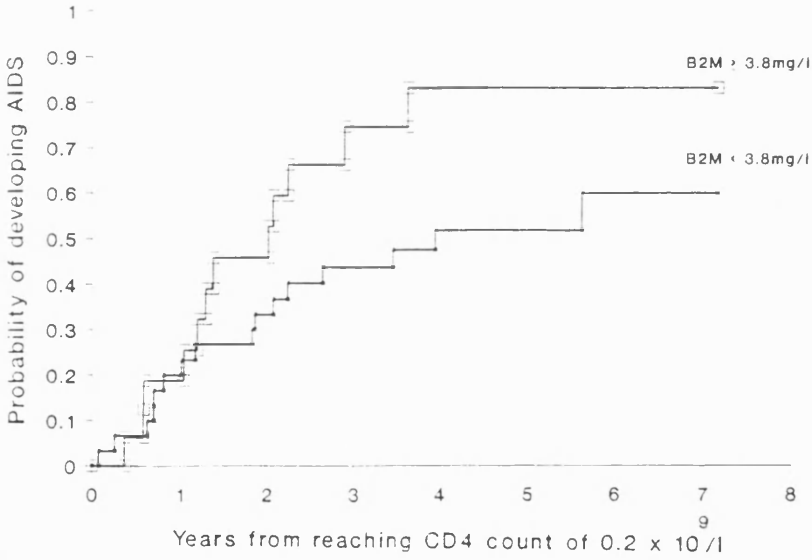

Fig 2. Kaplan-Meier plot of progression from a CD4 count of $0{\cdot}2 \times 10^9$/l to AIDS, stratified by s-β_2m level.

DISCUSSION

We have suggested in this paper a simple method of using the s-β_2m measurement in conjunction with the CD4 count. When the patient's CD4 count drops below $0{\cdot}5 \times 10^9$ l, the s-β_2m level should be measured. The observed value can then be compared to Fig 1 to give an idea of the patient's prognosis, given that they have a CD4 count of approximately $0{\cdot}5 \times 10^9$/l. When the CD4 count falls below $0{\cdot}2 \times 10^9$ l the s-β_2m levels should be measured again and the patient's prognosis, conditional on this low CD4 count, estimated by referring to Fig 2.

This method assigns a well-defined role to s-β_2m in clinical decision making. It is important, therefore, to emphasize that increases in the value of such parameters are not HIV-1 specific. Very high values of s-β_2m can be found in acute viral infections such as CMV, herpes, varicella and hepatitis (Cooper *et al.* 1984; Backman *et al.* 1986; Norfolk *et al.* 1987; Weistal *et al.* 1992), and other conditions associated with immune activation (Backman *et al.* 1986; Norfolk *et al.* 1987; Gianella *et al.* 1989), although such changes are usually temporary, not only in normal individuals but also in HIV-1 infected patients. These factors should be taken into consideration when interpreting individual s-β_2m values.

These results show that at the two CD4 counts chosen, patients with raised s-β_2m levels will tend to progress to AIDS more rapidly than those with lower s-β_2m levels. Additionally, the prognosis associated with a high s-β_2m measurement depends on the level of immunodeficiency. For example, at a CD4 count of $0{\cdot}5 \times 10^9$/l, a s-β_2m level of 3·2 mg/l would tend to indicate a patient with a relatively poor prognosis compared to other patients with similar CD4 counts. However, at a CD4 count of $0{\cdot}2 \times 10^9$/l, a s-β_2m level of 3·2 mg/l indicates a reasonable prognosis compared to most other patients with similar CD4 counts. We conclude that earlier treatment should be considered in patients whose s-β_2m levels have risen above 3 mg/l, without necessarily waiting for their CD4 count to fall below $0{\cdot}2 \times 10^9$/l. It is important to stress that there is a continuous relationship between s-β_2m levels and patient prognosis, and the stratification into two groups is simply for ease of clinical use. Whilst we have chosen the top 33rd percentile as the cut-off for this analysis.

any cut-off point could be chosen. Within each group there will be variability amongst the expected survival times of the patients between those with higher and lower s-β_2m levels.

By only including patients with a s-β_2m level measured within 6 months of CD4 counts of 0·5 and 0·2 × 10^9 l, many others are excluded from the analysis. However, this disadvantage is compensated for by the fact that the remaining groups are homogeneous in terms of their disease state, and therefore the results are useful in a clinical situation where decisions are often based on the CD4 count. Whilst our data has shown the prognostic value of s-β_2m over longer follow-up than almost all previous studies, there is a need for larger studies to repeat this analysis in order to stratify patients into a larger number of s-β_2m groups and to correlate the prognostic significance of s-β_2m with other markers such as neopterin and lymphocyte subsets (Prince *et al.* 1990; Bass *et al.* 1992).

These results are in agreement with other papers in which raised levels of s-β_2m have been shown to be indicative of a poor prognosis. Both Fahey *et al* (1990) and Hofmann *et al* (1990) have suggested that the predictive power of s-β_2m and CD4 counts are of a similar size and are independent (Anderson *et al.* 1990). Our results are very similar to those of Harrison & Skidmore (1990), who suggested that a s-β_2m level of 3·35 mg/l or more is predictive of imminent progression to AIDS, although no attempt was made to control for differences in CD4 count in their study. Schwartlander *et al* (1993) concluded that s-β_2m had additional predictive value whatever range the patient's CD4 count fell into at the time of measurement.

In this cohort, treatment is generally started at a CD4 count of 0·2 × 10^9/l. Recent papers assessing the relationship between zidovudine and s-β_2m levels have shown that the decrease in s-β_2m levels after the introduction of zidovudine is not correlated with changes in CD4 counts (Bass *et al.* 1992). We have seen that our conclusions are essentially unchanged if the data is censored at the end of November 1988, at the time when treatment became generally available to non-AIDS patients in the cohort. Hence, a high s-β_2m level indicates a poorer prognosis, regardless of any treatment effect.

The cohort is made up predominantly of heterosexual men whose only HIV risk was from treatment for their haemophilia. It is not yet known how these results can be generalized to other, non-haemophilic, patients, such as injecting drug-users (IDUs), homosexual men, heterosexual males and females. In particular, s-β_2m levels have been shown to be higher in HIV−ve patients with haemophilia than in non-haemophilic patients (Howard *et al.* 1988). Flegg *et al* (1991) found that s-β_2m levels were increased in HIV-1 negative IDUs when compared to normal controls. Gorter *et al* (1992) observed that levels were also higher in HIV-1 negative black IDUs than in white IDUs. Whilst the absolute levels may differ between risk groups, however, a raised s-β_2m level, relative to that of others in the same risk group, has been shown to be indicative of more rapid progression to AIDS in homosexual men, haemophilia patients and IVDU's (Cuthbert *et al.* 1990; Hofmann *et al.* 1990; Lifson *et al.* 1992; MAP workshop, 1993). Hence, care should be taken when applying our chosen cut-off values to non-haemophilic patients, and further studies into the most appropriate cut-off levels for other risk groups are essential. However, the general strategy for the use of s-β_2m remains unchanged.

Thus, a high s-β_2m measurement indicates patients who are more likely to develop AIDS rapidly irrespective of their CD4 count. The possibility of temporarily raised s-β_2m levels, due to the presence of acute viral infections such as CMV, EBV or influenza (Cooper *et al.* 1984; Backman *et al.* 1986; Norfolk *et al.* 1987; Wejstal *et al.* 1992), should be eliminated and the patients' levels retested if necessary. In the absence of such acute viral infections, our findings would support introduction of antiviral and prophylactic therapies at higher CD4 counts in patients with a raised s-β_2m level (3–3·5 mg/l). However, in view of possible long-term resistance to antiviral therapies, one might also consider delaying treatment for patients who have a low CD4 count, with near normal s-β_2m levels.

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CD4+ Counts before and after Switching to Monoclonal High-Purity Factor VIII Concentrate in HIV-Infected Haemophilic Patients

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Summary

Allogenic proteins that contaminate intermediate purity clotting factor concentrates may activate the immune system of HIV-infected haemophilic patients. In 37 haemophilic patients infected with HIV who had originally been treated with intermediate purity factor VIII concentrate and then changed to monoclonally-purified high purity factor VIII concentrate the rates of CD4+ decline (10^9/l per year) were 0.06 before and 0.02 after a switch to high purity products ($p = 0.01$). The median follow-up of patients after the switch to high purity products was 1.7 years (range 0.2 to 3 years). This significant change in the rate of CD4 decline was independent of the starting CD4 count, age and antiretroviral therapy. This result is consistent with those from randomised trials of the introduction of high-purity concentrate. Given the strong association between the CD4+ count and survival, treatment with high purity rather than intermediate purity clotting factor concentrate may confer a survival benefit for HIV-infected haemophilic patients.

Introduction

Selection of the clotting factor concentrate is a major consideration in the clinical management of patients with haemophilia. The purity of the concentrate is of concern for patients with HIV infection because of the potential detrimental impact of immune activation and dysfunction by alloantigens and other immunomodulatory materials which contaminate intermediate purity concentrates (1, 2).

A number of early studies suggested stabilisation of absolute CD4+ counts following treatment with monoclonally purified product, but these studies were uncontrolled (3–5). Rocino et al. first provided the evidence that avoiding the allogenic impurities present in intermediate purity concentrates resulted in stabilisation of CD4+ counts in a controlled study (6). This study was later extended to include 20 asymptomatic patients randomly assigned to either high purity monoclonal product or to continue on intermediate purity product: after 96 weeks there was a greater fall of CD4+ lymphocyte counts in those receiving the less pure concentrate (7). Another multicenter prospective, controlled trial compared 30 patients treated with monoclonal versus 30 patients treated with intermediate purity concentrate and there was a stabilisation of the CD4+ count in those receiving the monoclonal product (8). In a further study, Hilgartner et al. (9) showed that 36 patients treated with high purity concentrate had a smaller decline in CD4+ counts than 72 matched controls.

Although these studies have shown statistically significant benefits from high purity factor VIII concentrate, they have been criticised for their small numbers. Furthermore, the matter has been confused by the publication of a study showing that a high purity concentrate prepared by ion exchange chromatography (rather than monoclonal technology) does not confer such an advantage in HIV-positive patients (10).

On the basis of these reports the UK Regional Haemophilia Centre Directors have recommended that HIV-positive patients should be treated with high purity clotting factor concentrate (11). However, this treatment remains controversial, particularly because of the high cost involved (12) but also because a larger double-blind controlled trial would now be considered unethical.

In this paper we have estimated the change of CD4+ slope in HIV-positive haemophilic patients before and after switching to monoclonal high-purity factor VIII concentrate, and assess the impact of other cofactors, such as patient age and the introduction of antiretroviral therapy, on this.

Methods

Patients

Between the years 1979 and 1985, 111 men with haemophilia registered at The Royal Free Hospital Haemophilia Centre, became infected with HIV. Serum samples were stored on every patient visit. These serum samples were retrospectively for HIV seropositivity in order to estimate the time of HIV seroconversion (13–15). All patients were male, aged between 2 and 77 years (median 24 years) at the time of their first positive HIV test. The patients are regularly seen every 3–6 months for a complete clinical and laboratory review, including CD4 counts which have been measured since December 1982. By the end of 1992, a median of 17 CD4 counts had been measured on each individual (range 1–52 counts).

Until January 1989, all patients received intermediate purity products from a variety of manufacturers. From January 1989, patients whose CD4 counts dropped below 0.2×10^9/l received high purity products (either "Monoclate", Armour Pharmaceuticals Ltd., Eastbourne, UK, or "8SM", BPL, Elstree, UK). From late 1991, following the UK Regional Haemophilia Directors' guidelines (11), all HIV-positive patients were switched to these high purity products, irrespective of their CD4 count.

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In our protocol antiviral therapy is introduced at a CD4 count of 0.2 × 10^9/l and for AIDS or CDC IV disease (16). Since August 1987 to the end of 1992, 51 patients have been treated with zidovudine, including 10 patients who received active drug in the MRC/ANRS Concorde trial of immediate versus deferred zidovudine. Prophylaxis is also introduced at a CD4 count of 0.2 × 10^9/l. Prophylaxis for *Pneumocystis carinii* Pneumonia (PCP) with pentamidine or co-trimoxazole began in February 1988. By the end of 1992 36 patients (26 primary, nine secondary) had received it. Prophylaxis for candidiasis using fluconazole began in April 1988 and by the end of 1992 36 patients (13 primary, 26 secondary) were treated.

