CONCISE COMMUNICATION

Relationships among the Detection of p24 Antigen, Human Immunodeficiency Virus (HIV) RNA Level, CD4 Cell Count, and Disease Progression in HIV-Infected Individuals with Hemophilia

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The aim of this study was to assess the relationships among the detection of p24 antigen, human immunodeficiency virus (HIV) RNA level, CD4 cell count, and disease progression in 111 males with hemophilia who were infected with HIV for ≥20 years. Sixty-four individuals (58%) developed p24 antigenemia a median of 11.6 years after seroconversion. The time to first detection of p24 antigen was shorter among those who were older (P = .04) and those with a high initial HIV RNA level (P = .006). The median HIV RNA level and CD4 cell count at the time of the detection of p24 antigen were 4.95 log_{10} copies/mL and 100 cells/mm^3, respectively. In univariate analyses, p24 antigenemia was associated with more-rapid progression to AIDS (relative hazard [RH], 5.50; P = .001). The effect was reduced (RH, 1.85; P = .06) after adjusting for CD4 cell counts and HIV RNA levels during follow-up, age, and calendar year. A significant relationship between p24 antigenemia and death was nonsignificant after adjusting for CD4 cell count.

p24 Antigen is a core protein of human immunodeficiency virus (HIV) that is detectable just before HIV seroconversion but usually is not detectable after seroconversion. The reappearance of p24 antigen at later stages of infection is associated with a poor prognosis [1–3]. However, because this reappearance usually coincides with a declining CD4 cell count [1, 4], the question of whether the recurrence of p24 antigenemia adds any prognostic information to that derived from the CD4 cell count has been raised [1–3, 5].

Levels of p24 antigen also are correlated with HIV RNA levels [3, 6]. Thus, the introduction of routine HIV load testing has meant that p24 antigen testing now is used rarely as part of routine testing in developed countries. Virus load testing, however, is often prohibitively expensive in developing countries and requires specially trained laboratory staff and equipment. Thus, a need to assess the prognostic value of markers that are cheaper to measure still exists. The aim of this study is to describe patterns of detection of p24 antigen in a cohort of individuals with hemophilia who were infected with HIV for ≥20 years. In particular, we wish to describe the time, CD4 cell count, and HIV RNA level at which p24 antigen first becomes detectable and to assess the prognostic value of p24 antigenemia after adjusting for the other 2 markers.

Patients and Methods

Patients. After receiving nonvirucidally treated clotting-factor concentrates, 111 males with hemophilia who were registered at the Royal Free Hospital Haemophilia Centre (London) became infected with HIV between 1979 and 1985. This cohort has been studied extensively [2, 7]. Clinical, immunological, and virological data are extracted annually from patient records and are stored in a computerized database, with all personal identifiers removed. The patients have been followed for a median of 18.2 years (range, 7.3–20.9 years) after infection with HIV.

Laboratory methods. Since 1982, lymphocyte subsets have been measured routinely on all fresh serum samples obtained at the hospital. In 1996, HIV RNA levels were measured retrospectively on stored serum samples that had been collected at yearly intervals since HIV seroconversion, using the Amplicor HIV-1 Monitor v1.0 assay plus add-in non-B primers (Roche Diagnostic Systems). Since 1996, HIV RNA levels have been measured prospectively on fresh plasma samples, using the Roche Amplicor HIV-1 Monitor v1.5

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Detection of p24 antigen has been performed prospectively every 3–6 months, using a standard EIA (initially from Abbott Diagnostics and then from NEN Life Science Products). Although quantitative results were reported for short periods of time, most results for detection of p24 antigen are qualitative (i.e., positive or negative); thus, only qualitative results have been used for this analysis.

Statistical methods. Kaplan-Meier methods were used to estimate the cumulative proportion of patients who had developed p24 antigenemia by the start of each year after HIV seroconversion and according to the minimum CD4 cell count and maximum HIV RNA level measured before the detection of p24 antigen. For the former analysis, follow-up was considered from the time of seroconversion to the date of the first detection of p24 antigen. Follow-up was right censored on the date of death, on 31 August 2000 (the cut-off date for the analysis) for patients who were alive and under follow-up, or on the date when clinical status was last known for those patients who were lost to follow-up. For the analysis of the time to p24 antigen detection according to the minimum CD4 cell count and maximum HIV RNA level measured before p24 antigenemia was detected or over follow-up for those patients who tested negative for p24 antigen [8]. This analysis allows for the estimation of the median value of each marker at the time of p24 antigenemia, taking into account the evolution of these markers in all patients, including those who persistently tested negative for p24 antigen.

