containing less than 60% of the stated amount. This observation is much more important than the average loss of 5.8%. It should also be realised that the product had probably already lost some active ingredient before shipment and that further loss would be expected during inland transport and storage. The alarm recently expressed about ergometrine's stability in tropical climates⁷ is therefore justified. There is no obvious alternative to this life saving drug and it is not known whether methylergometrine is more stable. A WHO working group concluded recently that oxytocin is the best replacement,¹¹ but its stability in tropical climates is not known either.

None of the other drugs seemed unstable during international transport. From a medical and economic viewpoint this is particularly reassuring with regard to the common antibiotics. For most antibiotics the unprotected substance has shown signs of degradation⁴ as have unprotected capsules of chloramphenicol and tetracycline in simulation tests.⁵ For ampicillin¹² and benzylpenicillin^{13,14} high humidity or the presence of molecular water has been reported to be more damaging than high temperatures alone. These data suggest a high susceptibility of antibiotics to deterioration in hot and humid climates. However, two field studies from Sudan found no signs of instability in injections of procaine benzylpenicillin,^{7,15} benzylpenicillin,¹⁵ and ampicillin trihydrate¹⁵ and capsules of tetracycline^{7,15} and chloramphenicol¹⁵ and only a marginal loss in ampicillin trihydrate capsules.^{7,15} We found no stability problems with ampicillin trihydrate capsules and injection, benzylpenicillin injection, and tetracycline hydrochloride capsules. The stability of the common antibiotics in tropical climates therefore seems mainly

to depend on protection against humidity, even under exposure to high temperatures.

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Variability in serial CD4 counts and relation to progression of HIV-I infection to AIDS in haemophilic patients

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Abstract

Objective—To examine the CD4 count and its near term changes relative to progression to AIDS within 30 months and to subsequent CD4 counts.

Design—Longitudinal clinical and laboratory study.

Setting—Haemophilia treatment centres in six large American cities.

Patients—555 people with congenital clotting disorders who were infected with HIV, initially without AIDS, and seen at follow up for 6-30 months in 1986-9.

Main outcome measures—Absolute CD4 counts and incidence of AIDS.

Results—Outset CD4 count and age were independently related to progression to AIDS ($p < 0.0001$ and $p < 0.005$ respectively). Patients with CD4 counts of $0.30-0.49 \times 10^9$ cells/l had an age adjusted risk of AIDS within 30 months of only 9% that of patients with counts $< 0.20 \times 10^9$ /l. Children under 10 years old had only 16% of the CD4 adjusted risk of AIDS of people aged ≥ 45 years. Analysis of 149 patients' CD4 counts at the beginning and end of two successive six month intervals showed an average decrease of 11% in each six months regardless of the outset count ($\geq 0.20 \times 10^9$ /l). For individual patients the decrease in the second six month period was unaffected by the decrease in the first six month period.

Conclusions—Antiviral treatment of asymptomatic people, particularly children, with CD4 counts $\geq 0.3 \times 10^9$ /l is questionable if predicated on near term progression to AIDS. Because of individual CD4 count variability and the low rate of progression to AIDS near term declines in individual CD4 counts are a poor index for identifying people who will rapidly progress to AIDS.

Introduction

The absolute CD4 count has repeatedly been found to be either the best predictor or among the best predictors of the rapidity with which HIV-I infection progresses to clinical manifestations, especially AIDS disclosed by opportunistic infections.¹⁻⁴ As a consequence antiretroviral treatment is presently being administered to some people who are asymptomatic but whose CD4 counts are considered indicative of approaching AIDS.^{5,7} Values that have been suggested for initiation of AIDS prophylaxis are $0.2-0.5 \times 10^9$ cells/l, which if implemented could mean treatment for some 450 000 to 650 000 people in the United States alone.⁸

Although the appreciable toxic effects of zidovudine⁹ are now more manageable with regimens with reduced dosage, the residual incidence of bone marrow depression,¹⁰ the cost of the drug, the need for an increased amount of medical supervision, and the

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potential for later resistance to antiretroviral treatment¹¹ should be weighed against the still incomplete evidence for it postponing progression.

Many laboratory markers other than absolute CD4 count have a predictive value for progression to AIDS.¹²⁻¹⁴ There is no consensus, however, concerning which to use.¹⁵ Furthermore, many suggested tests are not well standardised or are unavailable outside research laboratories. Studies showing that decreases in CD4 counts preceding AIDS are often precipitous^{14 13 16} have suggested that an abrupt fall is a particular indication for treatment in asymptomatic people.¹⁷ Available data, however, do not establish that a sharp decline in CD4 count predicts an immediate further decline. We therefore investigated the value of visit to visit changes in CD4 counts.