Laboratory Methods

Between 1982 and 1986 the absolute CD4 counts were calculated from the lymphocyte count and CD4% values. The absolute lymphocyte count was determined by an automated whole blood counter (Ortho "ELT 800" with differential screen) and the percentages of CD4 lymphocytes were counted in Ficoll-Hypaque separated blood mononuclear cell suspensions using an EPICS V flow cytometer (Coulter Electronics) (14). Since 1986 a whole blood lysis method has been used, and percentage of CD4 lymphocytes analysed by flow cytometry, either using a FACScan (Becton Dickinson, Crawley, UK) (17). A monoclonal CD4 antibody, RFT4, to the p55 CD4 antigen was used in concentration with a monoclonal CD3 antibody (UCHT1) as described previously (17). Most recently absolute CD4 counts have been directly obtained on an ORTHO Cytoron-Absolute (ORTHO Diagnostics, High Wycombe, UK). Flow cytometer quality control was monitored in the UK National External Quality Assurance Scheme. We have compared CD4 counts from before and after the change in methods in 1986 and have seen no consistent difference.

Statistical Methods

Rates of CD4 decline have been estimated using linear regression methods. In order to remove the possible bias of the CD4+ counts "flattening out" as they reach a very low level, irrespective of high purity factor VIII usage, CD4+ counts were only included until the count fell below a threshold of 0.03 × 10^9/l; CD4+ counts after this measurement were excluded from the analysis. The choice of 0.03 × 10^9/l was arbitrary, to allow sufficient patient experience between CD4 counts of 0.2 and 0.03 × 10^9/l, but being high enough to remove as much bias as possible. In order not to place undue weight on estimates of slopes based on only two CD4 counts, rates of decline were estimated only in those patients who had three or more CD4+ counts before as well as after the introduction of high purity products. Follow-up was complete to 31st December 1993.

Tests for significance of differences between rates of decline were performed using non-parametric methods (Wilcoxon paired sign-rank test for assessing whether there was any overall slope change, and Kruskal-Wallis test for a comparison of the changes in rate of decline between subgroups). The patient's age and CD4+ count at introduction of high purity concentrate, zidovudine usage (before and after introduction) and calendar year of starting high purity products, were all assessed for association with the change in the rate of decline after the switch to high purity concentrate.

Results

By the end of 1992 61 of the 111 patients in the cohort had received high-purity products. In 31 of the 50 patients not receiving high-purity products death occurred before the switch to high purity products and in the remaining 19 individuals the information was incomplete (Fig. 1). Twelve patients (20%) receiving high purity products died during follow-up.

The 61 patients had three or more CD4+ counts measured prior to the switch to high purity products (median number of counts 14, range 5–32). CD4+ counts immediately before the switch to high purity products ranged from 0 to 1.19 × 10^9/l (median 0.3 × 10^9/l). After the switch to high purity products, 37 of the 61 patients had three or more CD4+ counts above the CD4+ threshold (median number of counts 6, range 3–25). The median follow-up of patients after the switch to high purity products was 1.7 years (range 0.2 to 3 years). The paired analysis of CD4 slopes before and after the introduction of high purity products is carried out in these 37 patients (Fig. 1).

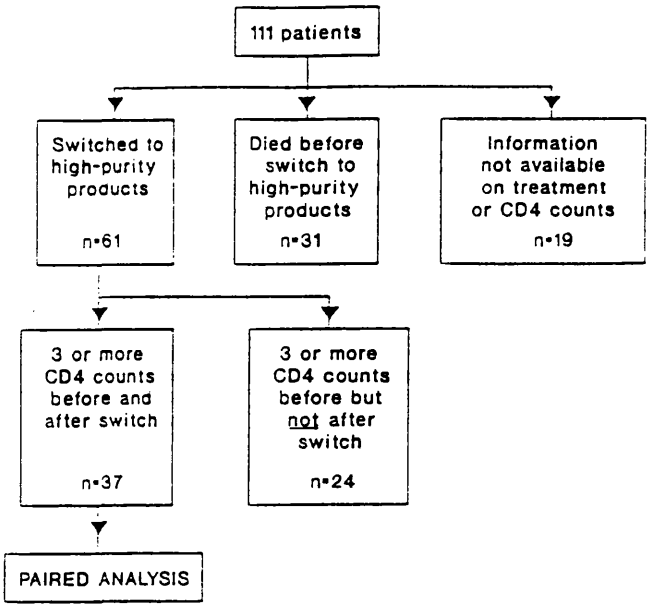

Fig. 1 Algorithm showing number of patients with available CD4 counts for the estimation of rates of decline before and after the introduction of high-purity products

Table 1 Median change in the rate of CD4+ decline (and range). A positive change indicates that the rate of decline has become less steep, whereas a negative value indicates that the rate of decline has become more steep

		Change in rate of CD4+ decline (×10^9/l per year) Median	Range	p-value*
All patients		0.04	0.74 to –0.28	0.01
CD4 count (×10^9/l)	≥0.5	0.02	0.25 to –0.28	
	<0.5, ≥0.2	0.02	0.74 to –0.19	
	<0.2	0.07	0.20 to –0.07	0.22
Age (years)	≤30	0.04	0.74 to –0.28	
	>30	0.05	0.20 to –0.07	0.75
total monoclate usage (units)	<64130	0.08	0.74 to –0.28	
	≥64130, <168910	0.03	0.13 to 0.00	
	≥168910	0.05	0.25 to –0.07	0.90
Zidovudine usage	Before monoclate	0.04	0.16 to –0.10	
	After monoclate	0.04	0.20 to –0.03	
	None	0.04	0.74 to –0.28	0.99
Calendar year of switch to high-purity products	Before 1991	0.02	0.25 to –0.10	
	1991	0.08	0.16 to –0.14	
	1992	0.07	0.74 to –0.28	0.56

* Based on results from paired Wilcoxon test for overall test of change in slope and Kruskal-Wallis test where differences are compared between subgroups.

Whilst on intermediate purity products CD4 counts in the 37 patients declined by, on average, 0.06 × 10^9/l per year (range 0.21 × 10^9/l decline to 0.01 × 10^9/l increase per year). In these same patients, after switching to high purity products CD4 counts fell by 0.02 × 10^9/l per year (range 0.28 × 10^9/l decline to 0.75 × 10^9/l increase per year). The change in the rate of CD4+ decline before and after the switch to high purity products was significant ($p = 0.01$, paired Wilcoxon test). CD4+ counts fell, on average, by 0.04 × 10^9/l less per year after the introduction of high purity products than before (range 0.74 × 10^9/l less to 0.28 × 10^9/l more per year).

Table 1 shows the change in the rate of CD4+ decline after the switch to high purity products in relation to other cofactors: CD4 count at time of switch, age at time of switch, the total amount of high purity product received, zidovudine usage and the calendar year of change of treatment. The p-values show no significant association between change in the rate of CD4+ decline and any of these parameters.

A further 24 patients had more than three CD4 counts prior to the introduction of high purity products, but had fewer than three after the introduction (see Fig. 1). By excluding these patients from the analysis a bias might have been introduced because there is the possibility that patients with more rapidly declining CD4 counts have been preferentially left out from the investigation. However, rates of decline whilst receiving intermediate purity products in the 24 patients excluded were similar to those in the 37 patients included (median rate of decline in 24 patients: 0.09 and 0.06 × 10^9/l per year respectively, $p = 0.1$, Wilcoxon test for differences between the two groups).

The analysis was also repeated using a number of different threshold levels (from 0.05 to 0.01 × 10^9/l) and the results remained essentially unchanged. As expected, the results also remain unchanged when no threshold is enforced.

Discussion

Our study has shown a significant decrease in the rate of CD4+ decline following a switch from intermediate to high purity monoclonal product using patients as their own controls, thus reducing the effect of between-patient variability which is often found in case-control studies. Furthermore, in univariate models, neither the CD4+ count, nor age at the introduction of treatment, nor the amount of high purity product received nor zidovudine usage either before or after switching to high purity products were found to be associated with the change in rate of CD4+ decline. Hence the possible confounding effects of these variables have been eliminated, as far as is possible outside a randomised trial setting. A further strength of our study is that CD4+ counts have been performed in the same laboratory, subject to rigorous quality controls since 1982 (15). However, it is important to stress that whilst in our study high purity products appear to confer a benefit for patients, the CD4+ counts did not fully stabilise: the rate of decline simply became less steep. Whilst we have not directly considered the effect of high purity products on either clinical endpoints or survival, this reduction in rate of CD4+ decline would be expected to result in an improvement in patient survival (18, 19).

We have chosen a CD4+ threshold of 0.03 × 10^9/l beyond which CD4+ counts have been censored. The reason for this was to remove the bias of CD4+ counts "flattening out" at very low levels, an effect which would only serve to make the change in CD4+ decline after the introduction of high-purity products even more extreme. The choice of 0.03 × 10^9/l as the threshold was arbitrary. By enforcing this threshold we may have removed some patients from the analysis whose CD4+ counts were declining very rapidly and fell to below 0.03 × 10^9/l in less than three measurements. Nevertheless, the rates of CD4+ decline pior to the introduction of high purity concentrates in the 24 patients without three CD4+ counts above the threshold after the switch, whildst slightly more rapid, were not significantly faster. CD4+ counts prior to the introduction of high purity products in these patients were low (median 0.04 × 10^9/l. 10/12 of the patient's counts <0.1 × 10^9/l), indicating that high purity was introduced at a late stage. If a higher threshold were chosen a greater number of patients would have been excluded from the analysis. An alternative approach would be to model the CD4+ decline in a non-linear way to take account of the "flattening out" effect. However, as there is little evidence to support the choice of such a transformation and the pattern of CD4+ decline seems approximately linear until the CD4 count falls to a very low level (15), this transformation would not be an appropriate model of CD4+ decline.

The beneficial effect of high purity monoclonal product is presumably due to the "removal" of alloantigens and other immunomodulatory components that are present in intermediate purity products. In HIV uninfected haemophiliacs these effects are probably of little or no clinical significance (2). Thus all HIV-negative haemophilic boys treated with intermediate purity product maintained consistently normal CD4+ counts (20). However, in HIV infected haemophiliacs the abnormalities of cellular and humoral immunity induced by residual components present in less pure clotting factor concentrates may act as a cofactor for CD4+ decline. Such components could include transforming growth factor-β (TGF-β) which is present in intermediate purity concentrates (21) and has been shown to activate HIV *in vitro* (22). Exclusion of TGF-β from concentrates by monoclonal technology would reduce HIV activation and further CD4+ decline.

These results pose obvious questions about the rate of progression of HIV disease in haemophilic patients compared to the rate in other risk groups. A recent comparative study has shown progression to HIV disease is faster in homosexuals, followed by haemophiliacs and intravenous drug users (23). There have been many differences between these risk groups such as differing common AIDS-defining conditions, the prevalence and effect of hepatitis C virus infection, other sexually transmitted diseases, intravenous drug use and different age distribution. It is inevitable that each risk group has exposure to many and differing antigenic challenges which influence the rate of progression.

Although longer follow-up is required to confirm the beneficial effects of monoclonal high-purity factor VIII concentrate, given the strong association between the CD4+ count and survival (19) it is possible that such treatment could confer a survival benefit for HIV infected haemophilic patients.

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The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion

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SUMMARY

The effect of prior infection with cytomegalovirus (CMV) on progression of HIV disease in a cohort of 111 men with haemophilia was studied after 13 years follow-up. The relative hazards associated with CMV positivity on progression to AIDS, death and a CD4 count of $0{\cdot}05 \times 10^9$/l were 2·28, 2·42 and 2·34, respectively. CMV seropositive patients were significantly older than the seronegative and this was controlled for by using a Cox proportional hazards model. The relative hazards for the three endpoints decreased to 1·89, 1·82 and 1·93 respectively and were marginally non-significant ($P = 0{\cdot}05$, 0·08 and 0·08 for the three endpoints respectively). We conclude that this cohort continues to show evidence of a 'co-factor' effect associated with prior infection with CMV which is confounded by age but not completely explained by age differences. The potential biological significance of these results is discussed in the context of recent controlled clinical trials which show a survival benefit from long-term high-dose acyclovir, a drug with activity *in vivo* against CMV and other herpesviruses.

INTRODUCTION

Although the human immunodeficiency virus (HIV) is the known cause of the acquired immune deficiency syndrome (AIDS), the incubation period from infection with HIV to the development of AIDS is long and variable [1–4]. This wide variation in the incubation period is far greater than that found for most other acute viral diseases, suggesting that other factors may contribute to disease progression. Among these, infectious agents, termed co-factors, could interact with HIV and increase its pathogenicity.