Finally, proportional hazards regression methods assessed the impact of p24 antigenemia on disease progression. Patients were followed until the development of AIDS or until death, with censoring as described above. The development of p24 antigenemia (coded as 0 before development and 1 thereafter), the CD4 cell count and HIV RNA level (both transformed on the log10 scale), and the calendar year of follow-up (included because of differences in the availability of antiretroviral treatment) were considered as time-updated covariates in this analysis, and age at seroconversion was included as a fixed covariate. All proportional hazards regression models were performed using the SAS statistical software package (SAS Institute). Use of the square-root transformation, rather than the log transformation, for CD4 cell count gave similar results, although the strength of the relationships between p24 antigenemia and the disease end points were reduced slightly.

Results

Sixty-four (57.7%) of the 111 patients developed p24 antigenemia a median of 11.6 years (95% confidence interval [CI], 10.6–12.6 years) after seroconversion, which represents a Kaplan-Meier rate of 71.3% by 18 years after seroconversion. Of the 64 patients who tested positive for p24 antigen, 17 (26.6%) did so ≤5 years after seroconversion, 21 (32.8%) did so 5–10 years after seroconversion, and 26 (40.6%) did so >10 years after seroconversion.

The patients were 1–77 years old (median, 22.6 years) at the time of seroconversion. Baseline CD4 cell counts, measured 0.0–6.5 years after seroconversion, were 40–1920 cells/mm³ (median, 600 cells/mm³). Baseline HIV RNA levels, measured 0.1–7.1 years after seroconversion, were 2.60–6.40 log10 copies/mL (median, 3.45 log10 copies/mL). The time to first detection of p24 antigen was significantly shorter for those who were older at seroconversion (P = .04, log-rank test) and for those whose initial HIV RNA level was high (P = .006) but was unrelated to the baseline CD4 cell count (P = .45).

By means of Kaplan-Meier methods, p24 antigen was first detected at a median CD4 cell count of 100 cells/mm³ (95% CI, 20–170 cells/mm³) and a median HIV RNA level of 4.9 log10 copies/mL (95% CI, 4.7–5.2 log10 copies/mL; figure 1). However, the cumulative proportion of individuals who had p24 antigenemia gradually increased as the CD4 cell count decreased and HIV RNA level increased. Thus, these results do not indicate a “threshold” level at which time the detection of p24 antigen first occurs.

Figure 1. Kaplan-Meier plot showing the cumulative percentage (with confidence intervals) of patients with p24 antigenemia, according to minimum CD4 cell count (A) and maximum human immunodeficiency virus RNA level (B), measured during follow-up. Dotted lines, 95% confidence limits.
During follow-up, 58 patients (52%) developed AIDS, and 69 patients (62%) died. In univariate analyses, the detection of p24 antigen was associated with more-rapid progression to AIDS (table 1); this effect remained significant after adjusting for CD4 cell counts over follow-up but was reduced after adjusting for HIV RNA levels, age, and calendar year of follow-up. In contrast, a significant relationship between the detection of p24 antigen and death became nonsignificant after adjusting for the CD4 cell counts and remained nonsignificant after adjusting for HIV RNA levels, age, and calendar year of follow-up.

We found some evidence that the relative hazard (RH) associated with the development of p24 antigenemia decreased over time. For example, the unadjusted RHs for the association of AIDS and the development of p24 antigenemia were 8.82 (95% CI, 2.54–30.56), 6.05 (95% CI, 3.01–12.17), 3.04 (95% CI, 0.86–10.69), and 1.75 (95% CI, 0.18–17.05) in the first 5, 6–10, 11–15, and ≥16 years after seroconversion, respectively. A similar, although less apparent, effect was seen for the association of death and p24 antigenemia. Thus, our overall estimates of the RHs may underestimate the true effect of p24 antigenemia in the early stages of HIV infection.

