Human Immunodeficiency Virus Type 1 p24 Concentration Measured by Boosted ELISA of Heat-Denatured Plasma Correlates with Decline in CD4 Cells, Progression to AIDS, and Survival: Comparison with Viral RNA Measurement

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Human immunodeficiency virus type 1 (HIV-1) RNA and p24 antigen concentrations were determined in plasma samples from 169 chronically infected patients (median CD4 cell count, 140 cells/µL; range, 0–1500 cells/µL). p24 quantification involved heat-mediated immune complex dissociation and tyramide signal amplification–boosted ELISA, which has a diagnostic sensitivity similar to that of RNA quantification by a commercial polymerase chain reaction kit. In Cox’s proportional hazard models adjusted for CD4 cell count, both RNA (P < .005) and p24 (P = .043) levels were significant predictors of progression to AIDS. Measurement of p24 was superior to measurement of RNA in the model for survival (P = .032 vs. P = .19). p24 level was a significant predictor of CD4 cell decline in models adjusted for CD4 cell counts and was superior or equivalent to RNA level, depending on the group analyzed. Stratification by CD4 cell counts at baseline showed that the superiority of p24 measurement was more pronounced at lower levels of CD4 cells (<200/µL). p24 level may be of interest as a simple and inexpensive predictive marker of disease progression.

Virus load measurements have become indispensable in the management of patients infected with the human immunodeficiency virus (HIV). Most virus is produced, and resides, in lymphatic tissues, where routine assessment is difficult [1–3]. Virus markers in plasma, specifically the viral RNA concentration, have therefore become a regularly used surrogate for the total virus load and are predictive of decline in CD4 cells, progression to AIDS, and survival [4–11]. In contrast, there is an agreement that quantification of the HIV-1 load by p24 antigen tests with native serum or after acid-mediated immune complex dissociation is unsuitable for this purpose, because these tests are relatively insensitive and therefore have a limited utility in clinical practice [12]. However, because many mechanisms of viral pathogenicity involve proteins as the direct or indirect mediators of disease, there is a theoretical possibility that the concentration of viral proteins in the various body compartments might be a better predictor of disease progression than is the particle-associated viral RNA and that the observed inferiority of antigen testing might thus be due merely to a technical inadequacy of the procedures used.

In previous work, we have demonstrated that p24 detection, by use of an ELISA coupled with tyramide signal amplification after heat-mediated immune complex dissociation [13], detects viral protein as sensitively as polymerase chain reaction detects viral DNA or RNA [14, 15]. The high diagnostic quality of the amplification-boosted p24 assay was also confirmed by others who used the procedure in a study of African children who were vertically exposed to HIV-1 [16]. A retrospective study of adults confirmed the high sensitivity of the procedure and demonstrated that treatment-induced changes in the concentrations of viral protein correlate very well with those in viral RNA, as measured by the Amplicor HIV-1 monitor (version 1.0; Roche Molecular Systems, Basel, Switzerland) with a detection limit of 200–400 copies/mL [17]. A prospective study with real-time analyses over 4 years among children born to HIV-positive mothers also demonstrated a diagnostic sensitivity and specificity of p24 testing equal to those of testing for RNA and demonstrated the suitability of this marker for monitoring antiretroviral treatment [18]. These results suggest that measurement of viral protein could be used as an alternative to RNA detection for the diagnosis of HIV-1 infection and treatment monitoring.
In the present study, we addressed the question of whether the p24 concentration in plasma is as predictive of clinical and laboratory progression as is the viral RNA concentration. A single plasma sample from each patient among a cohort representing all stages of chronic HIV-1 infection was drawn in 1993–1994 [15]. The concentrations of viral protein and RNA in this baseline sample were batchwise determined and related to the ensuing changes in CD4 cell count, progression to clinical AIDS, and survival.