Different viruses could interact with HIV by a variety of mechanisms [5]. For example, some viruses, including herpes simplex virus (HSV), cytomegalovirus (CMV), hepatitis B virus and Epstein–Barr virus have all been shown to

transactivate HIV *in vitro* [6–9]. However, there are only a few studies which assess the relative importance of these viral co-factors *in vivo*. It is well documented that older individuals experience more rapid progression of HIV disease than younger individuals, perhaps due to a more rapidly declining immune system in older individuals [10–12]. As the prevalence of antibodies to HSV and CMV increases with age [13], any assessment of the potential effect of these viruses on progression of HIV disease must take account of the possible confounding effect of age.

We have chosen to study whether CMV could act as a co-factor for HIV disease in a cohort of men with haemophilia in whom the prevalence of CMV infection is 60%, similar to that seen in young blood donors and attenders at antenatal clinics [14]. We have previously reported that in this cohort prior CMV infection is associated with more rapid progression to AIDS [15] but this has not been confirmed by others [16–19]. Here we extend follow-up to 13 years to assess whether this association remains after adjustment for age differences. Furthermore, sufficient follow-up is now available on the patients to analyse both survival and immune depletion as endpoints.

MATERIALS AND METHODS

Patients and their clinical management

A total of 111 men with haemophilia registered at the Royal Free Hospital Haemophilia Centre, London, became infected with HIV between 1979 and 1985 following treatment with unsterilized blood clotting factor concentrates [20, 21]. All patients are seen at the Centre approximately every 3–6 months when they undergo a clinical and laboratory review. Serum samples are regularly taken and stored at −20 °C. It has been possible retrospectively to test these serum samples for HIV seropositivity and hence dates of seroconversion have been estimated for all patients [20].

Zidovudine has been available for individuals with AIDS and AIDS-related complex (ARC) since 1987, and from October 1988 it has been available as part of the MRC/Agence Nationale de Recherches sur le SIDA (ANRS) Concorde trial of early versus deferred zidovudine. Secondary prophylaxis for *Pneumocystis carinii* pneumonia (PCP) with pentamidine or co-trimoxazole has been available since March 1988, and primary prophylaxis since February 1989. Secondary prophylaxis for candidiasis with fluconazole has been available since March 1988 and primary prophylaxis since April 1990. All patients are advised to start zidovudine and primary prophylaxis once their CD4 count is $< 0{\cdot}2 \times 10^9$/l. Patients developing either PCP or candidiasis are offered secondary prophylaxis regardless of their CD4 count. To date 51 patients have received zidovudine, 35 PCP prophylaxis (26 primary, 9 secondary) and 39 prophylaxis for candidiasis (13 primary, 26 secondary). Currently, whilst acyclovir may be given to patients experiencing herpes zoster repeatedly, it is not routinely prescribed for patients in the cohort.

Laboratory methods

Between 1982 and 1986 absolute CD4 counts were calculated from the lymphocyte count and CD4 percent values [20]. A whole blood lysis method has

been used since 1986, and the percentage of CD4 lymphocytes analysed by flow cytometry, using a FACScan (Becton Dickinson, Crawley, UK) [22]. A monoclonal CD4 antibody (RFT4) to the p55 CD4 antigen was used with a monoclonal CD3 antibody (UCHT1) as described previously [22]. More recently absolute CD4 counts have been directly obtained on an ORTHO Cytoron-Absolute (ORTHO Diagnostics, High Wycombe, UK). Quality control of flow cytometry was monitored as part of the UK National External Quality Assurance Scheme. We have compared CD4 counts in these patients from before and after the change in methods in 1986 and have seen no consistent difference. Antibodies to CMV were measured on early stored serum samples by radioimmunoassay as described elsewhere [23].

Statistical methods

Comparisons of patients with and without antibody to CMV were done using standard non-parametric methods (Mann–Whitney *U* test [24]). Analyses which assessed the effect of CMV on HIV disease progression were performed using standard survival methods. Plots of survival from seroconversion to endpoints of AIDS and death were estimated using Kaplan–Meier methods [25] and the univariate effect of CMV status on these were tested for significance using the log-rank test. Cox proportional hazards models [26] were used to assess the independent effect of CMV status on disease progression after adjusting for the patient's age and CD4 count in multivariate models using the procedure 'PROC PHREG' in the Statistical Analysis System (SAS) package [27]. For this analysis, progression to an endpoint of a CD4 count of $0{\cdot}05 \times 10^9$/l was also used. The low CD4 count of $0{\cdot}05 \times 10^9$/l was chosen as an endpoint due to the rapidly increasing risk of death once the CD4 count has fallen to this level [28]. The approximate date on which the CD4 count fell to $0{\cdot}05 \times 10^9$/l was estimated by linear interpolation. When studying progression to AIDS or a low CD4 count, patient follow-up was right-censored at death or at the cut-off date for the analysis (31 December 1992), if the patient had not developed AIDS or reached a low CD4 count by that time. When studying progression to death, patient follow-up was right-censored at December 1992 if still alive on that date. CMV status and age at seroconversion were considered to remain fixed throughout follow-up, and the patient's CD4 count was modelled as a time-dependent covariate. In order to assess the validity of the proportional hazards assumption, an interaction term between the logarithm of time and CMV status was added to the model and tested for significance.

In order to estimate the potential effect of CMV positivity on death at certain CD4 levels, the dates on which the CD4 count was estimated to fall below certain levels ($0{\cdot}2$, $0{\cdot}3$, $0{\cdot}4$, $0{\cdot}5$, $0{\cdot}6 \times 10^9$/l) were calculated using linear interpolation. Survival methods were used to assess the prognosis associated with CMV seropositivity, after adjustment for patient age at each CD4 baseline date.

In the UK, prophylaxis for PCP and candidiasis and antiretroviral therapy (zidovudine) for patients without AIDS became available from 1987 onwards. The direct modelling in this cohort of treatment effects is limited as few data are available on individual treatment usage in the cohort. Further, most treatment is instigated once the CD4 count falls below $0{\cdot}2 \times 10^9$/l, resulting in treatment effects

Table 1. *Comparison of CMV-positive and CMV-negative patients in the cohort*

Patient characteristics		CMV-positive	CMV-negative
Number of patients		59	50
Date of seroconversion*	Median	May 1982	April 1982
	Range	Oct 1979–Mar 1985	Nov 1979–Jul 1985
Age at seroconversion (years)†	Median	25·6	18·7
	Range	4·0–77·8	2·1–73·0
Number developing AIDS		30 (50·8%)	14 (28·0%)
Number of deaths		30 (50·8%)	12 (24·0%)

* P-value = 0·77.
† P-value = 0·03.

which are confounded with those of the CD4 count. Consequently, in order to assess whether these results were independent of the treatment, all analyses were repeated with the inclusion of an additional covariate to represent simply the availability of pre-AIDS prophylaxis and antiretroviral therapies. This was included in the model as a time-dependent covariate taking the value of zero before November 1987 and one after. This then allows for the fact that patients who have survived at least to November 1987 have the added survival advantage of any therapy available to them, whether or not they actually receive it.

RESULTS

Antibodies to CMV were measured on early blood samples from patients in the cohort. 59/109 (54%) were found to have antibody to CMV. CMV status is unknown for two patients. A comparison of the patients known to be seropositive and seronegative for CMV is shown in Table 1. CMV positive patients were older at seroconversion to HIV, but in general they did not seroconvert any earlier or later than those who were CMV negative.

By the end of 1992, a total of 44 of the patients had developed AIDS and 42 had died, with Kaplan–Meier progression rates of 47·1 and 48·3% by 13 years, respectively. CD4 counts had fallen below $0{\cdot}05 \times 10^9$/l on at least one occasion in 35 patients, a progression rate of 43·3% by 13 years after seroconversion. Unadjusted for age, CMV status was significantly associated with a faster progression to AIDS ($P = 0{\cdot}009$, log-rank test) and to death ($P = 0{\cdot}008$, log-rank test). Progression rates to AIDS and death, stratified by CMV status are shown in Figs 1 and 2. The effect of CMV status on progression to AIDS and death remains apparent, although reduced slightly, in both older and younger individuals.

Cox proportional hazards models were used to quantitate the effects of CMV seropositivity on the hazards of developing AIDS, of dying or of reaching a low CD4 count, after adjusting for age at HIV seroconversion (Table 2). Using this approach, before adjusting for age, patients known to be CMV positive were over twice as likely to develop AIDS, die or reach a low CD4 count as those known to be CMV negative. After adjusting for the patient's age at seroconversion, the effect of CMV positivity on progression to all three end points decreased slightly and became marginally non-significant ($P = 0{\cdot}05$, 0·08 and 0·08 for progression to AIDS, death and a CD4 count $< 0{\cdot}05 \times 10^9$/l respectively). In order to assess whether the effect of CMV status acts through the CD4 count, the relative hazards

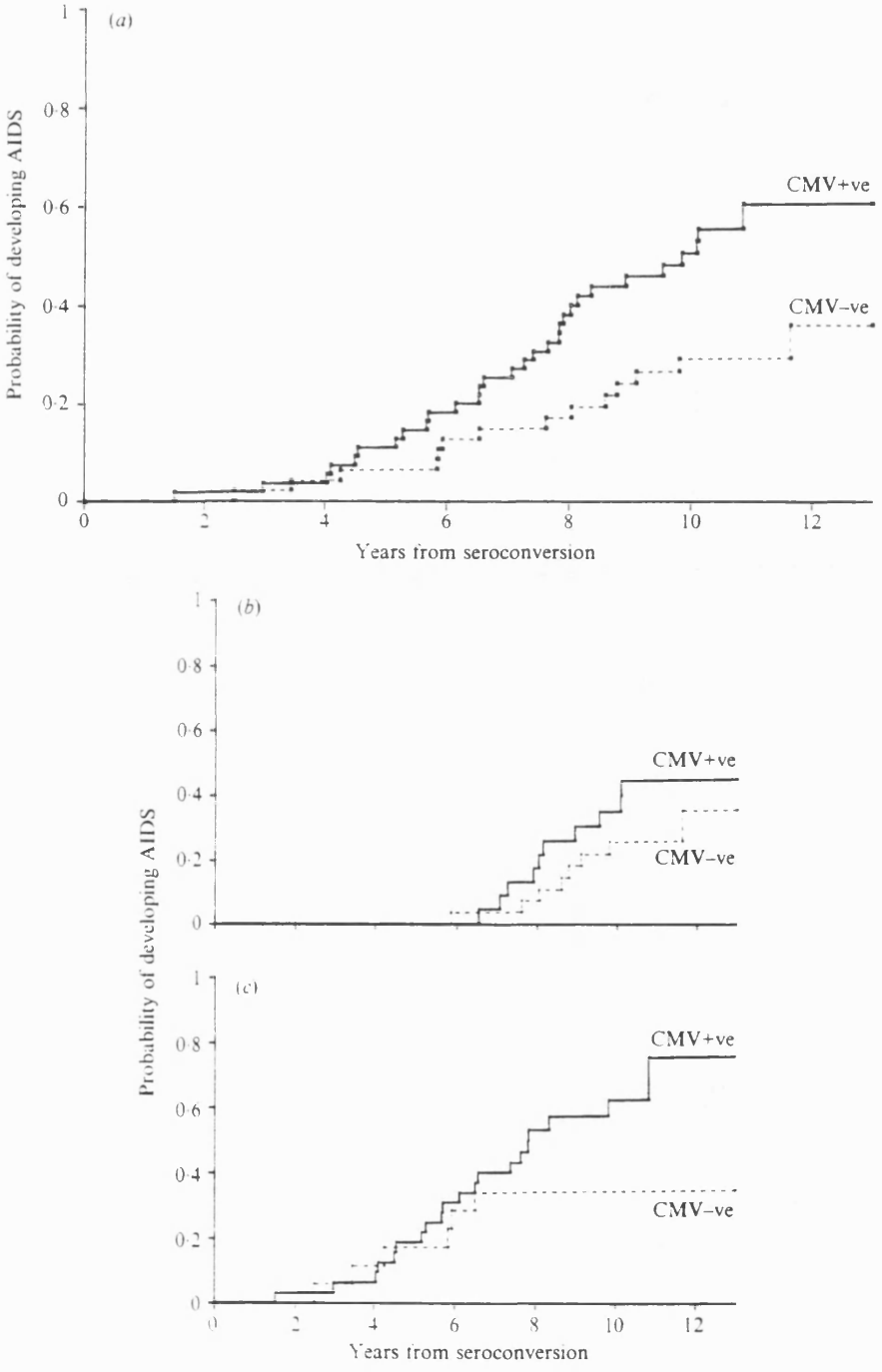

Fig. 1. Kaplan–Meier curves showing progression from HIV seroconversion to the development of AIDS, stratified by CMV status at seroconversion for (i) all patients, (ii) patients ≤ 35 years at seroconversion, and (iii) patients > 35 years at seroconversion.

