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The natural history of human immunodeficiency virus infection in a haemophilic cohort

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Summary. 112 haemophilic patients infected with HIV were followed up with clinical and laboratory assessment between 1 December 1979 and 30 November 1988. Sixty-six (59%) of the patients developed HIV-related clinical symptoms and 22 (20%) developed AIDS. Twenty (18%) of the patients developed p24 antigenaemia. Amongst the 59 patients whose date of seroconversion could be estimated the calculated 8-year cumulative incidence of AIDS was 40% (symptoms 73%). For the whole cohort of 112 patients, the median slope of linear regression of the absolute T4 lymphocyte count was steeper for those with AIDS ($-0{\cdot}113 \times 10^9$/l per year) than for those without AIDS ($-0{\cdot}054 \times 10^9$/l per year) ($P < 0{\cdot}02$). While 15 cases of AIDS developed during 58 patient-years of follow up after falling below a T4 lymphocyte count of $0{\cdot}2 \times 10^9$/l, only two cases occurred during 450 patient-years before reaching this count. Thus the decline of the T4 lymphocyte count to $0{\cdot}2 \times 10^9$/l may be an appropriate additional end-point for the assessment of new treatments for asymptomatic patients infected with HIV.

Prediction of the course of the AIDS epidemic requires knowledge of the time interval from infection with the human immunodeficiency virus (HIV) to the development of AIDS. Actuarial progression rates to AIDS have been calculated in studies of patients both with and without known or estimated seroconversion dates (Moss & Bacchetti, 1989). The former group includes haemophiliacs, homosexual men and transfusion recipients.

Haemophiliacs have a high prevalence of antibody to human immunodeficiency virus (anti-HIV) as a result of treatment with unheated contaminated clotting factor concentrates (United Kingdom Haemophilia Directors, 1988). Of 525 haemophiliacs registered at the Royal Free Hospital Haemophilia Centre, 112 have been found to be anti-HIV positive. We describe here the development of symptoms related to HIV and the progression to AIDS in this population where detailed clinical and laboratory follow-up has been possible in a majority of patients from the time of seroconversion.

PATIENTS AND METHODS

Patients. 112 anti-HIV positive patients were entered into the study using data from records and samples from the period 1 December 1979 to 30 November 1988. 101 patients had severe haemophilia A (factor VIII <2 u/dl), seven moderate/mild haemophilia A (factor VIII >2 u/dl), one severe haemophilia B (factor IX <2 u/dl) and two von Willebrand's disease. One patient was the wife of a severely-affected haemophiliac and all other patients were male. Of the male patients, all except two had received treatment with unheated factor VIII concentrates; one had been treated with unheated factor IX concentrate and the other with cryoprecipitate. One patient regularly injected heroin intravenously. Treatment with heated concentrates was introduced in late 1984. Patients were reviewed for clinical and laboratory assessment at least every 6 months. Retrospective analyses were made of clinical records and stored serum samples.

From July 1987, 20 patients were started on treatment with zidovudine for advanced symptomatic HIV-related disease but the study period ended before any patients were entered into the Medical Research Council double-blind placebo controlled trial of zidovudine for asymptomatic patients (the Concorde 1 trial).

Laboratory methods. Antibody to HIV was measured using a competitive enzyme immunoassay (Wellcozyme, Wellcome Diagnostics, Dartford, England). HIV core antigen (p24) was measured by enzyme immunoassay (Abbott Diagnostics Division, Maidenhead, England). The absolute lymphocyte