Patients and Methods

Patients and samples. The 169 patients (131 men and 38 women) were derived from a group of 245 patients from the Zurich center of the Swiss HIV Cohort Study, whose plasma was used for a comparison of various antigen test procedures between 5 January 1993 and 27 May 1994 [15]. The study design and recent results of the Swiss HIV Cohort Study have been published elsewhere [19–21]. In brief, enrollment of HIV-1-infected persons aged >16 years into the cohort is independent of disease stage or degree of immunosuppression, and information is collected prospectively according to standardized criteria on structured forms at registration and at follow-up visits, which are scheduled at 6-month intervals. Routine laboratory results from intermediate visits are included. For the present study, a single EDTA-anticoagulated plasma specimen from each patient was analyzed, and the sole inclusion criterion was whether sufficient material was available for testing. In March 1998, clinical and laboratory follow-up data from the 169 patients were extracted from the database of the Swiss HIV Cohort Study, which included information up to 9 February 1998. Clinical progression to AIDS was strictly defined on the basis of AIDS indicator diseases [22].

Among the 169 patients, the risk factors for contracting HIV-1 were homosexual contact (51%), injection drug use (31%), heterosexual contact (14%), or unknown (4%). Participants with a history of injection drug use who also had heterosexual contacts were classified as injection drug users. Fifty-nine (35%) of the patients were in CDC disease stage A, 49 (29%) in stage B, and 61 (36%) in stage C. The median CD4 cell count at baseline was 140 cells/μL (range, 0–1,500 cells/μL), with the 10th, 25th, 75th, and 90th percentiles at 10, 30, 312.5, and 510 cells/μL, respectively. Ninety (53.3%) of the patients were receiving no antiretroviral treatment, 69 (40.8%) were being treated with a single nucleoside analogue reverse-trascriptase inhibitor, and 10 (5.9%) were receiving a combination of 2 nucleoside analogue reverse-trascriptase inhibitors. No patient was receiving treatment with protease inhibitors (PIs) at baseline.

Quantification of viral components. Viral protein p24 concentration in heat-denatured plasma was assessed by batch analysis of blinded samples. In brief, 100 μL of plasma was diluted with 500 μL of 0.5% Triton X-100 in 1.5-mL Eppendorf tubes, subjected to 5 min of heat denaturation at 100°C on a Techne (Cambridge, UK) dry heat block, and tested in duplicate with the NEN/Du Pont HIV-1 Core Profile ELISA in combination with the ELAST ELISA Amplification System (both from NEN Life Science Products, Geneva, Switzerland), as described elsewhere [15]. The reaction product was quantified on a Dynatech MR5000 ELISA reader (Microtech Produkte, Embrach, Switzerland). Samples with protein concentrations above the assay range were retested by use of kinetic analysis with the Quanti-Kine Detection System (Tib Molbiol, Genoa, Italy). This permitted quantification in a range of ~400–6,250,000 fg/mL with a single sample dilution.

HIV-1 RNA concentrations were determined by batch analysis in plasma samples that had been frozen at −70°C no later than 4 h after the sample was drawn and had not been thawed before testing. For testing, the AmpliC orth HIV monitor, version 1.0 (Roche Molecular Systems), with a detection limit of 200–400 copies/mL, was used.

Data analysis. The StatView 5.0 program for Macintosh (SAS Institute, Cary, NC) was used for plotting figure 1 and for calculating this figure’s linear and locally weighted regression curves, by means of a locally weighted scatter plot smoother (lowess) based on a least-squares fit, with a tension of 66%.

For analysis of predictive values, data were analyzed by use of SAS software, version 6.11 (SAS Institute). Differences between groups were analyzed by χ2 tests, analysis of variance (ANOVA), or t tests.

Prediction of CD4 cell slopes. Individual CD4 cell slopes were determined by use of linear regressions of CD4 cell counts (cells × 10^9/L) for all patients with ≥2 CD4 cell measurements over time (n = 136). To control for the possible effects of antiretroviral therapies on the evolution of CD4 cell values, several subgroups of CD4 cell values were analyzed separately: (1) all available CD4 cell values with dates greater than or equal to the baseline evaluation; (2) CD4 cell values restricted to dates before a therapy including a PI was started for an individual patient; (3) CD4 cell values restricted to dates before any antiretroviral therapy was started; and (4) CD4 cell values restricted to a period after baseline with unchanged antiretroviral therapy for >6 months, antiretroviral therapy discontinued ≥2 months before baseline, or never having received antiretroviral therapy. Associations between individual CD4 cell slopes and baseline values of p24 antigen and plasma HIV RNA levels were compared by use of Spearman’s rank correlation coefficients.