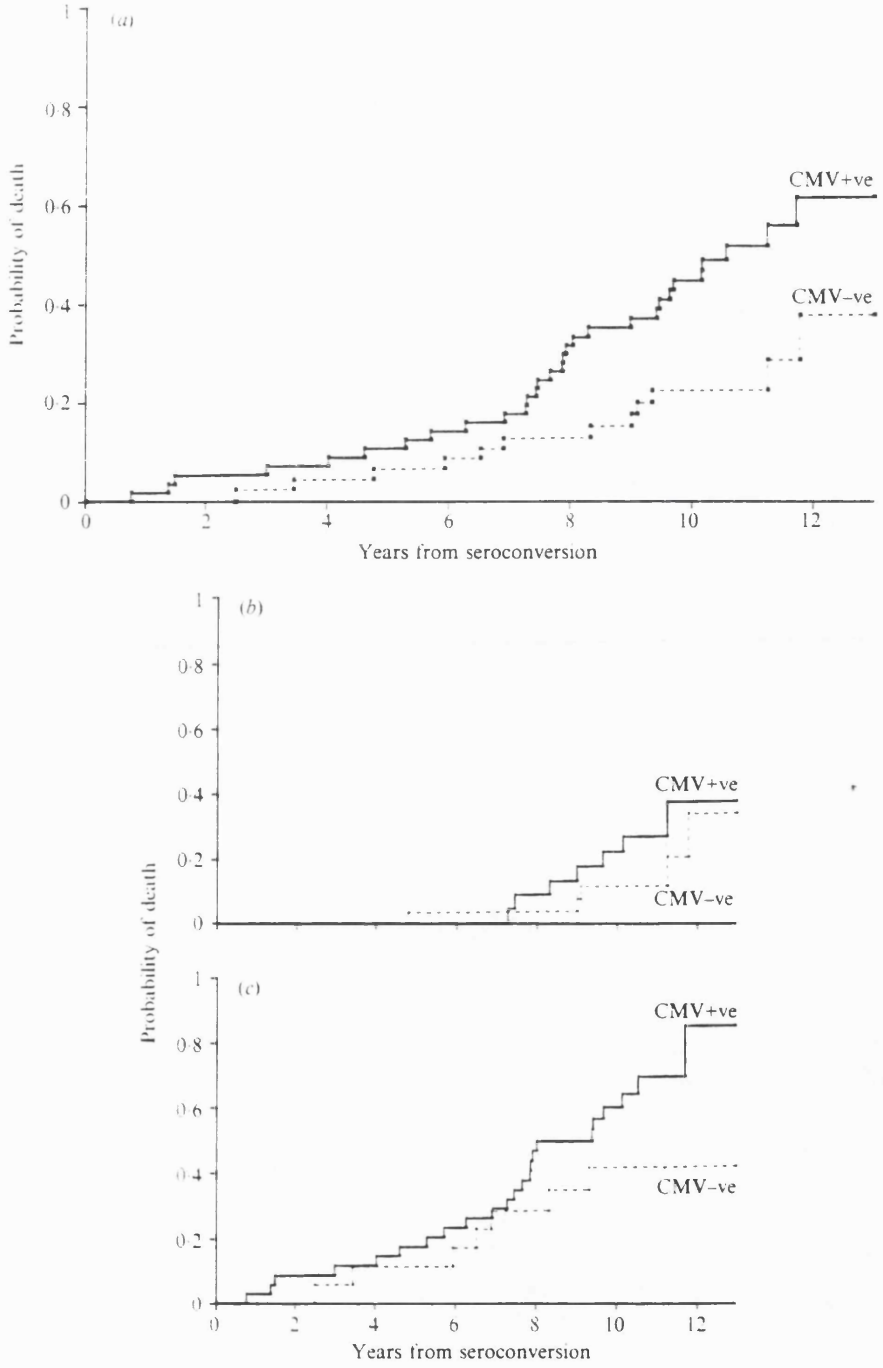

Fig. 2. Kaplan–Meier curves showing progression from HIV seroconversion to death, stratified by CMV status at seroconversion for (i) all patients, (ii) patients ≤ 35 years at seroconversion, and (iii) patients > 35 years at seroconversion.

Table 2. *Relative hazards (and 95% confidence intervals) of progression to AIDS, death or a CD4 count of 0·05 × 10⁹/l associated with CMV positivity unadjusted for other factors, and with CMV positivity after adjustment for patients' age at seroconversion and most recent CD4 count*

	Progression to		
	AIDS	Death	CD4 0·05 × 10⁹/l
Unadjusted	2·28 (1·20–4·31)	2·42 (1·24–4·73)	2·34 (1·14–4·79)
Adjusted for age	1·89 (0·99–3·60)	1·82 (0·93–3·58)	1·93 (0·93–4·01)
Adjusted for age and CD4 count*	2·17 (1·13–4·16)	1·73 (0·88–3·39)	— —

* Progression to CD4 count of 0·05 × 10⁹/l is not adjusted for most recent CD4 count.

Table 3. *Relative hazards (and 95% confidence intervals) of death associated with CMV status. Baseline dates are defined as the date on which the CD4 count first fell below certain levels. Relative hazards for CMV status are adjusted for age at baseline*

Baseline CD4 count (× 10⁹/l)	No. of patients	No. of events	Relative hazard	95% CI
0·2	60	30	1·79	0·80–4·04
0·3	66	26	2·83	1·12–7·14
0·4	69	23	2·40	0·92–6·27
0·5	59	21	2·71	0·95–7·77
0·6	54	19	1·74	0·61–4·92

of developing AIDS and dying associated with CMV status are also shown in Table 2 after adjustment for the patients' most recent CD4 count, updated as a time-dependent covariate. After this additional adjustment the effect of CMV on survival was further reduced, whilst the effect of CMV on progression to AIDS returned towards its original value and attained statistical significance. Because of its obvious relationship with the most recent CD4 count, the relative hazard associated with CMV positivity on progression to a CD4 count of 0·05 × 10⁹/l was not adjusted for the patients' CD4 counts during follow up.

The addition of an interaction term between CMV status and log(time) to the model did not significantly improve the fit of the model ($P = 0·71$), suggesting that the proportional hazards assumption was reasonable.

Examination of the Kaplan–Meier plots suggested that the CMV effect may be smaller in younger patients than in older patients. However, the addition of an interaction term between CMV status and age was not significant for any of the three endpoints ($P = 0·85$, 0·78 and 0·88 for progression to AIDS, death and a CD4 count of 0·05 × 10⁹/l respectively).

In order to assess whether the hazard of death associated with CMV seropositivity was dependent on the CD4 count of the patient, the proportional hazards model was fitted with baseline dates defined as the first time a patients CD4 count fell to a certain level (0·2, 0·3, 0·4, 0·5, 0·6 × 10⁹/l). Results from this

analysis are shown in Table 3 and indicate that the size of effect of CMV status remains reasonably constant, after adjustment for age at CD4 baseline at all CD4 baselines.

The analyses were repeated with the addition of a term representing the availability of pre-AIDS prophylaxis and antiretroviral therapies. With the inclusion of this term the results were essentially unchanged.

DISCUSSION

The results presented here show that there is an association between CMV seropositivity and HIV disease progression measured by the time to AIDS, and also time to a low CD4 count and time to death. Whilst some of these effects may be explained by differences in age at seroconversion in the two groups, there remains a residual effect of CMV status on progression to AIDS which is unexplained by adjustment for this factor. We will discuss our findings and those of others for each of the three endpoints assessed.

Time to AIDS

For the time to AIDS we find an effect of CMV status (relative hazard 2·28) which is somewhat lower (relative hazard 3·2) than we reported in 1989 [15]. After controlling for age the relative hazard has also declined from 2·5 to 1·9. The results depicted in Figs 1 and 2 show that CMV seropositive patients progress to AIDS more rapidly than seronegative patients, even when considering younger and older individuals separately. After adjustment for patient age in the Cox proportional hazard model, the relative hazard fails to reach the conventional 5% level of significance (Table 2). However, the fact that the lower limit of the confidence interval is only marginally below 1 suggests that strict adherence to this convention is not sensible: a slight change in either the length of follow-up or an extra AIDS case could esily lead to a relative hazard which was the same but statistically significant. We conclude that prior CMV infection approximately doubles the risk of progression to AIDS. Comparisons of the Kaplan–Meier plots within the two age groups with those generated from all patients show that age differences do not completely explain the CMV effect. Unfortunately we do not have information on when patients seroconverted to CMV. There is a suggestion that the CMV effect is smaller in younger individuals than in older individuals. If any effect of CMV status on progression of HIV disease exists, then those who have been infected with CMV for longest and who are also likely to be those who were older at HIV seroconversion, may experience the greatest effect of CMV on the progression of their disease. However, there is no evidence of an interaction between age and CMV status. This suggests that this is unlikely to be the case so that the differences which are apparent from the Kaplan–Meier plots are more likely to be the result of random variation due to the small numbers of individuals who are younger and who have antibody to CMV. Two of the AIDS-defining conditions, CMV retinitis and other CMV disease, are CMV-related [29] and so this might be one possible explanation for our findings. However, in our cohort only one patient developed either of these two conditions as his initial AIDS-defining condition, showing that this relationship cannot expain our results.

A number of other studies have reported findings on the potential effect of CMV positivity on HIV disease progression. Three studies [16–18] found no statistically significant effect of CMV on progression to symptomatic HIV infection or AIDS. However, these studies had flaws either in design or conduct [30]. Results from a recent study from the United States of men with haemophilia with known dates of HIV seroconversion [19] did not find a significantly raised risk of AIDS associated with CMV seropositivity after adjustment for age, and offered the strongest evidence against the hypothesis that CMV status is associated with rapid disease progression. The patients included in that study were of a similar age range to those in our study and the proportion who were CMV positive was also similar. Serum samples were tested for CMV antibody under code in London using the same radioimmunoassay, thus removing the effect of laboratory differences. Because both studies are carried out in haemophilic patients, the effects of other possible co-factors (e.g. gender, HIV exposure category, intravenous drug use, etc.) on progression rates between the two studies are unlikely to explain these differences. However, it may be possible that differences in covariates other than those currently known to be associated with progression of HIV disease could explain the contrasting results, and we are continuing to collaborate with the USA investigators to study this with the aim of identifying other previously unrecognized factors which may explain the differences. These may include differences in patient selection methods or in the background prevalence rates of antibodies to other viruses in the patient populations.

Survival

Sufficient follow-up has now occurred in our cohort to allow an analysis of survival which shows a raised risk of death associated with CMV seropositivity. The association of CMV status with death is not attributable to clinically recognized CMV disease. Thus there is no evidence that CMV is causing opportunistic disease and we have argued elsewhere that this supports the concept of CMV as a 'co-factor', increasing the burden of disease without declaring itself clinically [5]. This concept is supported by autopsy findings that CMV is present in at least one tissue in 66% of AIDS patients [31].

It is interesting to speculate on the clinical effects of anti-CMV therapy administered to AIDS patients. Recently, two controlled trials used acyclovir in homosexual men with advanced HIV disease [32, 33]. In both trials an approximately 40% reduction in the number of deaths was seen in patients receiving acyclovir compared to those in the control group. As acyclovir is known to inhibit the DNA polymerase enzyme of several herpesviruses, such a virus could be involved in the pathogenesis of death in AIDS patients. CMV is a strong candidate in this setting because CMV has been found by cell culture in the majority of AIDS patients coming to autopsy [31] and because controlled trials in transplant patients have shown that high-dose acyclovir can decrease CMV replication and disease [34–36]. All patients in these trials were CMV positive and had low CD4 counts. Results from Table 3 suggest that at low CD4 counts a relative hazard value < 2 associated with death is reasonable and consistent with the approximate halving of the death rate in these two trials, which again suggests that the inhibition of CMV replication is a plausible reason for the reduced

mortality seen. Further studies of quantitative CMV virology during such trials will be required to determine if suppression of CMV, and/or other herpesviruses, correlates with improved survival. This work is in progress under the auspices of the AIDS Clinical Trials Group.

