Temporal Trends of Initial CD4 Cell Counts Following Human Immunodeficiency Virus Seroconversion in Italy, 1985–1992

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To determine whether initial CD4 cell counts after human immunodeficiency virus (HIV) seroconversion have decreased over calendar time among participants in the Italian Seroconversion Study, HIV seroconverters who between 1985 and 1992 had a documented negative serology followed by a positive serology within 12 months and a first CD4 cell measurement within 24 months of seroconversion (defined as midpoint of negative and positive HIV tests) were cross-tabulated by year of seroconversion. Linear regression methods were used to examine temporal trends in initial CD4 level after adjustment for age, lag time of seroconversion, lag time of CD4 cell measurement, risk group, and clinical center. Between 1985 and 1992, the overall median initial CD4 cell level after seroconversion was 660 μl with a median lag time of 212 days and 137 days for seroconversion and first CD4 cell measurement, respectively. In univariate and multivariate models, the CD4 cell count increases of 4.3 and 4.2 cells μl/year, respectively, were not statistically significant. These data do not identify a trend of lower CD4 counts following HIV seroconversion in Italy and suggest indirectly that HIV has probably not become more virulent between 1985 and 1992. Am J Epidemiol 1996;143:278–82.

HIV; incidence; substance abuse, intravenous

Two reports from investigators at the Naval Health Research Center in San Diego examined CD4 T-lymphocyte counts following seroconversion. With 40 percent having counts <500 cells/μl within 24 months after seroconversion, the authors suggested that there may be a growing population with rapid CD4 cell loss (1, 2). If the proportion of recently infected persons with low CD4 cell counts is increasing over time, this would raise the troubling suggestion that more virulent strains of human immunodeficiency virus (HIV) may be circulating and infecting the population. Documentation of such trends would portend potentially disastrous implications on the course of the acquired immunodeficiency syndrome epidemic.

To adequately address and evaluate the findings and conclusions of the Navy studies, data are needed from other populations in which ascertainment of HIV seroconversion and T-cell subset studies have been ongoing. In addition, there is the issue of accounting for other variables that could affect the observed results of CD4 cell counts among HIV seroconverters by calendar year. First, because date of seroconversion is usually not known but is estimated as a value (usually the midpoint) between the last seronegative and first seropositive visits (i.e., the lag time), and because CD4 cell counts are known to drop precipitously in the first 12–18 months following seroconversion (3), studies that examine temporal trends in decline of CD4 cell level should verify that the observed results are not due to variation in lag times. For example, if the proportion of HIV seroconverters with low CD4 increases over time, and the lag time increases (due to flagging interest), then the trend could simply be due to the fact that the more recent seroconverters are first measured a longer time after seroconversion than earlier seroconverters for whom counts could be expected to be lower. Hence, analyses of temporal trends need to statistically adjust for lag time. Second, inasmuch as
several studies have reported an association between age and rate of CD4 cell decline (4), future studies of temporal trends should account for differing age distributions of HIV seroconverters over time. Although differing distributions of other demographic characteristics (e.g., sex, risk group) are probably less important than age (5), they should be considered in analysis of temporal trends. Third, because measurement of CD4 absolute count is variable between laboratories and over time within laboratories, it is important to account for this interlaboratory heterogeneity and the potential for "laboratory drift" of measurement. Finally, antiretroviral therapies introduced in 1988 are known to produce transient increases in CD4 cell levels (6) that in temporal trend analyses could mask a shift toward more virulent strains. One approach is to restrict analyses to the first CD4 cell level measurement after seroconversion because this measurement will dictate, rather than follow, any decision for antiretroviral therapy.

To examine temporal trends of CD4 cell measurement following HIV seroconversion and to adjust simultaneously for potential confounders of lag times, demographic and risk group characteristics, and potential laboratory variation, we collected data on 684 HIV seroconverters identified between 1985 and 1992 in the Italian Seroconversion Study.

METHODS

Study populations

We derived data from a cohort of injection drug users, homosexual men, and heterosexual contacts of HIV-infected persons who were recruited from 16 clinical centers in several Italian cities, as described elsewhere (7). The most important inclusion criterion for this analysis was the availability of a documented HIV-antibody seronegative test followed by a positive one within 12 months. Because the first CD4 cell measurement was sometimes obtained at a date later than the first HIV-positive test, we further required that the first CD4 cell measurement be available within 24 months from the estimated date of HIV seroconversion; 24 months was selected to remove outliers yet retain a maximum sample size for analysis. Estimated date of HIV seroconversion was defined as the midpoint between the last negative and the first seropositive test. Two centers that contributed fewer than 10 seroconverters were excluded from analysis. Individuals with an unknown risk group or those infected through blood transfusion were also excluded ($n = 16$). Analysis compared the years 1985–1992, since 1992 was when documentation of HIV seroconversion was complete at the time of analysis. No patients were started on antiretroviral therapy prior to the first measurement of CD4 that was used for this analysis.