Subjects and methods

SUBJECTS

The transfusion safety study is a cooperative investigation of circumstances influencing the occurrence and expression of HIV infection and other virus infections among people receiving blood components and plasma derivatives. All subjects give written consent for participation.

On entry and at each follow up visit, usually at six month intervals, the patient's clinical status is determined and blood obtained for laboratory assessments. Evaluation includes questions and review of records with regard to the decision whether to give antiretroviral drugs.

HIV antibody status was determined by enzyme linked immunoassay supplemented by western immunoblotting.¹⁸ The diagnosis of AIDS was accepted if recorded by the physician with principal responsibility for the patient's care and if it conformed to the criteria of the United States Centers for Disease Control.¹⁹

From August 1985 to mid-February 1989 we enrolled 1230 people with congenital clotting disorders, 682 of whom were positive for HIV antibody on entry. Of those who were positive, 555 were free of AIDS and had a valid CD4 count when seen on the first occasion from 1 July 1986 to 30 June 1989. The present analyses use data collected for the ensuing 6-30 months (to 31 December 1989). The first measurement during the period is defined as the outset CD4 count.

We considered progression to AIDS in terms of CD4 counts at the first visit in the study interval, and also in terms of age, which is known to be a prognostic factor.^{1 20-22} We then analysed the predictiveness of any given CD4 count for a specific patient's CD4 count six months later for subjects who had three successive CD4 measurements at intervals of about six months in 1986-7. We investigated how well the first count at the outset of these observations and the second count six months later, individually and in combination, would predict the third count 12 months after the outset of observations.

LYMPHOCYTE IMMUNOPHENOTYPING

At each visit the patient's lymphocyte populations were analysed. All laboratories used the same model flow cytometer (EPICS-C), monoclonal antibodies from the same batch, and standardised testing procedures. Study specific intralaboratory and interlaboratory quality controls were added to controls routinely used* by the clinic.²³ Duplicate or triplicate specimens were routinely submitted under different study identification numbers along with the routinely collected specimens. For interlaboratory control the standardisation laboratory sent specimens by air-mail for parallel processing in all laboratories the next day.

STATISTICAL ANALYSES

For analysis of the rate of progression to AIDS standard life table and Cox logistic methods were used²⁴⁻²⁶; calculations were made with the EPILOG statistical package (Epicenter Software, Pasadena, California, United States). For analysis of changes in CD4 count over time standard regression methods using log(CD4) (all logarithmic values are to base 10) were used²⁷; these calculations were made with the SAS statistical package (SAS Institute, Cary, North Carolina, United States). All p values are two sided. Confidence intervals are for 95% coverage.

Results

RATE OF PROGRESSION TO AIDS

Among the 555 patients, 57 (10%) had developed AIDS on or before 31 December 1989. The cumulative probability of progression to AIDS within 30 months was estimated to be 50% for subjects with CD4 counts $<0.2 \times 10^9$ cells/l, 22% for patients with counts of $0.20-0.29 \times 10^9$ /l, and 3-6% for counts of $\geq 0.3 \times 10^9$ /l (fig 1).

FIG 1—Kaplan-Meier plot showing proportions of patients infected with HIV (n=555) with various outset CD4 counts who developed AIDS during period of observation

Thus the relative risk of AIDS was strongly dependent on the outset CD4 count (table I).

The relative risk of AIDS was also strongly dependent on age (table I). Outset CD4 count and age were inversely related (table II). Outset CD4 counts $<0.2 \times 10^9$ /l were found in only 8% of the patients aged under 10, but in 27% of patients aged ≥ 45 . The mean CD4 count decreased steadily from 0.67×10^9 /l in patients aged under 10 to 0.39×10^9 /l in patients aged ≥ 45 . The prognostic implication of each variable, however, was only slightly diminished after adjustment for the other (table I); individual 95% confidence intervals for adjusted relative risks for age categories

TABLE 1—Life table analysis of progression to AIDS by CD4 count and age

Characteristic	No of patients		Relative risk	
	Total	Progressed to AIDS	Crude	Adjusted (95% confidence interval)*
CD4 count ($\times 10^9$ /l)				
≥ 0.50	228	9	0.07	0.09 (0.04 to 0.18)
$0.30-0.49$	178	7	0.08	0.09 (0.04 to 0.20)
$0.20-0.29$	60	10	0.36	0.37 (0.18 to 0.76)
<0.20 †	89	31	1.00‡	1.00‡
Age (years)				
<10	53	1	0.08	0.16 (0.02 to 1.29)
10-24	209	16	0.32	0.52 (0.25 to 1.12)
25-44	242	28	0.52	0.61 (0.31 to 1.21)
≥ 45 †	51	12	1.00‡	1.00‡

*Values are adjusted for the other categorised variable in the table. All calculations for significance were done by using a Cox analysis with continuous variables.