Low CD4 count

The hypothesis that the CMV effect might be mediated through loss of CD4 cells was investigated. The results presented here suggest that CMV status has a significant effect on the rate of CD4 decline, as shown by a faster progression to a CD4 count of $0{\cdot}05 \times 10^9/l$ in CMV seropositive individuals than in seronegative. It has previously been shown that there is an effect of age on progression to AIDS which is not completely explained by more rapidly declining CD4 counts [12]. Certainly, the effects of CMV, age and CD4 count do not act totally independently of each other. However, results from Table 2 suggest that whilst CMV positives do indeed progress to a CD4 count of $0{\cdot}05 \times 10^9/l$ more rapidly than CMV negatives, largely independently of their age at seroconversion, this rapid drop in CD4 cells does not fully explain the CMV effect on progression to AIDS. Further functional studies of activated CD8+ T lymphocytes are required to answer these questions.

In summary, our results show a continued association between CMV and progression of HIV disease which cannot entirely be explained by age. These results have implications for both our understanding of the pathogenesis of HIV and for therapy, which are being actively pursued in clinical trials of anti-herpes drugs.

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Use of CD4 lymphocyte count to predict long term survival free of AIDS after HIV infection

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Abstract

Objective—To estimate the probability of remaining free of AIDS for up to 25 years after infection with HIV by extrapolation of changes in CD4 lymphocyte count.

Design—Cohort study of subjects followed from time of HIV seroconversion until 1 January 1993. Creation of model by using extrapolated linear regression slopes of CD4 count to predict development of AIDS after 1993.

Setting—Regional haemophilia centre in teaching hospital.

Subjects—111 men with haemophilia infected with HIV during 1979-85. Median length of follow up 10·1 years, median number of CD4 counts 17. The model was not fitted for three men because only one CD4 measurement was available.

Main outcome measures—Development of AIDS.

Interventions—From 1989 prophylaxis against candida and *Pneumocystis carinii* pneumonia and antiretroviral drugs when CD4 count fell below 200×10^6/l.

Results—44 men developed AIDS up to 1 January 1993. When AIDS was defined as a CD4 count of 50×10^6/l the model predicted that 25% (95% confidence interval 16% to 34%) would survive for 20 years after seroconversion and 18% (11% to 25%) for 25 years. Changing the CD4 count at which AIDS was assumed to occur did not alter the results. Younger patients had a higher chance of 20 year survival than older patients (32% (12% to 52%) for those aged <15, 26% (14% to 38%) for those aged 15-29, and 15% (0% to 31%) for those aged ≥30).

Conclusions—These results suggest that even with currently available treatment up to a quarter of patients with HIV infection will survive for 20 years after seroconversion without developing AIDS.

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Introduction

Current knowledge of the natural course of infection with the HIV type 1 is limited by the length of follow up studies. The longest running studies have followed infected subjects for a maximum of around 14 years since seroconversion, although few studies have followed many patients beyond 10 years.[1-11] Depending on the age of the groups studied current estimates suggest that between 25% and 60% of people do not develop AIDS over this period.[1-11] Since AIDS tends to develop only after patients' CD4 lymphocyte counts have reached low levels[12-19] useful projections can now be made of its long term course. These provide a background against which to make decisions concerning early intervention with antiretroviral drugs. In this paper, we update analyses done four years ago[20] and use serial CD4 lymphocyte counts measured in a cohort of 111 haemophilic men followed for up to 13 years from seroconversion to estimate the probability of not developing AIDS for up to 25 years after seroconversion.

Patients and methods

The cohort which has been described,[15 20] consisted of 111 haemophilic men infected with HIV who were under the care of our haemophilia centre and haemostasis unit. All except two patients had received unheated factor VIII concentrates; one had been treated with unheated factor IX concentrate and the other with cryoprecipitate.

The dates of the last negative and first positive HIV antibody test results were available for 63 patients. The date of HIV seroconversion was estimated as the midpoint between these two dates (median difference between the dates 11 months; 10th centile 41 days, 90th centile 21·5 months; range 15 days-24 months). The earliest seroconversion was estimated as October 1979 (difference between dates of last negative and first positive result=seven days) and the latest July 1985 (difference between dates of last negative and first positive result=10·1 months). These dates roughly agree with data from other sources on the period in which contaminated blood products were infused.[6] Patients' estimated dates of seroconversion showed a roughly uniform distribution.[21] For 36 of the 48 patients in whom no negative HIV test result was available, the date of seroconversion was estimated as the midpoint between October 1979 (the presumed first possible date of infection) and the date of the first HIV test (median difference 44 months; 10th centile 5·5 months, 90th centile 64·3 months; range 1·5-67 months). This approach minimises the maximum error in seroconversion date. For the remaining 12 patients the first positive HIV test result was after the presumed last possible date of infection (July 1985), and the date of seroconversion was estimated as the midpoint between October 1979 and July 1985—that is, September 1982 (difference 68 months).

We analysed information on patients up to 1 January 1993. The median length of follow up from seroconversion to this date was 10·1 years. A series of CD4 lymphocyte counts was recorded for each patient.[15] The median number of counts was 17 (range 1 to 50). Five or more counts were available for 90% of patients. The median age at seroconversion was 24 years (range 2 to 77). AIDS was defined according to the 1987 Centers for Disease Control definition.[22]

Zidovudine has been used to treat AIDS and people with Centers for Disease Control group IV disease since August 1987. The current protocol for treating asymptomatic patients is to start antiretroviral therapy with zidovudine and primary prophylaxis with pentamidine or co-trimoxazole and fluconazole at a CD4 lymphocyte count of 200×10^6/l. Treatment of asymptomatic patients began in November 1988, when 25 patients were recruited into the Medical Research Council and Agence Nationale de Recherches sur le SIDA Concorde trial of early versus deferred zidovudine.[23] Thirteen of these patients are now known to have been treated with zidovudine in the trial. Forty four patients have been openly treated with zidovudine (some of whom were formerly in the Concorde trial), 40 with prophylaxis against *Pneumocystis carinii* pneumonia, and 36 with prophylactic fluconazole. Since 1989 patients have also been given monoclonal high purity factor VIII concentrate instead of intermediate purity product when the CD4 count fell below 200×10^6/l. After 1991 all patients were switched to this product, which may slow the fall in CD4 count in HIV infected men with haemophilia.[24 25]

STATISTICAL METHODS

We fitted linear regression slopes through CD4 lymphocyte counts on time for each patient using least squares. We also used square root and square transformations for the CD4 count. The square root transformation is consistent with the rate of CD4 cell loss decreasing with time, while the square transformation is consistent with a more rapid loss with time. Since the average CD4 count at the development of AIDS has been found to be close to 50×10^6/l, the "modelled" date to develop AIDS was the time at which the negatively sloped linear regression line crossed 50×10^6/l (square root of 50 and square of 50 when using these transformations) on the y axis (see fig 1). When the slope was positive it was assumed the patient would not develop AIDS within 25 years from seroconversion. This was also assumed for patients in whom the slope of the linear regression line was zero (that is, a horizontal line) unless their CD4 count was less than 50×10^6/l, in which case the modelled date of AIDS was the date of the first CD4 count. For four patients only one CD4 count had been measured. These patients were excluded unless the count was below 50×10^6/l (one patient), in which case the date of this count was the modelled date of AIDS. We also did analyses using 30×10^6/l and 80×10^6/l as the count at which AIDS occurs and another in which the count for AIDS was taken as 80×10^6/l before routine prophylaxis and antiviral therapy was started (November 1988) and 50×10^6/l thereafter. This last analysis was also done with counts of 50×10^6/l and 30×10^6/l instead of 80×10^6/l and 50×10^6/l. This analysis was done because AIDS may occur at lower CD4 counts in those given prophylaxis and antiviral therapies. We also allowed patients' CD4 count at the time AIDS developed to be determined by sampling from the following probability distribution: 100×10^6/l=5%, 75×10^6/l=25%, 50×10^6/l=30%, 25×10^6/l=25%, 0×10^6/l=15%.

Kaplan-Meier estimates of the probability of remaining free of AIDS up to 25 years after seroconversion were made as follows. For those in whom AIDS had developed by 1 January 1993 the survival time free of AIDS was taken as the observed time to AIDS. For those who did not have AIDS on 1 January 1993 (median of 20 CD4 counts per subject over a median of nine years) the time to AIDS was taken as the time to the modelled date of AIDS. Follow up was censored in seven who died before AIDS was diagnosed. Weibull and Gamma distributions[20] were fitted to the observed survival times free of AIDS (by using the program PROC LIFEREG in SAS) to compare the projected survival rates with those obtained by our CD4 count modelling.

Results

Figure 1 gives the CD4 lymphocyte counts for one patient together with the linear regression slope to show how the modelled date of AIDS was obtained. When this was done for all patients, regardless of whether they actually have developed AIDS, the timing of the modelled dates of AIDS corresponded well with the actual dates (table I). Forty one of the 108 patients included in the modelling had developed AIDS by 1 January 1993. The modelled date was before the actual date of development for 36 (sensitivity 88% (36/41)). The modelled date of AIDS was after 1 January 1993 for 57 of the 67 patients who had not developed AIDS by this date (specificity 85%). Of the 36 patients who developed AIDS before 1 January 1993 as the model had predicted, 33 had a modelled date of AIDS within three years of the actual date and 29 had a modelled date within two years of the actual date.

Results were similar when the square root of the CD4 count was assumed to fall linearly over time

TABLE I—*Comparison of observed and modelled date of development of AIDS*

Modelled date of AIDS	AIDS by 1 January 1993		
	Yes	No	Total
Before 1993	36	10	46
1993 Onwards	5	57	62
Total	41	67	108

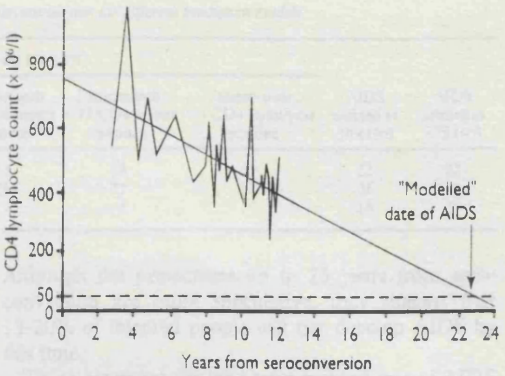

FIG 1—*Serial CD4 lymphocyte counts for an HIV infected man with haemophilia. The modelled time of AIDS is the time at which the linear regression slope reaches a CD4 lymphocyte count of 50×10^6/l*

(sensitivity 83%, specificity 88%). The model that used the square of the CD4 count, however, did not provide such good agreement (sensitivity 88%, specificity 63%). The results were not greatly affected by changing the CD4 count of AIDS from 50×10^6/l to 30×10^6/l (sensitivity 83%, specificity 88%) or 80×10^6/l (sensitivity 90%, specificity 81%). The results were also unaffected by using the CD4 count at AIDS sampled from the probability distribution rather than a fixed value for all subjects. The models in which a higher CD4 count was used to define AIDS before November 1988 (when prophylaxis was started) also did not greatly improve fit (sensitivity 90%, specificity 76% for model with 80×10^6/l and 50×10^6/l; sensitivity 90%, specificity 84% for model 50×10^6/l and 30×10^6/l). The results were essentially the same when patients with fewer than five CD4 counts were excluded (sensitivity 88%, specificity 87%) or when those aged below 15 at seroconversion were excluded (sensitivity 91%, specificity 84%).