Data collection

Using a standardized form, we collected the following information at the first visits after the individual was known to be seropositive: 1) demographics (age, sex, risk group); 2) laboratory parameters (white cells, lymphocytes, CD4+ cells, CD8+ cells).

Laboratory procedures

Antibody to HIV was analyzed using a commercial enzyme-linked immunosorbent assay with Western blot confirmation. T-cell subset studies were performed by flow cytometry using OKT4 monoclonal antibodies (Ortho diagnostic, Raritan, New Jersey).

Statistical methods

Various methods of descriptive statistics were used to describe the cohort and the raw CD4 data. Demographic characteristics of the cohort by risk group were described using frequency tables and univariate distribution parameters such as median and range. The distributions of the first CD4 cell count taken after seroconversion by calendar year are shown in figure 1. In addition, the proportion of individuals with the first CD4 count less than 500 and 350 cells/ml was calculated for each calendar year after seroconversion, and a $\chi^2$ test for trend in proportions was used.

To evaluate the temporal trends in CD4 count measured soon after seroconversion, linear regression methods were applied with CD4 count as the dependent variable. Since the distribution of absolute CD4 count is known to be skewed toward high values, we have repeated all analyses with the square root of CD4 count as well as the CD4 percent of total lymphocytes. Because results were very consistent, we present only the analyses for the absolute count of CD4, which is readily interpretable.

Potential confounders entered as independent variables in the regression analysis included the following: lag time between the last HIV-negative and the first HIV-positive test (lag time for seroconversion); lag time between the first CD4 measurement and the estimated time of seroconversion (lag CD4); age at seroconversion (as a continuous variable); and risk group (injection drug users, homosexual men, heterosexuals). In the final model, we have added clinical center as a random effect factor to directly account for the contribution of interlaboratory differences to the overall variability.
RESULTS

During 1985–1992, 683 HIV seroconverters were identified from the Italian Seroconversion Study as meeting the following study criteria: lag time for seroconversion less than 12 months; lag time for estimated date of seroconversion to first CD4 cell measurement less than 24 months; from a clinical center that identified at least 10 HIV seroconverters; were injection drug users, homosexual men, or heterosexual contacts of HIV-seropositive individuals; and had complete demographic information. The median age for this study population was 26 years (range, 16–61 years): 53 percent were injection drug users, 29 percent were homosexual men, and 18 percent were heterosexual contacts. The overall median lag time for seroconversion (last negative to first seropositive test result) was 212 days (range, 15–365 days), and the median lag time for first CD4 cell measurement (estimated date of seroconversion to first CD4 cell count) was 137 days (range, 14–700 days). The overall median for the first CD4 cell count after seroconversion was 660 μl (range, 20–2,611 μl). The median CD4 percent was 29 (range, 4–58 percent).

Table 1 shows the number of seroconverters and the proportions with CD4 cell counts less than 500 μl and less than 350 μl by calendar year. The overall propor-
tions with less than 500 CD4 cells/μl and 350 μl were 27.4 percent and 11.1 percent, respectively, which did not vary systematically by year of seroconversion. Both tests for linear trends were nonsignificant.

Table 1 also shows the number of seroconverters, the median initial CD4 cell counts, the initial CD4 percent, the median lag time for seroconversion, and the median lag time for CD4 cell determination, each by calendar year of seroconversion. For CD4 cell count, the values range from 580 μl in 1988 to 725 μl in 1990; no obvious consistent trend is apparent. The median lag time for seroconversion ranged from 182 days in 1992 to 214 days in both 1986 and 1987; no obvious linear trend is apparent. The median lag times for first CD4 measurement were 106 days in 1985 and 137-152 days for the subsequent years, again with no apparent trend.

Table 2 shows the linear regression analysis of initial CD4 cell count by calendar year of HIV seroconversion. The model shows a nonsignificant tendency of initial CD4 cell counts to have increased by 4.3 cells/μl/year during 1985-1992. To adjust for potential confounders of lag time for seroconversion, lag time of CD4 cell measurement, age at seroconversion, and risk group, we added each variable one at a time to the basic model of initial CD4 cell count by year of seroconversion. We then constructed a final model that adjusted for all covariates simultaneously and considered center differences as a random effect factor. The results of the final multivariate regression analysis (table 2) showed that the slope estimate for first CD4 measurement were 106 days in 1985 and 137-152 days for the subsequent years, again with no apparent trend.

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**DISCUSSION**

The major finding of this paper is that the initial measurement of CD4 cell level following HIV seroconversion was virtually stable across 8 consecutive years (1985-1992) in Italy. In fact, there was a hint of a very slight increase of initial CD4 cell measurement over calendar time, although the trend was not statistically significant. The finding of no change could be considered spurious if the distribution of demographic variables or lag times that were used to estimate date of seroconversion, or the interval lag between seroconversion date and initial CD4 cell measurement, differed across the sequential cohorts defined by calendar year. However, in multivariate analyses that statistically controlled for these potential confounders, no change in initial CD4 cell measurement was observed over calendar time.