†Reference category.

‡p<0.0001.

§p=0.0044.

overlap 1.0 but the trend test remains highly significant ($p=0.0044$).

Exclusion of patients given zidovudine before their outset CD4 count was measured ($n=2$) or during the period of observation ($n=85$) did not change these relations.

CHANGE IN CD4 COUNT WITH TIME

Thirty seven of the 57 patients with AIDS had CD4 counts measured less than six months before diagnosis of AIDS; 32 (86%) had values $<0.2 \times 10^9/l$. Thus a low CD4 count immediately preceded AIDS in most patients. Patients who progressed to AIDS had a more precipitous rate of fall in CD4 count, and both the absolute and proportionate declines were greatest among those patients whose outset CD4 counts were highest.

To determine prospectively the predictiveness of successive CD4 counts (that is, when the AIDS outcome is still unknown) we looked at 196 patients with three successive CD4 counts at six month intervals in the period 1 July 1986 to 31 December 1987. We denote the three counts as $CD4_{1st}$, $CD4_{2nd}$, and $CD4_{3rd}$. Excluded from further analysis in this group were 37 patients with $CD4_{2nd}$ counts $<0.2 \times 10^9/l$ (a level at which fluctuations are disproportionately wide); eight patients who received zidovudine any time before the $CD4_{3rd}$ count; and two patients whose large changes in CD4 count were "outliers" in the statistical analysis (neither developed AIDS). We studied the remaining 149 patients for changes in CD4 count over time.

The relation between $CD4_{2nd}$ and $CD4_{3rd}$ was expressed logarithmically. This mathematical transformation achieved approximate linearity and stability of variance (after model fitting) over the given range of CD4 counts ($\geq 0.2 \times 10^9/l$ for $CD4_{2nd}$). As expected $CD4_{2nd}$ was highly predictive of $CD4_{3rd}$ ($r=0.756$,

TABLE II—Relation of CD4 count with age in 555 patients infected with HIV at outset of observational period (1 July 1986 to 30 June 1989). Figures are numbers (percentages) of patients

Age (years)	CD4 count ($\times 10^9/l$)				Mean CD4 count ($\times 10^9/l$)
	<0.20 (n=89)	0.20-0.29 (n=60)	0.30-0.49 (n=178)	≥ 0.50 (n=228)	
<10 (n=53)	4 (8)	1 (2)	14 (26)	34 (64)	0.67
10-24 (n=209)	29 (14)	12 (6)	73 (35)	95 (45)	0.49
25-44 (n=242)	42 (17)	40 (16)	77 (32)	83 (34)	0.45
≥ 45 (n=51)	14 (27)	7 (14)	14 (27)	16 (31)	0.39

FIG 2—Plot of $\log(CD4_{3rd})$ against $\log(CD4_{2nd})$ for 149 patients infected with HIV with $CD4_{2nd}$ count $\geq 0.2 \times 10^9/l$ in 1986-7. Regression line has slope fixed at 1.0

TABLE III—Changes in CD4 count over about six months among 149 patients with $CD4_{2nd}$ counts $\geq 0.2 \times 10^9/l$. Figures are numbers of patients

CD4 _{2nd} count ($\times 10^9/l$)	CD4 _{3rd} count ($\times 10^9/l$) (six months later)					
	<0.20	0.20-0.29	0.30-0.39	0.40-0.49	0.50-0.69	≥ 0.70
0.20-0.29	4	8	4	2	0	0
0.30-0.39	3	8	8	5	2	0
0.40-0.49	1	2	9	11	8	1
0.50-0.69	0	5	4	8	13	7
≥ 0.70	0	0	1	3	13	19

FIG 3—Proportion of patients with outset CD4 counts of $0.3-0.9 \times 10^9/l$ predicted to decrease to $<0.2 \times 10^9/l$ within 30 months. Lines (a), (b), and (c) indicate proportions for those with outset counts of 0.3 , 0.4 , and $0.5 \times 10^9/l$

$p=0.0001$). The fitted line with its slope set to 1.0 is given by the simple equation:

$$\log(CD4_{3rd}) = \log(CD4_{2nd}) - 0.050 \times t_{2,3} \quad (1)$$

where $t_{2,3}$ is the time interval from $CD4_{2nd}$ to $CD4_{3rd}$ (measured in units of six months (fig 2); $r=0.749$, $p=0.0001$). Equation 1 estimates a mean fall in CD4 count of 11% (95% confidence interval 7% to 18%) in a six month period at all CD4 values $\geq 0.2 \times 10^9/l$.