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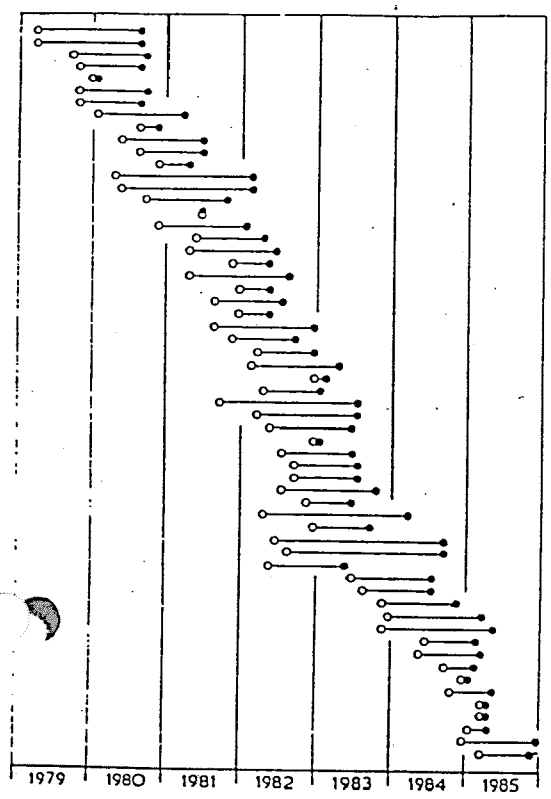

Fig 1. 59 haemophiliacs with estimated dates of seroconversion. Open and closed circles denote negative and positive anti-HIV results, respectively.

count was determined using an automated whole blood counter (Ortho ELT 800 with a differential screen). The proportion of T4 lymphocytes was determined using a fluorescence activated cell sorter (Coulter EPICS V) after labelling the lymphocytes with monoclonal antibodies in the CD4 group: Leu-3a from Becton-Dickinson and RFT4 from the Royal Free Hospital Department of Immunology. Absolute T4 lymphocyte counting started in November 1982.

RESULTS

Seroconversion dates and clinical HIV related disease

In 59 patients for whom a previously negative anti-HIV result was available, the date of seroconversion could be estimated as the mid-point between the last negative and the first positive result. The median time interval between these two events was 11 months (range 16 d to 25 months), estimated dates of seroconversion spanning the years 1980–85 inclusive (Fig 1). In the remaining 53 patients the earliest available serum sample tested was anti-HIV positive. The earliest serum found to be anti-HIV positive was dated 12 December 1979; the last 'new' seroconversion was estimated to be July 1985. For the whole cohort, the median age at time of initial seropositivity was 24 years (range 2–77 years). The median time of follow-up was 5 years 5 months (range 1–9 years).

By 30 November 1988, 66/112 (59%) patients had developed HIV related clinical symptoms (Table I). Thrombocytopenia, defined as a platelet count $< 50 \times 10^9$/l, occurred in seven (6%) patients and two of these had developed AIDS. Bacterial infections included tonsillitis (6), otitis media (3), appendicitis (2), *E. coli* septicaemia (2), sinusitis (1), tooth abscess (1) and pneumococcal pneumonia (1). AIDS, classified according to the Centres for Diseases Control (CDC, 1987), had occurred in 22 (20%) patients (Table II). Eleven of the 16 deaths were due to AIDS (Table III).

Table I. HIV-related clinical symptoms in 66/112 (59%) anti-HIV positive haemophiliacs

Seborrhoeic dermatitis	22 (20%)
Oral candidiasis	17 (15%)
Bacterial infections	16 (14%)
Herpes zoster	11 (10%)
Thrombocytopenia	7 (6%)
Lymphadenopathy	7 (6%)

Table II. AIDS-presenting clinical features in 22/112 (20%) anti-HIV positive haemophiliacs

Pneumocystis pneumonia	11
HIV wasting syndrome	2
Non-Hodgkins lymphoma	3
Oesophageal candida	2
Aspergillus pneumonia	1
Cerebral toxoplasmosis	1
Chickenpox pneumonia (heroin addict)	1
Staphylococcal septicaemia (multiple cerebral abscesses)	1

Table III. Causes of death in 16 anti-HIV positive haemophiliacs

AIDS (11)	
Pneumocystis pneumonia	4
Non-Hodgkins lymphoma	2
Mycobacterium avium intracellulare (abdominal infection)	1
Aspergillus pneumonia	1
Chicken pox pneumonia (heroin addict)	1
HIV wasting syndrome	1
Staphylococcal septicaemia	1
Cirrhosis, liver failure	2
Cerebral haemorrhage	1
Suicide	1
Aspiration of vomit following an epileptic fit	1

p24 Antigenaemia

p24 antigenaemia was detected in 20 patients (18%) (Fig 2). Seven were p24 positive at the time of their first anti-HIV