Figure 2 shows the observed probability of surviving free of AIDS up to 13 years from seroconversion, This is based on the development of AIDS not on predicted

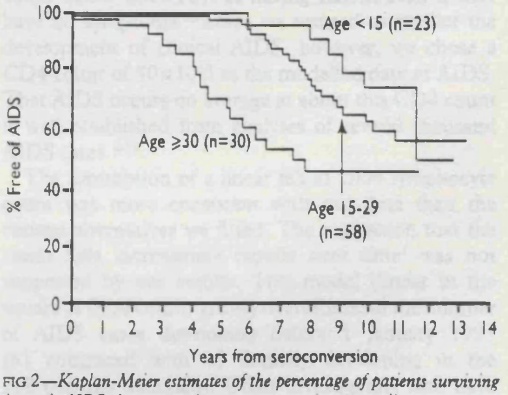

FIG 2—*Kaplan-Meier estimates of the percentage of patients surviving free of AIDS by years from seroconversion according to age at seroconversion. Estimates are based on actual times of occurrence of AIDS*

development from the CD4 count model. Eleven year AIDS free survival rates were 74% (95% confidence interval 55% to 93%) for patients aged <15, 57% (43% to 71%) for those aged 15-29, and 46% (27% to 65%) for those aged ≥30 (P=0·002, log rank test). Figure 3 shows the Kaplan-Meier curves based on the modelled date of AIDS in those free of AIDS on 1 January 1993. The probability of remaining free of AIDS 20 years after seroconversion was 32% (12% to 52%) in those aged <15 at seroconversion, 26% (14% to 38%) in those aged 15-29, and 15% (0% to 31%) in those aged >30 (table II). Over all age groups together the predicted percentage remaining free of AIDS for 20 years was 25% (16% to 34%). In all three age groups

TABLE II—*Percentage likelihood of survival free of AIDS for 20 years after seroconversion for different prediction models*

Age group (years)	AIDS defined as CD4 count 50×10^6/l: No transformation of CD4 count	Square root transformation of CD4 count	Square transformation of CD4 count	Patients with < 5 CD4 counts excluded	Patients with < 10 CD4 counts excluded	Patients with < 1 CD4 count/year excluded	AIDS defined as 30×10^6/l	AIDS defined as 80×10^6/l
<15	32	45	9	32	26	29	32	27
15-29	26	39	16	25	27	27	26	25
≥30	15	26	12	7	8	7	15	16

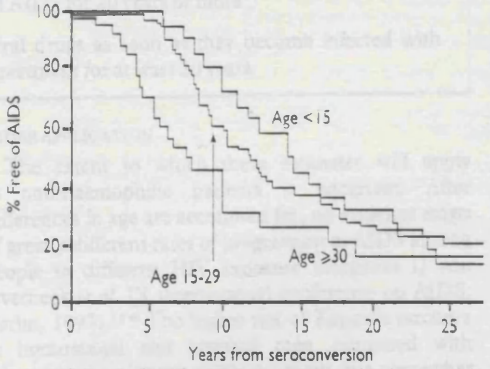

FIG 3—*Kaplan-Meier estimates of the percentage of patients surviving free of AIDS by years from seroconversion according to age at seroconversion. For those patients alive and free of AIDS on 1 January 1993, the modelled date of AIDS was used*

there was a 15-20% probability of remaining free of AIDS 25 years after seroconversion (over all age groups 18%; 11% to 25%), although the projections are less certain than for 20 years. Table II shows the effects of varying the model for estimating survival.

To give further detail on the 25 patients whom the model suggests will remain free of AIDS 20 years after seroconversion figure 4 shows the geometric mean CD4 lymphocyte count by years from seroconversion. Two of these men received zidovudine openly and a further three received zidovudine as part of the Concorde trial. The slow average rate of loss of CD4 lymphocytes is clearly seen. Twenty year survival free of AIDS seems highly plausible for these men.

Although clinical AIDS is unlikely to develop at 200×10^6/l we also used this value in our model as this is the new definition of the Centers for Disease Control AIDS surveillance.[27] The resulting estimates of 20 and 25 year survival free of AIDS over all age groups were 18% and 15%, respectively.

Table III compares estimates of survival free of AIDS obtained by our methods with those obtained from fitting Weibull and Gamma distributions through the observed survival times for all subjects aged 15 and over at seroconversion. The Gamma distribution seems to give results much closer to ours than the Weibull distribution.

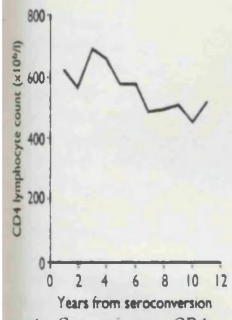

FIG 4—*Geometric mean CD4 lymphocyte count by years from seroconversion in 25 patients for whom the modelled time of AIDS is more than 20 years after seroconversion*

TABLE III—*Estimated percentages surviving free of AIDS by years from seroconversion obtained by assuming that the times from seroconversion to AIDS fit Weibull and Gamma distributions and by CD4 count model. All subjects aged 15 or over at seroconversion*

	Years from seroconversion: 5	10	15	20	25
Weibull (median 11·5, index 2·0)	88	60	30	11	3
Gamma (median 11·8, index 1·3)	88	60	38	25	17
CD4 count model	90	51	33	23	19

Discussion

We have used the well recognised ability of the CD4 lymphocyte count to predict the development of AIDS to assess the long term prospects for survival free of AIDS in patients with HIV infection. The projections suggest that there is roughly a 25% chance of remaining free of AIDS 20 years after infection with HIV. Although the projections up to 25 years from seroconversion are more speculative, they suggest that 15-20% of infected people will not develop AIDS by this time.

These estimates for long term survival free of AIDS are based on a cohort of men most of whom attend regularly for care and since 1989 have been offered prophylaxis against *P carinii* pneumonia and candida and antiviral drugs, particularly zidovudine, when their CD4 lymphocyte count reaches 200×10^6/l. It seems reasonable to assume that those patients in whom AIDS developed before 1989 (that is, at most within 10 years of seroconversion) would have developed AIDS within 20 years after seroconversion even if they had received these therapies. The long term survival estimates given in this paper therefore apply to people receiving treatment when the CD4 count falls below 200×10^6/l. Further advances in treatment may increase the proportion surviving free of AIDS for at least 20 years. Our estimates suggest that use of antiretroviral drugs as soon as HIV infection is diagnosed may in some patients entail over 20 years of therapy with drugs of uncertain long term risk to benefit ratios.[23 28 29]

PREDICTIVE VALUE OF CD4 COUNT

The use of the CD4 lymphocyte count to predict the progression to AIDS has a firm statistical basis. Many studies have shown the predictive value of the CD4 count in different risk groups and different settings.[12-19] Indeed, the Centers for Disease Control AIDS surveillance definition now classes people with a CD4 count below 200×10^6/l as having AIDS, even if they have no symptoms.[27] Since we wanted to predict the development of clinical AIDS, however, we chose a CD4 count of 50×10^6/l as the modelled date of AIDS. That AIDS occurs on average at about this CD4 count is well established from analyses of several thousand AIDS cases.[30-33]

The assumption of a linear fall in CD4 lymphocyte count was more consistent with our data than the various alternatives we fitted. The suggestion that the count falls increasingly rapidly over time[4] was not supported by our results. This model (linear in the square of CD4 count) greatly overestimated the number of AIDS cases developing before 1 January 1993 (61 compared with 41 actually developing in the 108 patients included). Other investigators who have modelled the fall in CD4 count in HIV infection have found that a linear fall in the untransformed, square root, or logarithmic scales provide the best fit.[34-36] When we used either of the last two models our projections of long term survival free of AIDS were more favourable (data for logarithmic model not shown).

CD4 lymphocyte counts tend to fall naturally from birth in uninfected children before stabilising at about age 13.[37] This phenomenon could result in underestimation of the length of time before AIDS develops in our linear model and hence underestimation of the proportion of children surviving free of AIDS for 20-25 years. Nevertheless, the agreement between actual and modelled date of AIDS was still close even in these young patients (data not shown).

Clinical implications

- The time of development of AIDS after HIV infection varies widely and few data exist on long term prognosis
- Older people tend to develop AIDS more rapidly after HIV infection than younger people
- Predictions from this study suggest that one quarter of people infected with HIV may remain free of AIDS for 20 years or more
- Patients given antiretroviral drugs as soon as they become infected with HIV may therefore require treatment for at least 20 years

WIDER APPLICATION

The extent to which these estimates will apply to non-haemophilic patients is uncertain. After differences in age are accounted for, no evidence exists of greatly different rates of progression to AIDS among people in different HIV exposure categories (J von Overbeck *et al*, IX international conference on AIDS, Berlin, 1993).[2 8 10] The higher risk of Kaposi's sarcoma in homosexual and bisexual men compared with other exposure groups seems to result in a somewhat poorer survival free of AIDS in this group.[10] Nevertheless, the incidence of Kaposi's sarcoma seems to be falling,[38] and so differences in AIDS rates between homosexual men and those in other transmission categories may become smaller.

There is increasing evidence that haemophilic patients who are coinfected with HIV and hepatitis C virus are at greater risk of liver failure than those infected with hepatitis C virus only (M E Eyster *et al*, IX international conference on AIDS, Berlin, 1993). Furthermore, the development of liver disease may be related to the degree of immunosuppression. In our cohort four patients without AIDS have died of liver disease. Thus liver disease may become important in the long term prognosis of patients infected with HIV and hepatitis C virus, and survival rates could be lower in coinfected patients.

VALIDITY OF MODEL

Predicting disease is always uncertain, and the modelled date of AIDS for an individual patient can differ substantially from the actual date of AIDS. Our model is therefore probably not clinically useful for predicting individual patients' prognosis. We believe, however, that our model is sufficiently accurate to provide useful estimates of the average experience of a whole group. Although we have given confidence intervals for our 20 and 25 year projected AIDS-free survival rates, these do not reflect all sources of uncertainty in the estimates. In particular, they do not reflect the uncertainty concerning the validity of the specified model. This uncertainty can be evaluated partly by studying the results obtained with alternative models. The results we obtained for long term survival free of AIDS were little affected by plausible changes in the formulation of the model. Further uncertainty concerns the accuracy of seroconversion dates. In almost all subjects, however, the maximum error in this date was less than two years, and thus, at worst, our estimates for 20 year survival free of AIDS would relate instead to 18 year survival.

Methods for projections of future numbers of AIDS cases in a country or community rely heavily on knowledge of the distribution of survival times free of AIDS, commonly termed the "incubation period."[39 40] Since data are available for at most only 13-14 years from seroconversion, predictions of the shape of the remainder of the cumulative distribution curve are usually made by fitting either a Weibull or Gamma distribution.[39 40] Table III shows that if our projections are correct the Weibull distribution underestimates the proportion surviving without AIDS for 15 years after seroconversion whereas the Gamma distribution gives a much better fit.

We previously used a similar approach to project the probability of remaining free of AIDS up to 15 years from seroconversion.[20] That analysis gave an estimate of 27% for the whole patient group compared with 36% in this analysis. The first estimate was based on experience before antiretroviral therapy or prophylaxis against *P carinii* pneumonia was given to patients with a CD4 count below 200×10^6/l (November 1988) and before the introduction of high purity factor VIII concentrate. This probably largely accounts for the difference and also gives some indication of the effect of such treatment policies on survival free of AIDS.

In conclusion, we have used 11 years of CD4 lymphocyte count experience in 111 haemophilic men to forecast the probability of survival free of AIDS up to 25 years after infection with HIV. The results suggest that such prolonged survival is likely in about a quarter of patients.

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C4B*Q0 allotype as risk factor for myocardial infarction

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The prevalence of the deficient, silent allotype of the C4B gene (C4B*Q0) is lower in elderly than in young healthy people, particularly in men.[1] This may reflect increased mortality from some disease in middle aged carriers of the C4B*Q0 gene. We determined the presence of the gene in patients with acute myocardial infarction because myocardial infarction is the leading cause of death among middle aged Hungarians.

Patients, methods, and results

We studied 181 consecutive patients with confirmed Q wave myocardial infarction admitted to four hospital departments between June 1992 and January 1993 (125 men, 56 women, aged 42-78), 93 consecutive patients with symptoms of angina pectoris (65 men, 28 women; aged 43-62) who were examined by coronary angiography (coronarography), and 737 previously tested healthy controls (252 young people aged 22-45 and 485 elderly people aged 60-99).[1] Myocardial infarction was diagnosed as typical chest pain lasting at least one hour, an ST segment elevation of at least 1 mm in an electrocardiogram, and typical cardiac enzyme values. We diagnosed inferior and anterior wall infarction in 103 and 70 patients, respectively; in eight patients the localisation of the infarct was uncertain.

We took blood samples from the patients with myocardial infarction within 24 hours of admission and sent them immediately to the laboratory in tubes containing EDTA. Plasma samples were stored at −70°C until tested. C4 allotyping was performed with high voltage electrophoresis, followed by immunofixation with human C4 antibody (Atlantic Antibodies).[2,3] We determined aspartate aminotransferase and alanine aminotransferase values serially with commercially available kits (Boehringer Mannheim, Germany). In order to exclude patients with enzyme elevations unrelated to myocardial infarction, we evaluated peak aspartate aminotransferase values only in patients whose alanine aminotransferase values had not increased concomitantly. Patients with raised aspartate aminotransferase values at the first determination were also excluded from the further evaluation.

The prevalence of C4 allotypes was significantly higher in patients with myocardial infarction than in the healthy elderly controls (27·6% *v* 10·7%; $P<0·0001$)—the only significant difference between the patients and the controls. After age matching, which was possible only in those aged 60-79, 38% (24/63) of male patients and 8% (10/133) of healthy men carried the C4B*Q0 allotype ($P<0·0001$). The odds ratio of a 60-79 year old man with acute myocardial infarction being a C4B*Q0 carrier compared with his healthy counterpart was 7·57 (95% confidence interval 3·31 to 17·2); in women this odds ratio was 0·84 (0·33 to 2·16).