The variability in the proportionate change in $CD4_{2nd}$ to $CD4_{3rd}$ over the six month period may be seen in the wide scatter of points in figure 2 around the fitted line. The standard deviation around the line is 0.14—that is, one standard deviation represents a 38% increase or decrease in CD4 count. CD4 counts in 54 (36%) of the 149 patients actually increased during the six month period; in 29 (19%) of these patients their CD4 count increased by one or more of the divisions in table III.

We added a term to equation 1 for the rate of change in the six month period before measurement of the $CD4_{2nd}$ count:

$$\log(CD4_{3rd}) = \log(CD4_{2nd}) - \alpha t_{2,3} + \beta [(\log(CD4_{2nd}) - \log(CD4_{1st})) / t_{1,2}] \quad (2)$$

where $t_{1,2}$ is the time interval from $CD4_{1st}$ to $CD4_{2nd}$. This increased r slightly from 0.749 to 0.779, but the sign of β is negative, so that for a given $CD4_{2nd}$ count the larger the drop between the $CD4_{1st}$ count and the $CD4_{2nd}$ count the higher the predicted $CD4_{3rd}$ count. Adding a term to equation 1 for the age of the patient barely increased r (from 0.749 to 0.751).

Equation 1 can be used, together with knowledge of the scatter of the points around the fitted line in figure 2, to estimate the statistical distribution of CD4 counts over time. Figure 3 shows the estimated proportion of people with outset CD4 counts of 0.3 , 0.4 , 0.5 , 0.7 , and 0.9×10^9 cells/l expected to have their CD4 count fall to $<0.2 \times 10^9/l$ over a 30 month period. In all, 58% of those with an outset CD4 value of $0.3 \times 10^9/l$ would be expected to fall to $<0.2 \times 10^9/l$ by 30 months, decreasing to 9% when the outset CD4 count is

$0.9 \times 10^9/l$. For the first six months of the period the equation predicts a fall to $<0.2 \times 10^9/l$ for 18% of those who start at $0.3 \times 10^9/l$ (fig 3, level (a)), 4% who start at $0.4 \times 10^9/l$ (level (b)), and $<1\%$ for those who start at $0.5 \times 10^9/l$ (level (c)).

Discussion

People with CD4 counts of $<0.2 \times 10^9/l$ progress to AIDS at a high rate. Interrupting the decline of CD4 count toward this level seems at least temporarily possible with antiretroviral drugs. The major trial of zidovudine in asymptomatic people, however, did not distinguish the rate of progression among those with before treatment CD4 counts of $0.20-0.29 \times 10^9/l$ and $0.30-0.49 \times 10^9/l$.⁵ The latter asymptomatic people in our study had an incidence of AIDS over 30 months of only 4.5% (fig 1), little different from the group with a CD4 count $\geq 0.5 \times 10^9/l$. It seems premature, therefore, to recommend routinely prescribing zidovudine or similarly toxic drugs entirely on the basis of a CD4 count of $<0.5 \times 10^9/l$. The proportion of people with potential short term postponement of AIDS is particularly small among children infected through transfusion.^{27 28} In contrast to vertically transmitted HIV infection the rate of progression among haemophilic patients under 10 years of age is very low even when adjustment is made for their higher CD4 count (table I).²⁹

Furthermore, the low near term risk of AIDS in people with a CD4 count of $0.30-0.49 \times 10^9/l$ (table I) means that therapeutic trials to evaluate prophylaxis against AIDS among people in that range will require large numbers of patients and lengthy follow up. Surrogate end points must be validated, particularly among children.^{16 30}

The marker of progression most widely used as a surrogate for AIDS is a CD4 count of $<0.2 \times 10^9$ cells/l. As seen from figure 3 the equation we derived for the overall CD4 count trend with time suggests that only in people with outset CD4 counts around 0.3×10^9 cells/l would the count fall to a level of $<0.2 \times 10^9/l$ with sufficient frequency over six months for this to have any possibility of meaningfulness. In people with outset CD4 counts of around $0.3-0.5 \times 10^9$ cells/l, however, a trial should be feasible in 24-30 months despite individual and interlaboratory variability.

In managing particular patients fluctuations in CD4 count (table III) make the physician's task difficult. Even the more reliable long term trends⁴ may not apply to individuals.³¹ Sources of error are measurement of total leukocyte counts and differential counts, specimen handling, and flow cytometric analyses. Aside from laboratory variability, CD4 counts may fluctuate from visit to visit because of factors other than HIV infection; these should be more systematically explored.