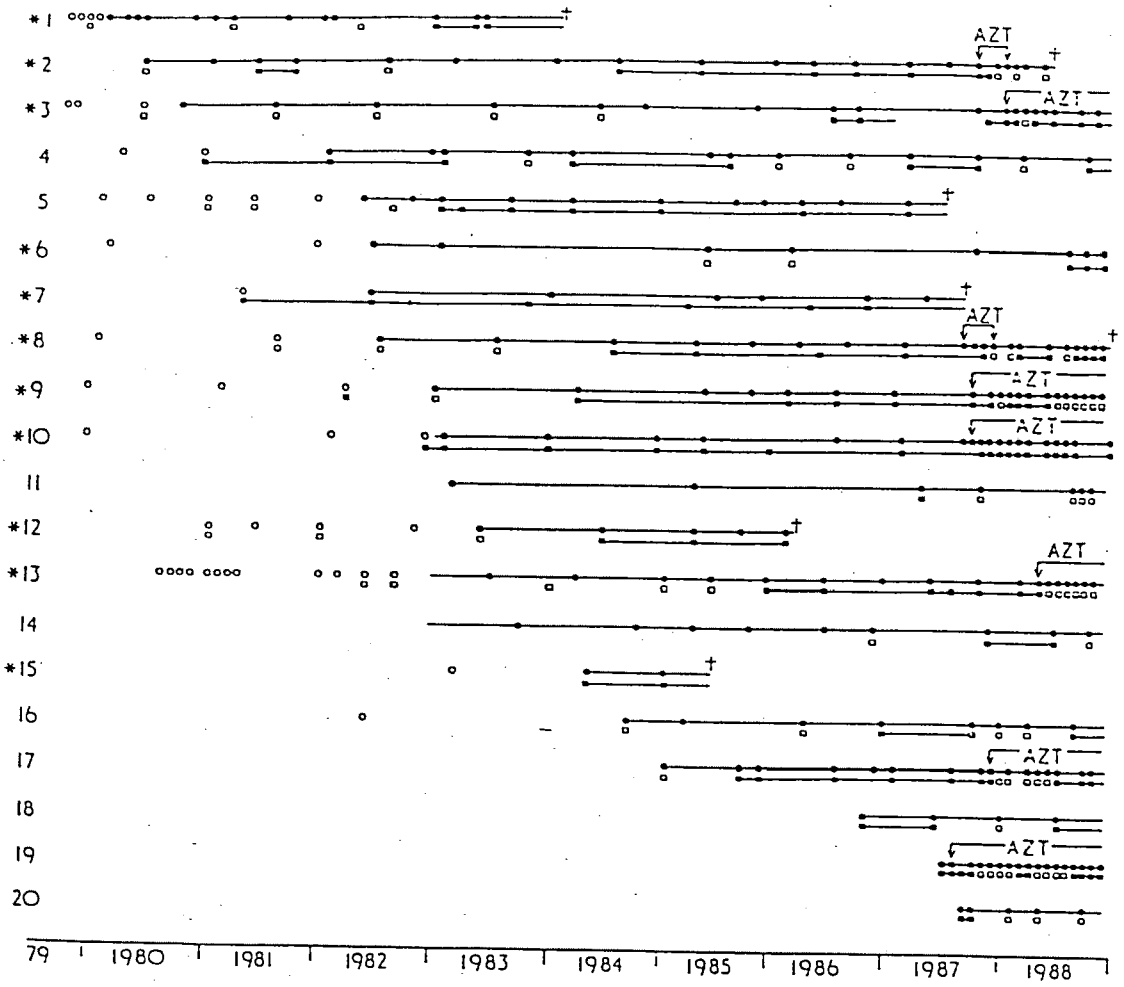

Fig 2. p24 antigenaemia in 20 haemophiliacs. Open and closed squares denote negative and positive p24 results respectively. Open and closed circles denote negative and positive anti-HIV results. Patients with AIDS are indicated by an asterisk and those who have died are indicated †.

positive result, but the time to develop p24 after seroconversion was variable in the remaining 13 patients. Eleven (55%) p24 positive patients developed AIDS at a median interval of 2 years 3 months (range 2 months to 6 years 3 months) after first detection of p24 antigen. In contrast, only 11/92 (12%) p24 negative patients developed AIDS ($P<0{\cdot}001$, chi-square test).