The C4B*Q0 carrier state influenced the outcome of myocardial infarction (table). The odds ratio of dying was significantly higher for men who carried the gene compared with those who did not (18·0 (2·1 to 153) in homozygous men and 5·53 (1·21 to 25·4) in heterozygous men). Data on women were insufficient to calculate odds ratios.

Average peak aspartate aminotransferase values were significantly higher in patients who carried the C4B*Q0 gene than in those who did not (218 U/ml (median 195 U/ml, range 20-635 U/ml) *v* 145 U/ml (median 120 U/ml, range 12-506 U/ml; $P=0·040$ by Mann-Whitney U test). Similarly, the proportion of patients with a peak aspartate aminotransferase value greater than 200 U/ml was significantly higher in

*Outcome of Q wave myocardial infarction in patients with or without C4B*Q0 allotype*

Group	No (%) who died	No (%) who survived
Carrier of C4B*Q0 gene:		
Homozygous (n=6)	3 (50)	3 (50)
Heterozygous (n=38)	8 (21)	30 (79)
Non-carrier (n=137)	17 (12)	120 (88)
Total	28 (15)	153 (85)

$P<0·05$ for difference between patients with and without C4B*Q0 by χ^2 test.

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Prophylaxis for *Pneumocystis carinii* pneumonia: its impact on the natural history of HIV infection in men with haemophilia

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Summary. It has been suggested that the range of AIDS-defining conditions witnessed in patients with HIV infection has changed since the early years of the HIV epidemic. In this paper we consider the range of AIDS-defining conditions in a cohort of 111 HIV-positive men with haemophilia registered at the Royal Free Hospital Haemophilia Centre. In particular we assess whether the incidence of *Pneumocystis carinii* pneumonia (PCP) has changed over time. The men were all infected between 1979 and 1985 after treatment with infected blood products and have now been followed prospectively for up to 13 years from HIV seroconversion. By the end of 1992, 44/111 patients had developed AIDS. Of the 44 men, 18 (41%) presented with PCP as their first AIDS-defining condition (ADC), mainly before the initiation of primary prophylaxis in 1989. The remaining 26 patients presented with a range of conditions as their first ADC, but there were no more than four cases in any one disease category. It is estimated that patients suffer from 0.7 further ADCs per year after being diagnosed with AIDS. After taking account of the increased levels of immunosuppression in the cohort with time, it appears that the incidence of PCP, both as the first ADC or as any ADC, has declined since the introduction of primary prophylaxis for the disease in 1989. However, non-compliance with prophylaxis for PCP appears to have played a major role in the continuing occurrence of PCP since 1988. Improvements in compliance with therapy should result in a further reduction in the incidence of PCP both as a first ADC and as any ADC.

Keywords: AIDS-defining conditions, haemophilia, HIV, non-compliance, *Pneumocystis carinii* pneumonia, prophylaxis.

In the early days of the AIDS epidemic, when little treatment was available for individuals with opportunistic infections, survival after diagnosis with an AIDS-defining condition was relatively short. However, with the introduction of new therapies for people with AIDS, prospects for survival have improved [1–3]. Whereas increased age at diagnosis has almost universally been associated with poorer survival from AIDS, uncertainty remains as to the effects of other factors, such as the patient's CD4 count, gender, ethnicity or HIV exposure category [1, 2, 4–7].

From 1988 a number of prophylactic treatments became available for HIV-infected individuals with low CD4 counts. These have included the use of pentamidine or co-trimoxazole as prophylaxis for *Pneumocystis carinii* pneumonia (PCP) [8–10] and, in some treatment centres, fluconazole as prophylaxis against candidiasis [11]. Prior to the introduction of therapy, PCP was the most common AIDS-defining condition in many groups of patients [12]. A number of authors have suggested that the introduction of prophylaxis for PCP has reduced the incidence of this infection as the primary AIDS-defining condition [12, 13] and that survival has increased, possibly as a result of this treatment. The effects of the introduction of the antiretroviral drug, zidovudine, on survival for patients initially AIDS-free are still not confirmed [14–16].

Kaposi's sarcoma (KS), a common AIDS-defining condition, has been reported in around 6–24% of men developing AIDS [1, 4, 6, 17, 18]. However, the incidence of KS as an AIDS-defining condition declined between 1981 and 1990 [4, 16, 19]. The reduction in PCP and KS as AIDS-defining conditions has been matched by a corresponding increase in the incidence of several other

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AIDS-defining conditions. Whilst KS has been a frequent AIDS-defining condition amongst homosexual men, it is rarely seen outside this group. Because many studies on the incidence of different AIDS-defining conditions over time have been carried out in homosexual men, the information from these studies has been of limited use for clinicians treating patients from other risk groups, in whom KS is rare. In this paper we consider the AIDS-defining conditions (ADCs) in a cohort of men with haemophilia, infected with HIV between 1979 and 1985, paying particular attention to the changing incidence of PCP over time. We also compare survival after an AIDS diagnosis within different subgroups, defined by whether the initial ADC was PCP or some other condition, the year of diagnosis, age and CD4 count at diagnosis, and zidovudine usage prior to an AIDS diagnosis.

Methods

Patients

One hundred and eleven men with haemophilia registered at the Royal Free Hospital Haemophilia Centre became infected with HIV between 1979 and 1985 following treatment with unsterilized blood products. These patients have been well described [20–22]. All patients are seen at the Centre every 3–6 months, at which time they undergo complete clinical and laboratory review. AIDS is classified according to the most recent CDC classification accepted for use in the UK at the time of diagnosis. Patients are not given a retrospective diagnosis of AIDS if a change in the case-definition occurs. Data on AIDS-defining events are abstracted on a yearly basis from medical notes.

Since 1987 zidovudine has been available for individuals with AIDS and ARC, and from October 1988 it has been available as part of the MRC/ANRS Concorde trial of early versus deferred zidovudine in asymptomatic HIV+ve individuals. Secondary prophylaxis for PCP (with pentamidine) has been available since March 1988 (300 mg 2-weekly) and primary prophylaxis since February 1989 (300 mg monthly). Patients with haemophilia are used to administering home therapy with factor VIII. In the majority of cases, therefore, pentamidine was self-administered at home. From 1992 it became apparent that co-trimoxazole was more effective as prophylaxis than pentamidine. Where possible, patients were changed to co-trimoxazole from 1992 onwards (960 mg three times a week). However, a large proportion of individuals receiving co-trimoxazole develop skin sensitivity and, where this was the case, patients were switched back onto pentamidine. Secondary prophylaxis for candidiasis (fluconazole) has been available since March 1988 and primary prophylaxis since April 1990 (both 150 mg weekly). Patients developing either PCP or candidiasis are considered at risk of recurrence of the condition and are therefore offered secondary prophylaxis regardless of their CD4 count. All patients are started on zidovudine and primary prophylaxis once their CD4 count falls below 0.2×10^9/l. To date, a total of 51 patients have received zidovudine, 35 PCP prophylaxis (26 primary, nine secondary) and 39 prophylaxis for candidiasis (13 primary, 26 secondary). The dose of fluconazole received is increased (50 mg daily) either when the patient's CD4 count falls below 0.01×10^9/l, or while the patient is receiving antibiotics. Compliance with prophylaxis is assessed at each patient's clinical review by careful questioning of the patient. Further information about the patient, e.g. whether they are a drug user or suffer from mental illness, is also taken into account when assessing compliance.

Laboratory methods

Between 1982 and 1986 absolute CD4 counts were calculated from the lymphocyte count and CD4% values. Absolute lymphocyte counts were determined by an automated whole blood counter (Ortho 'ELT 800' with differential screen) and percentages of CD4 lymphocytes were counted in Ficoll-Hypaque-separated blood mononuclear cell suspensions [20]. Since 1986 a whole blood lysis method has been used, and the percentage of CD4 lymphocytes analysed by flow cytometry using a FACScan (Becton Dickinson, Crawley, UK) [23]. A monoclonal CD4 antibody, RFT4, to the p 55 CD4 antigen was used in concentration with a monoclonal CD3 antibody (UCHT1) as described previously [23]. More recently, absolute CD4 counts have been directly obtained on an Ortho Cytoron-Absolute (Ortho Diagnostics, High Wycombe, UK). Flow cytometer quality control was monitored in the UK National External Quality Assurance Scheme. We have compared CD4 counts from before and after the change in methods in 1986 and have seen no consistent difference.

Statistical methods

All ADCs were divided according to whether they were PCP or some other condition. Calendar year of diagnosis has been dichotomized (prior to 1989, 1989 onwards) to reflect the introduction of therapy for AIDS patients and pre-AIDS patients in the late 1980s. Due to the expected non-normality of the continuous variables studied (patients' age and CD4 count at AIDS diagnosis), comparisons of these with year of diagnosis and the patient's first ADC were carried out using standard non-parametric methods (Wilcoxon Mann-Whitney test [24]). For all further analyses, the patients' age and CD4 count at AIDS diagnosis were dichotomized by stratifying above

and below the median values (32 years and 0.09×10^9/l respectively). The date on which a patient's CD4 count fell below 0.2×10^9/l was estimated by linear interpolation between the dates of the measurement immediately preceding and the measurement after the count had fallen below this level.

All rates were compared using standard Poisson modelling procedures in GLIM [25, 26]. In each case the logarithm of the patient-years of follow-up was calculated and offset in the model. The number of events (e.g. further episodes of PCP, deaths) was modelled as the dependent variable. Tests of significance were performed by calculating the difference between the scaled deviances from models with and without the factor of interest [27]. This was compared to tabulated values of the chi-squared distribution with degrees of freedom equal to the difference in modelled degrees of freedom. When considering the incidence of PCP as an initial ADC, patient follow-up was censored at an initial non-PCP ADC. Patient follow-up was censored at death when considering the incidence of PCP as any ADC.

A large number of those diagnosed with AIDS prior to 1989 were diagnosed with AIDS posthumously, possibly due to lack of diagnostic experience of HIV in the early years of the epidemic. Further, AIDS patients diagnosed in more recent years have limited follow-up and heavy censoring occurs in this group of patients. Consequently, the estimation of survival probabilities using standard survival methods is problematic. For this analysis, the death rate was calculated as the death rate per 100 person-years of follow-up after an AIDS diagnosis, and differences in this rate between categorical variables (year of diagnosis, initial ADC, age at diagnosis) were compared for statistical significance using Poisson modelling, as described above. In order to remove some of the potential bias associated with early cases of AIDS remaining unrecognized until after death, the above analyses were repeated, excluding all patients with zero survival times from the data set.

Results

The 111 men in the cohort have previously been described in detail [20–22]. By 1 January 1993, 44/111 (40%) of the cohort had developed AIDS, with a Kaplan-Meier progression rate of 47% at 13 years after seroconversion. 25 (57%) developed AIDS prior to 1989, while 19 (43%) were diagnosed in 1989 or later. At the time of AIDS diagnosis, CD4 counts ranged from 0 to 1.07×10^9/l (median 0.09×10^9/l), and the patients' age from 8 years to 82 years (median 32 years). There is some evidence of a change in the CD4 count at which AIDS develops according to the year of AIDS diagnosis although this is non-significant (median CD4 counts of 0.13 and 0.03×10^9/l in patients diagnosed prior to 1989 and from 1989 onwards respectively, $P = 0.13$, Mann-Whitney test). However, as expected, due to the known association between age and HIV disease progression, patients developing AIDS since 1989 are, on average, younger at diagnosis than those developing AIDS earlier on in the epidemic (median age at diagnosis, 27.3 and 35.0 years respectively, $P = 0.04$, Mann-Whitney test).

The initial AIDS-defining conditions are shown in Table 1. PCP was the initial ADC in 18 (41%, 95% CI 26–56%) of patients developing AIDS. In the remaining patients, oesophageal candida, lymphoma and wasting syndrome were the most frequent initial ADCS. No patient in the cohort has developed Kaposi's sarcoma during follow-up. 22 (50%) of the patients developed only one ADC. The remaining patients either experienced a single recurrence of their initial ADC (two patients), had a recurrence of their initial ADC and developed at least one further condition (three patients) or developed at least one further condition but not a recurrence of their initial ADC (17 patients).

Table 2 shows the pattern of illnesses amongst the 44 patients with AIDS, according to whether their initial ADC was PCP (18 patients) or not (26 patients). During follow-up there have been a total of 83 episodes of any

Table 1. Initial AIDS-defining conditions observed in cohort.