Discussion

The detection of p24 antigen remains associated with a more rapid progression to AIDS and death in this cohort [2]. In early studies of HIV progression, the detection of p24 antigen often was shown to be associated with more-rapid HIV disease progression [1, 5, 9], although, after adjusting for CD4 cell count and other markers of immune activation, this effect usually became nonsignificant [5, 10]. In many of these studies, however, changes in p24 antigen status were not determined, because testing was done at only 1 point in time, rather than at various time points during follow-up. Thus, the interpretation of the results from many of these early studies was limited.

A correlation between the detection of p24 antigen and CD4 cell count has been reported in some [1, 4], but not all [6, 11], studies. In one study, rates of p24 antigen detection ranged from 7% among those with CD4 cell counts >600 cells/mm$^3$ to 75% among those with CD4 cell counts <200 cells/mm$^3$ [1], with a mean CD4 cell count at first detection of p24 antigen of 406 cells/mm$^3$. In a Brazilian study [4], rates of p24 antigen detection varied between 27% among those with CD4 cell counts >500 cells/mm$^3$ to 72% among those with CD4 cell counts <200 cells/mm$^3$. One explanation for the higher mean value in the first study is that, in the calculation of the mean value, the history of CD4 cell counts for individuals who tested negative for p24 antigen throughout the study was not considered. However, this does not explain why the detection rates in these 2 studies were generally higher than those in our study, in which the cumulative rate of detection of p24 antigen at a CD4 cell count of 200 cells/mm$^3$ was only 38%. The increased use of highly active antiretroviral therapy (HAART) cannot explain these differences. First, our calculations are based on the minimum CD4 cell count measured during follow-up, which was usually before the introduction of HAART. Second, the decision to initiate HAART is made relatively conservatively in the United Kingdom, compared with other countries, and many patients therefore remain untreated until their CD4 cell counts fall to levels <350 cells/mm$^3$. However, in these other studies, rates of p24 antigen detection were generally higher at all CD4 counts.

The detection of p24 antigen often is hindered by the presence of p24 antigen–antibody complexes. Thus, a criticism of the use of p24 antigen as a prognostic marker is that it often is undetectable in patients with advanced disease [1]. The use of acid- or heat-based immune-complex dissociation before testing for p24 antigen enables the detection of p24 antigen at an earlier stage of disease, which results in a more sensitive, but less specific, marker for disease progression [12]. At our hospital, only the standard EIA method has been used, and we therefore cannot compare the prognostic values of the two methods. One additional limitation of our results is that most p24 antigen results are qualitative (i.e., positive or negative). The use of quantitative values may provide additional prognostic information, although analyses using quantitative results have been inconsistent [3, 11].

We found a stronger relationship between p24 antigenemia and the development of AIDS than between p24 antigenemia and survival, which is in contrast with recent findings that suggest the opposite [3]. In our cohort, there were a large number of deaths from non-AIDS causes—in particular, from liver failure and other hemophilia-related causes. Thus, the finding that, in this cohort, p24 antigenemia is a weaker marker of survival than of progression to AIDS was not surprising.

Although p24 antigen has been proposed as a marker for HIV disease progression in patients who, for the most part, have not been treated, its value as a marker for patients who have been treated with antiretroviral therapy has been less well documented [13, 14]. We made the assumption that p24 antigen detection is irreversible—that is, once a patient tests positive,
the patient continues to test positive. Thus, we may have overestimated the risk of disease progression in patients treated with HAART who may test negative for p24 antigen. Although we adjusted for calendar year in our analysis, to account for the differing availability of antiretroviral treatment, the comparison of the prognostic value of p24 antigenemia before versus after the introduction of HAART was difficult, because few individuals have developed p24 antigenemia since the introduction of HAART. However, because our recommendation is for the use of testing for p24 antigenemia in developing countries with limited access to antiretroviral treatment, our results, which are based largely on clinical progression before the introduction of HAART, will have relevance.

We have reported that the detection of p24 antigen is associated with a faster progression to AIDS and death. As in other studies, most of the association can be explained by increased HIV RNA levels in those with p24 antigenemia. However, an association between p24 antigenemia and progression to AIDS that cannot be explained fully by changes in either HIV RNA level or CD4 cell count remains, which suggests that detection of p24 antigen still may have an important role in assessing patient prognosis, even in countries where virus load testing is performed routinely.

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References