Progression to HIV-related clinical symptoms and AIDS

The methods of Kaplan-Meier were used to calculate cumulative incidence of clinical disease in the 59 patients with estimated dates of seroconversion. At 8 years the estimated cumulative probability of developing symptoms was 73% (95% confidence limits 58% and 88%) (Fig 3), and of developing AIDS 40% (95% confidence limits 30% and 50%) (Fig 4). These estimates were made for 8 years after seroconversion because only two patients had been seropositive for 9 years. Lymphadenopathy as a symptom was excluded from the analysis because of difficulty in timing onset. The respective estimates for symptoms and AIDS are also shown for the cohort stratified for age. The older group (≥ 5 years) had a significantly higher chance of developing symptoms ($P=0{\cdot}002$ log rank test) and AIDS ($P=0{\cdot}005$ log rank test).

T4 lymphocyte count and disease progression

The median number of measurements of absolute T4 lymphocytes for each of the 112 patients was seven (range 0–31). The relationship between the median T4 lymphocyte count and time since seroconversion is shown in Fig 5. When a linear regression model was fitted for each patient the median slope for the whole population was $-0{\cdot}068 \times 10^9$/l per year. The respective slopes for patients with and without AIDS were $-0{\cdot}113 \times 10^9$/l per year and $-0{\cdot}054 \times 10^9$/l per year ($P<0{\cdot}02$, Wilcoxon two-sample test).

In an analysis of covariance the difference in mean minimum T4 lymphocyte counts between those patients with and without AIDS remained large and significant after adjusting for years of sero-positivity ($0{\cdot}21 \times 10^9$/l, $P=0{\cdot}04$). In contrast, the difference in the mean number of years of

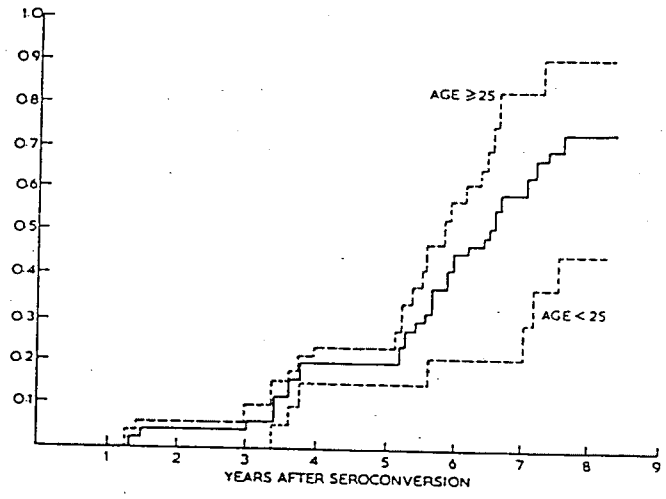

Fig 3. Estimated cumulative probability of developing HIV-related clinical symptoms in 59 anti-HIV positive haemophiliacs with known date of seroconversion.

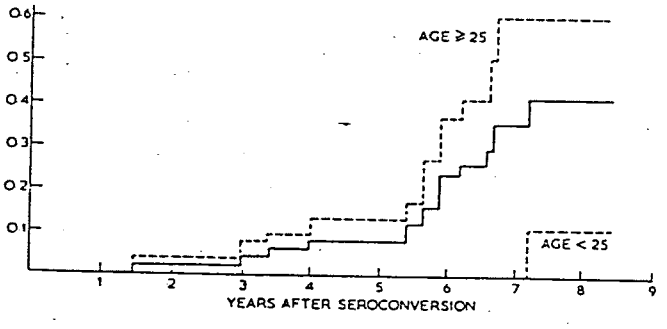

Fig 4. Estimated cumulative probability of developing AIDS in 59 anti-HIV positive haemophiliacs with known date of seroconversion.

eropositivity between those with and without AIDS was small and non-significant after standardizing for T4 lymphocyte count (0·7 years, $P=0·39$).