	No of occurrences		Prior to 1989		1989 onwards	
	No.	%	No.	%	No.	%
PCP	18	41	12	48	6	32
Oesophageal candida	4	9	2	8	2	11
Lymphoma	4	9	3	12	1	5
Wasting syndrome	4	9	3	12	1	5
Toxoplasmosis of brain	3	7	1	4	2	11
HIV encephalopathy	3	7	1	4	2	11
Cryptosporidiosis	2	5	0	0	2	11
Salmonella septicaemia	2	5	1	4	1	5
Other	4	9	2	8	2	11
Total	44	100	25	100	19	100

Table 2. Initial and subsequent AIDS-defining conditions (ADC) seen in cohort, person-years after an AIDS diagnosis and the rate of further conditions per person-year of follow-up.

	Initial AIDS-defining event		
No. of events	PCP (n = 18)	Other (n = 26)	Total (n = 44)
1	6	16	22
2	5	7	12
3	4	2	6
4	2	0	2
5	0	1	1
6	1	0	1
Initial ADC	18	26	44
Other ADC	24	15	39
Person-years after AIDS diagnosis	34.54	21.31	55.85
Rate of other ADC per person-year	0.69	0.70	0.70

ADC, either as an initial condition (44 episodes) or as subsequent ADCs (39 episodes). On average, each man experienced 0.70 further episodes of ADCs per year after an AIDS diagnosis.

There has been no apparent decrease in the 'crude' incidence of PCP as an initial ADC since 1989 (Table 3, 'Total' columns, $P = 0.54$). However, as CD4 counts have been shown to decline in individuals throughout HIV infection, the patients followed later in the epidemic would be expected to be more immunosuppressed than patients followed prior to 1989. After taking account of the level of immunosuppression (P-value for inclusion of CD4 category < 0.0001), it is clear that the incidence of PCP as an initial ADC has declined (P-value for effect of calendar year after adjusting for CD4 category = 0.04). In particular, the incidence of PCP at CD4 counts below 0.2×10^9/l has declined dramatically, from 17.65 per 100 patient-years prior to 1989 to 6.91 per 100 patient-years from 1989 onwards. A similar pattern is seen when considering the incidence of PCP as any ADC (data not shown). After stratifying for level of immunosuppression, a significant increase in the 'crude' incidence is reduced to a small, although non-significant, decrease in the rate over time (P-value for effect of calendar time after adjusting for CD4 category = 0.16). Again, this effect is most noticeable amongst patients when their CD4 count falls below 0.2×10^9/l.

Of the 44 patients developing AIDS in the cohort, 35 have died with survival times ranging from 0 to 4.1 years. 10 of these patients received their diagnosis at death, nine of whom died prior to 1989. These 'zero' survival times have resulted in a low median survival time of 0.1 years amongst the patients who have died. A further seven patients have died without an AIDS diagnosis. In those patients who have developed AIDS but are still alive, follow-up times range from 0.3 to 4.9 years (median 1.7 years). Table 4 shows survival patterns for the patients developing AIDS in the cohort. 23 of the patients diagnosed prior to 1989 have died, with a total of 38.13 patient-years of follow-up after an AIDS diagnosis (60.32 deaths per 100 years of follow-up). There have been 12 deaths in the 19 patients diagnosed from 1989 onwards over 17.72 years of follow-up of follow-up (67.72 deaths per 100 years, $P = 0.75$). Similar analyses were performed where patients were grouped according to their initial ADC (PCP or not), age and CD4 count at AIDS diagnosis and whether they had received zidovudine prior to their AIDS diagnosis. Only the patient's initial ADC was significantly associated with survival with a lower death rate in patients presenting with PCP as their initial ADC

Table 3. The incidence of PCP as an intial AIDS-defining condition (ADC) by patient-years of follow-up since seroconversion, stratified according to year of AIDS diagnosis and level of immunosuppression. Patient follow-up censored at non-PCP ADC, death, or December 1992.

Year of AIDS diagnosis:	Prior to 1989			1989 onwards		
	>0.2*	<0.2	Total	>0.2	<0.2	Total
Episodes of PCP	3	9	12	0	6	6
Patient-years after seroconversion	653.4	51.0	704.4	170.8	86.8	257.6
Rate per 100 patient-years	0.46	17.65	1.70	0.00	6.91	2.33

*CD4 count ($\times 10^9$/l).

Table 4. Survival following an AIDS diagnosis.

	AIDS cases	Deaths	Patient-years of follow-up after AIDS	Death rate per 100 patient years	P-value
Year of AIDS diagnosis					
Prior to 1989	25	23	38.13	60.32	
1989 onwards	19	12	17.72	67.72	0.75
Initial ADC					
PCP	18	13	34.54	37.64	
Other conditions	26	22	21.31	103.24	0.003
Age at AIDS					
≤32	22	15	30.51	49.16	
>32	22	20	25.34	78.93	0.16
CD4 count at AIDS ($\times 10^9$/l)					
>0.09	22	16	29.10	54.99	
≤0.09	22	19	26.75	71.02	0.45
Zidovudine usage prior to AIDS					
No	31	25	44.25	56.49	
Yes	13	10	11.59	86.24	0.27

($P = 0.003$) than in those presenting with other conditions. Whilst no statistically significant differences were found when stratifying by the patient's CD4 count at diagnosis, age at diagnosis, and zidovudine usage prior to an AIDS diagnosis, the differences were large and in the expected direction.

To remove some of the effect of any potential bias associated with the large numbers of AIDS diagnoses made posthumously early in the epidemic, the 10 patients with zero survival times were excluded from the data set and the above analyses were repeated. All but one of these patients were diagnosed with AIDS and died prior to 1989. After excluding the 10 patients most of the results were essentially unchanged. However, zidovudine usage prior to an AIDS diagnosis, whilst previously non-significant, was now significantly associated with an increased death rate (rates per 100 person-years after AIDS diagnosis; 86.2 and 33.9 in those receiving zidovudine prior to diagnosis and those not, respectively, $P = 0.03$).

A total of 40 patients had CD4 counts which fell below 0.2×10^9/l on at least one occasion, were still alive and had not developed PCP by February 1988. These patients were eligible for primary prophylaxis for PCP. Four of these patients had persistently high CD4 counts, and their one low count was thought to be misrepresentative of their general level of immunosuppression. These patients were not therefore considered eligible for prophylaxis. The remaining 36 patients were eligible for primary prophylaxis for a total of 95.97 years (i.e. from the date the CD4 count first fell below 0.2×10^9/l or February 1988, whichever was earlier, to the date of first occurrence of PCP, death or the end of the study). Whilst eligible, eight of the patients experienced an episode of PCP. 22 patients were thought to be compliant with therapy, seven were known to be non-compliant and six were not started on prophylaxis, because they did not visit the centre on enough occasions before dying, either because they moved to another centre or were frequent non-attenders. Information about compliance and prophylaxis is unavailable for one patient. All eight episodes of PCP occurred in patients known to be known to be non-compliant, or in whom prophylaxis was not started (rates of development of PCP 0.0 and 50.0 per 100 patient-years in those compliant and those non-compliant respectively, $P < 0.0001$).

A total of 19 patients experienced episodes of PCP and were still alive when secondary prophylaxis for PCP was introduced in March 1988. These patients were therefore eligible to receive secondary prophylaxis. Total patient-years of eligibility for secondary prophylaxis (i.e. from the onset of their first episode of PCP or March 1988, whichever was later, to their death or the end of the study) was 33.56 years. Over this period, five of these patients experienced a recurrence of PCP (four patients experienced one further episode, one patient experienced two further episodes). Whereas the patients thought to be non-compliant or those in whom secondary prophylaxis had not been started had a higher risk of recurrence of PCP than those thought to be compliant, this difference was not statistically significant (rates of 10.2 and 28.6 per 100 patient-years in those compliant and those non-compliant respectively, $P = 0.22$).

Two patients developed PCP while eligible to receive both primary and secondary prophylaxis, i.e. they

Table 5. Clinical details of patients who have developed PCP after eligible to receive prophylaxis for PCP.

Patient	Type of prophylaxis receiving when PCP episode occurred*	First date of eligibility for PCP prophylaxis	Comments
1	1	November 1989	Non-attender
2	1	February 1989	Non-compliant
3	1	Janurary 1992	Non-compliant/drug addict
4	1	February 1989	Only received one dose
5	1	July 1990	Non-compliant
6	1	March 1989	Non-compliant/in mental hospital
7	1 and 2	February 1989	Non-compliant
8	1 and 2	February 1989	Not started on prophylaxis, presented with PCP from different country
9	2	March 1988	Compliant
10	2	September 1988	Non-compliant
11	2	April 1988	No known reason

*1 = Primary prophylaxis; 2 = secondary prophylaxis.

developed PCP while eligible for primary prophylaxis and then experienced a further episode while eligible for secondary prophylaxis. Information about compliance on all 11 patients who experienced episodes of PCP while eligible for either primary or secondary prophylaxis is shown in Table 5.

Discussion

We have shown that in our cohort of men with haemophilia infected with HIV, PCP is the most common initial AIDS-defining condition (ADC), with almost half the men being diagnosed with this as their initial ADC. Whereas the overall 'crude' incidence of PCP, both as an initial ADC and as any ADC, appears to have increased since 1989, the patients are now, in general, much more immunosuppressed than they were earlier in the epidemic. Once the increased levels of immunodeficiency seen in the cohort in more recent years are taken into account, there is evidence to suggest that the incidence of PCP is decreasing. Whereas the decrease was significant for PCP as an initial ADC it did not reach statistical significance for PCP as any ADC. Nevertheless, the result is consistent with those from other, larger cohort studies which have shown a decrease in the incidence of PCP since 1989 [12, 13]. The decrease in the incidence of PCP seen in our cohort is almost certainly due to the introduction of primary prophylaxis in 1989. There is no room for complacency, however, as PCP, whilst declining in incidence, has not been completely eliminated. A large proportion of patients who were believed to be non-compliant with their prophylaxis went on to develop either an initial episode of PCP or a recurrence, whereas the majority of patients known to be compliant with therapy remain free of PCP. This suggests that non-compliance is a major contributory factor in the cases of PCP seen in the cohort since the introduction of primary prophylaxis in 1989.

There is little evidence that survival from an AIDS diagnosis is improving in our cohort with time, although follow-up continues in many of those diagnosed since 1989. Survival appears to be associated with the patient's initial ADC, with lower death rates in patients developing PCP as their initial ADC, suggesting that treatment of PCP is usually successful. Some authors have suggested that survival from an AIDS diagnosis is improving [1–3]. However, the association between survival and initial ADC appears to be paradoxical, with those patients presenting with PCP having both lower CD4 counts at diagnosis [28] but improved survival [4, 7]. Other studies have reported that survival from an AIDS diagnosis ranged from 5 to 17 months [1, 4, 6, 29], and many of these studies have shown that the probability of survival is reduced with increasing age at AIDS diagnosis [1, 2, 4–7]. There is the suggestion that much of the effect of age on survival could be due to the fact that different age groups present with a different range of ADCs [4], and that the effect of age simply reflects the distribution of PCP, for example, in the group of patients studied. We also found improved survival in patients whose CD4 counts were higher at diagnosis, in those who were younger, and in patients who had not received zidovudine prior to their AIDS diagnosis. Whereas none of these differences reached statistical significance, the effects were large and in the direction expected, given other results in some, but not all, published research [1, 2, 4–7, 15].

Whereas the proportion of patients in the cohort who have PCP as their initial ADC is higher than in other studies, confidence limits are wide and include estimates from other cohorts which range from 20% to 46% [4, 6, 13, 17]. Because we have not seen any cases of KS in our cohort, the proportion presenting with PCP may be expected to be larger than in studies including homosexual men. However, because the incidence of KS as an initial ADC is declining in other HIV exposure categories, the information derived from this cohort will become increasingly important, as the differences in morbidity between risk groups diminish. It has been suggested that survival from an AIDS diagnosis is slightly longer in intravenous drug users [30] than in other risk groups, although this may be explained by differences in age between the risk groups. With the exception of KS, however, patterns of AIDS-defining illness within different exposure categories are likely to be broadly similar [31] and we therefore believe that the information from this cohort is applicable to other exposure categories.

In conclusion, we have shown that in this cohort of men with haemophilia infected with HIV, the incidence of PCP, either as an ADC or as any ADC, is declining after controlling for changing levels of immunosuppression. However, the condition is still relatively common, possibly due to high levels of non-compliance amongst patients when taking their prophylaxis. By preventing patients from developing PCP it is possible to delay the onset of AIDS. Steps to improve compliance, including continual education of the benefits of prophylaxis and treatment at the centre rather than at home, could be taken to reduce the incidence of PCP even further, both as an initial or any ADC.

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