The fact that patients infected with HIV who develop symptoms of AIDS have more rapid declines in CD4 counts than those who remain asymptomatic causes an abrupt decrease to be negatively interpreted by both the patient and the physician. A more rapid long term decline in CD4 count can be seen retrospectively to have occurred in patients who progress to AIDS, but at any point before the development of AIDS the near term variability precludes assigning any prognostic significance to precipitous changes other than that associated with a low CD4 count. The data in this study show that abrupt decreases are frequent and often temporary.

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Controlled low protein diets in chronic renal insufficiency: meta-analysis

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Abstract

Objective—To determine whether low protein diets retard the development of end stage renal disease.

Design—Meta-analysis of 46 trials since 1975, from which six randomised controlled trials were selected.

Setting—Five trials in Europe and one in Australia between 1982 and 1991.

Subjects—890 patients with mild to severe chronic renal failure who were followed up for at least one year. 450 patients received a low protein diet and 440 a control diet.

Intervention—Difference in protein intake between control and treated groups of at least 0.2 g protein/kg/day.

Main outcome measure—Number of renal deaths (the necessity to start dialysis or death of patient during study).

Results—156 renal deaths were recorded, 61 in the low protein diet group and 95 in the control group, leading to an odds ratio of low protein to control of 0.54 with a 95% confidence interval of 0.37 to 0.79.

Conclusions—This result, obtained on a large population of patients suffering from chronic renal insufficiency, strongly supports the effectiveness of low protein diets in delaying the onset of end stage renal disease.

Introduction

During the past 10 years a lot of experimental data have suggested that dietary protein restriction may retard or even halt the development of non-specific glomerular lesions and hence the progression of end stage renal disease. Despite the many studies on dietary interventions that were performed a few decades ago, some questions have re-emerged¹—What kind of proteins? What degree of restriction? Is a supplement with essential amino acids or keto-analogues of amino acids required? When is the most appropriate moment to start the diet?²⁻⁴ These questions have been poorly answered by numerous studies showing that low protein diets reduce the time related increase in plasma creatinine concentration. However, the validity of plasma creatinine concentration as an estimate of renal function,⁵ especially during reduced protein intake, has not really been investigated in terms of extrarenal creatinine metabolism and the tubular secretion of creatinine, which is dependent on protein intake.⁶ The physician's main goal is to maintain clinical improvement in patients who wish to delay starting dialysis for as long as possible, and so we have reanalysed research data to determine the number of patients reaching end stage renal disease during follow up. This meta-analysis, should enable the strength of the association between low protein diet and reduction in end stage

renal failure to be assessed, taking into consideration the bias due to the various confounding factors (such as regression to the mean) and avoiding the use of intermediate criteria (such as the slope of the time course for the reciprocal values of serum creatinine concentration and the rate of decay of creatinine clearance).

Methods

The principle of meta-analysis entails the adjusted analysis of results in a collection of studies, with a unique criterion for all studies. Meta-analysis is particularly suitable for studies of rare events in, for example, ponderous or longlasting trials that do not include many patients.⁷⁻⁹ The quality and impact of a meta-analysis depends on the selection process for the criterion, which must be well defined at the beginning of the meta-analysis, and easily collected by non-specialist observers.

Definition of a common event—We defined renal death as either the necessity to begin dialysis or the death of patients during the study. Patients receiving grafts before starting dialysis were counted as having had renal death. Patients who were lost to follow up or who stopped taking the diet were not counted as having had renal death. These criteria were applied independently of the randomised treatment by the authors on the basis of published data or complementary data kindly provided by the investigators.

Collection of data and study selection—To avoid omission of results from less well known studies we scanned research reports with a computer search of Medline files and abstract books from the International Society of Nephrology and the American Society of Nephrology. We also asked many investigators for complete or incomplete, published or unpublished randomised studies. We restricted the review to randomised trials because without randomisation lack of bias cannot be guaranteed. Trials that used other methods of allocating treatments (such as retrospective controls and non-randomised, crossover, or prospective uncontrolled protocols) were therefore not included. Trials studying only diabetic nephropathy were not selected, because the equilibrium of diabetes is of major importance in the progression of renal failure and cannot be adequately measured between control and treated groups. Table I summarises 46 studies on protein restriction and its effects on the progression of chronic renal insufficiency. Individual data and extra details if not mentioned in the original papers, were collected from investigators who kindly agreed to send us their raw data.

Statistical methods—Intention to treat analysis was made on the selected populations, randomly assigned to control or treated groups (a restricted protein diet). Standard statistical methods were used (percentage

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