The cumulative probability of developing HIV-related clinical symptoms or AIDS before falling to a given T4 count is shown in Table IV. For a given T4 count, the numerator for the probability is the number of patients diagnosed as having symptoms or AIDS while having a T4 count above this level. The denominator consists of all those in the numerator plus the number of patients who reached the T4 cut-off value symptom or AIDS-free. There was only a 50% chance of being symptom free at a T4 lymphocyte count of $0·2 \times 10^9$/l and AIDS-free at a T4 lymphocyte count of $0·05 \times 10^9$/l.

The relationship between the T4 lymphocyte count and progression to AIDS was studied in relation to the total number of patient years of follow-up. There were 450 years before and 58 years after reaching a T4 lymphocyte count of $0·2 \times 10^9$/l. The numbers of AIDS cases in these groups were two and 15. This difference is highly significant ($P<0·0001$, Poisson model (IARC, 1987)).

The median T4 count by years from seroconversion is shown separately for patients aged less or more than 25 years in Fig 6. The rate of fall of T4 count is similar in the two age groups, but the older patients had lower T4 counts at the time of seroconversion and therefore reached very low T4 counts more rapidly.

The fall in T4 lymphocyte count for patients who were p24 antigen positive at any time during follow-up in comparison with p24 antigen negative patients is shown in Fig 7. The fall was more rapid in the p24 positive patients.

DISCUSSION

The timing of the first seroconversion (1979) in this cohort correlated well with the beginning of the AIDS epidemic in homosexuals and intravenous drug users in the United States and similar observations have been made in American (Evatt *et al.* 1985) and French (Mathez *et al.* 1986) haemophilic populations. Heat-treated concentrates were introduced into our centre in late 1984 and there have been no new seroconversions since July 1985. As the period between HIV exposure and seroconversion is usually less than 14 weeks (Simmonds *et al.* 1988) and only occasionally exceeds 6 months (Ranki *et al.* 1987) we are optimistic that no new seroconversions will occur.

Previous estimates of the proportion of haemophiliacs

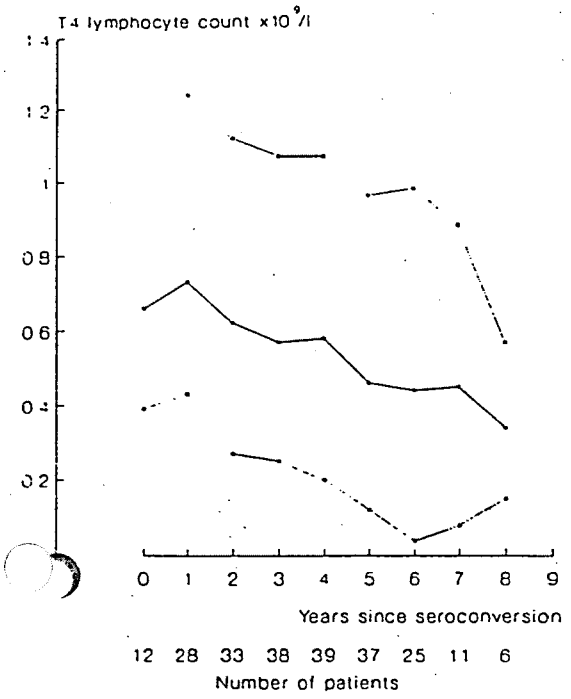

Fig 5. Median (10% and 90% percentiles) T4 lymphocyte counts from time of seroconversion.