Because CD4 cell measurements were not performed in a single laboratory, it is possible that the observed results might be affected by interlaboratory variation. In response to this concern, we added the variable “center” to the multivariate analysis as a random effect. Although the results indicated that there was statistically significant interlaboratory variation, the results show no significant change in initial CD4 cell measurement over time even after accounting for this variation.

Because the distribution of CD4 absolute count is skewed, we repeated the analysis using the square root transformation as well as the percentage of CD4 lymphocytes, both of which have more symmetric distributions with stable variances over time. Nonetheless, the results of these analyses were very consistent with the results using CD4 count and showed no significant temporal trend in levels. Thus, these data do not support the suggestion of increasing HIV virulence over time.

Another potential confounder that could mask trends over time is the phenomenon of intralaboratory variation or “laboratory drift,” wherein a systematic shift in the measurement occurs over time. We obtained data on HIV-seronegative healthy individuals from the largest clinical center. We examined the 703 CD4 measurements by calendar year and noted no

**TABLE 2. Estimated temporal trend of first CD4 count following seroconversion among 683 human immunodeficiency virus seroconverters in Italy, 1985-1992**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Linear model coefficient</th>
<th>Unadjusted†</th>
<th>Adjusted‡</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(Mean ± SE)</td>
<td>(Mean ± SE)</td>
<td></td>
</tr>
<tr>
<td>Intercept (year = 1985)</td>
<td>696.4 ± 25.2</td>
<td>905.1 ± 61.0</td>
<td></td>
</tr>
<tr>
<td>Calendar year (CD4+ cells)</td>
<td>4.3 ± 6.3</td>
<td>4.2 ± 6.4</td>
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<tr>
<td>per year</td>
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<td></td>
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</tr>
<tr>
<td>Lag for seroconversion (months)</td>
<td>-2.8 ± 4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lag for CD4 (months)</td>
<td>-1.0 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-4.8 ± 1.7*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual men†</td>
<td>-82.1 ± 35.3*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual†</td>
<td>-90.2 ± 37.6*</td>
<td></td>
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</table>

* p < 0.05.
† Univariate model including only calendar year.
‡ Multivariate model adjusted for the listed covariate and for center differences considered as a random effect factor.
§ SE, standard error.
† Relative to injection drug users.
systematic trend over the period 1986–1992 with medians ranging from 1,281 to 974 cells/μl. In view of these findings, it is unlikely that a laboratory drift accounts for the observed results.

The apparent discrepancy between our conclusions and those of the Naval Health Research Center (1, 2) requires clarification. In fact, the proportion of HIV seroconverters with CD4 cell levels less than 500 cells/μl was similar across these studies. However, our data, which cover a broader time span, show that an apparent increase in the proportion with CD4 less than 500 cells/μl between 1990 and 1992 needs to be considered in light of values from earlier years. In addition, we extended our analyses to account for multiple sources of variability. Thus, while the Naval Health Research Center reports are provocative, the broader time span and additional analyses of the data from the Italian Seroconversion Study argue against concluding that there is a growing population of HIV-infected persons with rapid CD4 cell loss.

Before firm conclusions are drawn, several study limitations should be acknowledged. First, our analysis used only the first CD4 cell measurement following HIV seroconversion. The rationale for this strategy was to permit inclusion of the more recent years for comparison (because a requirement of 2 or more years of follow-up in addition to permissible windows of lag times would have restricted analysis of trends only to earlier calendar years that would provide complete information). The second rationale is that the first CD4 cell measurement following HIV seroconversion precedes the opportunity to prescribe antiretroviral therapies (e.g., zidovudine), which are known to cause transient elevations in CD4 cell measurements. Therefore, the observed results are not affected by the introduction of antiretroviral therapies.

Using only the first CD4 cell measurement following HIV seroconversion to evaluate virulence involves an assumption that the effects of changes in virulence would be manifest earlier in HIV infection. Definitive data that examine longitudinal patterns of CD4 cell decline by viral strains are lacking. Although data have been published to show tracking of CD4 cell counts (whereby those HIV seroconverters who have higher CD4 cell levels tend to remain higher over time) (8), it is possible that the initial effect of infection by different strains of the virus may in no way be as severe as the more long-term effects. Therefore, the link between CD4 cell levels following HIV seroconversions over time and virulence awaits additional, more detailed longitudinal study.

Another factor to consider is that because US Naval personnel serve throughout the world, they may have been more frequently exposed to the advancing edge of the Far Eastern epidemic on their return from duty in the Pacific. However, naval personnel also serve in the United States and Italy. The data from two studies conducted in the United States (9, 10) and the data from this study suggest that in these two countries, there has not been an important temporal change in CD4 cell levels following HIV seroconversion. Similar studies from the Far East are needed.

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REFERENCES