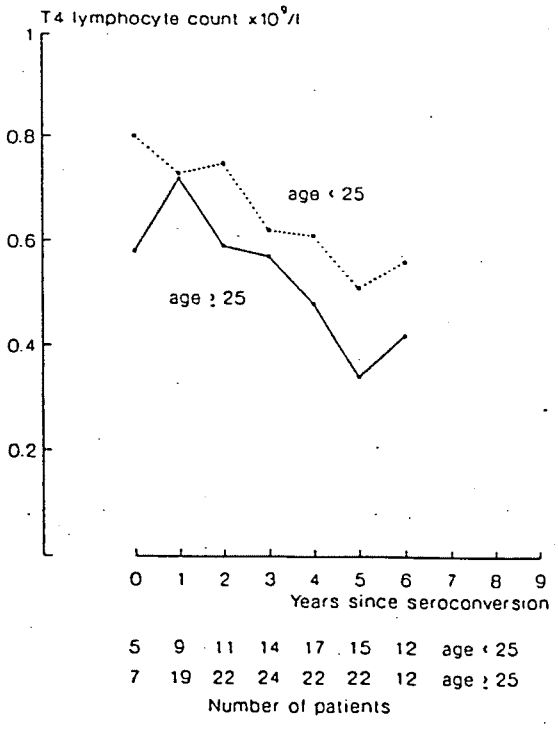

Fig 6. Effect of age on median T4 lymphocyte count from time of seroconversion.

Table IV. Estimated cumulative probability of developing HIV-related symptoms and AIDS before falling to given T4 count levels

T4 count × 10^9/l	Symptoms	AIDS
0·30	32% (18/56)	0% (0/48)
0·25	39% (21/54)	0% (0/43)
0·20	52% (29/56)	5% (2/39)
0·15	63% (34/54)	13% (4/32)
0·10	86% (42/49)	38% (9/24)
0·05	90% (43/48)	50% (10/20)
0·03	94% (47/50)	68% (13/19)
0·02	96% (47/49)	76% (13/17)
0·01	98% (47/48)	81% (13/16)

infected with HIV who progress to serious disease have been made difficult by the uncertain date of seroconversion (Eyster *et al.* 1987; Darby *et al.* 1989). Sophisticated statistical methods have therefore been used to estimate the time of infection (Giesecke *et al.* 1988) including the use of Weibull distributions (Medley *et al.* 1987). We followed the more usual approach of estimating the mid-point between the last negative and first positive anti-HIV results and limited the Kaplan-Meier analysis to the 59 patients in whom this was possible.

Haemophilic cohorts often permit a more precise definition

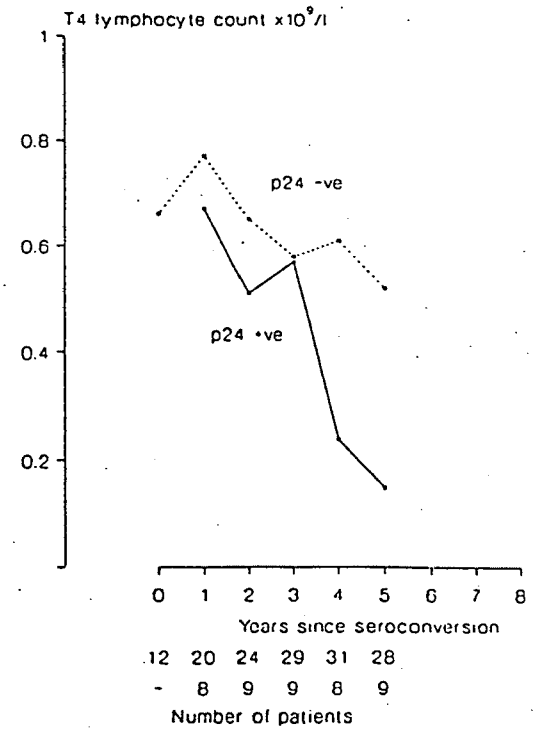

Fig 7. Median T4 lymphocyte count from time of seroconversion in p24 negative and positive haemophiliacs.

of the onset of clinical signs and symptoms of HIV infection han other at risk groups because haemophiliacs are usually nder at least 6-monthly surveillance of their haemophilia Eyster *et al.* 1987; Giesecke *et al.* 1988). Our estimate of the umulative incidence of symptoms is similar to that for wedish haemophiliacs, with 45% of patients developing ymptoms at 5 years after seroconversion (Giesecke *et al.* 988). Many of these symptoms require therapeutic interention, underlining the enormous potential cost of health are in patients infected with HIV who have not yet rogressed to AIDS. In contrast to others (Eyster *et al.* 1987), ve did not find thrombocytopenia to be a strongly associated isk factor. The high incidence of bacterial infections, particularly of the head and neck, may reflect poor humoral nmunity (Lane *et al.* 1983) and indicates the importance of mpt use of antibiotics in this patient group.

ne estimated cumulative incidence of AIDS in our patient roup, 40% at 8 years, was similar to that of Hessol *et al* 88) for male homosexuals (37% at 8 years) but was newhat higher than that of Goedert *et al* (1988) for haemophiliacs (29% at 8 years) (Moss & Bacchetti. evertheless, all these studies confirm that the rate of rogression from infection to AIDS is slow.

The p24 core protein of HIV is usually transiently detectble soon after initial infection, reappearing late in the disease Allain *et al.* 1986; Goudsmit *et al.* 1986). In a minority of atients, however, HIV antigenaemia has persisted after rimary infection (Pedersen *et al.* 1987). Our patients have emonstrated a varied pattern of p24 antigenaemia: occurence at any time of seroconversion, persistence of antigenemia, and recurrence both early and late in disease. It has een postulated that disappearance of HIV antigenaemia ight follow the development of antibodies to p24 (Allain *et l.* 1986) individuals with persistent antigenaemia having ffective humoral immunity (Lane *et al.* 1983). The recurence of p24 antigenaemia during the course of HIV infection be due to loss of anti-p24 (Pedersen *et al.* 1987). The nsition from latent to active HIV infection or AIDS could lso result from increased p24 antigen production which y depend on other co-factors such as cytomegalovirus MV) (Polk *et al.* 1987). In another study we have shown haemophiliacs infected with CMV progress more rapidly (Webster *et al.* 1989). Clearly, in our patients the resence of p24 antigen at any stage of HIV infection is ssociated with a worse prognosis.

We have not only confirmed the decline in the absolute T4 mphocyte count with time lapsed since seroconversion Moss & Bacchetti, 1989) but also established that this ecline is greater in patients who develop AIDS. This, gether with the analysis of covariance, suggests that the T4 mphocyte count may be of greater predictive power than he duration of seropositivity. Although the T4 lymphocyte ount is a coincident measure and may not predict well as a ingle measurement in individual situations we have found hat the T4 counts taken over time provide a reliable basis for ssessing the risk of developing symptoms of immune eficiency and AIDS. The more rapid fall of T4 lymphocyte ounts in p24 positive patients may reflect faster replication f the virus in these individuals. We have shown, like others (Moss & Bacchetti, 1989), that a depressed T4 lymphocyte count is essential for progression to AIDS. In particular, it is clear that the risk of developing AIDS is negligible until T4 lymphocyte counts fall to $0{\cdot}2 \times 10^9$/l. For this reason, when new treatments are assessed for asymptomatic patients the decline of the T4 lymphocyte count to $0{\cdot}2 \times 10^9$/l should be considered as an important indicator of the patient's condition, and the efficacy or otherwise of therapy.

In common with some other studies in haemophiliacs we found the cumulative incidence of symptoms and AIDS to be greater in older patients (Eyster *et al.* 1987; Darby *et al.* 1989); however, this is not a universal finding (Moss & Bacchetti, 1989). In our older patients the T4 lymphocyte count was lower at time of seroconversion, possibly reflecting a decline in T4 lymphocyte count with age irrespective of HIV infection (Falcao *et al.* 1987). Interestingly, the rates of fall of T4 lymphocyte count were similar in the two age groups.

This study was completed before any patients had entered the Medical Research Council double-blind placebo controlled trial of zidovudine in asymptomatic patients. The detailed description of HIV-related disease in this cohort will therefore provide a useful control for future drug trials in other asymptomatic at-risk groups. The cohort may also provide a basis for calculation of future health service needs in terms of care-provision and cost for HIV infected individuals.

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