Prediction of clinical progression. Kaplan-Meier analysis and Cox’s proportional hazards regression models were used to analyze the risk of progression to a first diagnosis of AIDS or death across quartiles of baseline plasma levels for p24 antigen and HIV RNA [20, 21]. We measured times until either the attainment of the respective end point or the date of the most recent follow-up visit. The analyses of progression to clinical AIDS were restricted to 108 patients in clinical stage A or B. To compare the predictive values of plasma p24 protein and plasma HIV RNA levels, these were entered in separate proportional hazard models that were adjusted for disease stage, transmission category, sex, age in increments of 10 years, and CD4 cell count. CD4 cell counts (+1 [added to avoid log, in patients with no detectable CD4 cells]) were log-transformed and entered as a continuous variable. In addition, beginning PI-containing therapy was entered as a time-dependent covariate. Results are presented as risk ratios with 95% confidence intervals. Proportional hazard assumptions were tested on the basis of Schoenfeld residuals [23].

Results

Up to February 1998, the 169 patients (131 men and 38 women) selected for this study contributed 431 person-years of...
follow-up (median, 2.7 years; range, 0.09–4.9 years). Eighty-three patients died. At baseline and during follow-up, a total of 1291 CD4 cell counts were recorded, with a median of 7 determinations (range, 1–29 determinations) per patient. Baseline viral RNA and protein concentrations were determined batchwise in a blinded fashion from 169 plasma samples (1 per patient) collected in 1993–1994. All samples had detectable viral protein, and all but 2 were positive for viral RNA. For quantitative evaluations, the copy number of the 2 RNA-negative samples (both from patients in stage A2 with 600 and 1000 fg/mL of viral protein, respectively) was set to 100/mL. Expressed as quartiles, median baseline RNA concentrations were 3293 copies/mL for quartile 1; 29,262 copies/mL for quartile 2; 83,389 copies/mL for quartile 3; and 204,558 copies/mL for quartile 4. The respective concentrations of p24 antigen were 1700 fg/mL for quartile 1; 15,000 fg/mL for quartile 2; 31,000 fg/mL for quartile 3; and 200,500 fg/mL for quartile 4. For the 108 patients free of clinical AIDS at baseline, the corresponding values for quartiles 1–4 were 2394, 23,766, 82,933, and 175,155 copies/mL, respectively, for RNA and 1550, 16,400, 41,500 and 149,000 fg/mL, respectively, for viral p24 antigen.

Viral protein and RNA concentrations at baseline were significantly correlated in the 169 samples (Spearman $R = .525; P < .0001$). Correlations were similar for patients in stages A–C (as defined by the CDC 1993 classification) [22], as well as in CD4 cell categories 1–3 (data not shown). Mean viral RNA levels, as shown by the lowess regression curve, increased little as CD4 cell counts went down to 400 but rose with an increasing angle when CD4 cell counts dropped below this mark (figure 1A). Linear regression showed a highly significant correlation with CD4 cell count (Spearman $R = -.578; P < .0001$), although the curve did not fit well with lowess regression. A log representation of the CD4 cell counts, which gives a better resolution in the range of low counts, showed that the mean RNA concentration, as assessed by lowess regression, reached a plateau at ~1.5 logs (CD4 cell count, 30 cells/μL) and did not increase any further at lower CD4 cell counts (figure 1B).

Mean p24 levels increased very gradually from early to late
stages of disease, and the lowest regression curve followed the
linear regression curve very closely (linear regression = −.183;
P = .0178). p24 concentrations in early immunodeficiency (CD4
cell count, 200–500 cells/μL), compared with the cross-sectional
distributions, tended to be relatively higher than the corre-
sponding RNA levels, whereas the reverse situation was ob-
served at low CD4 cell counts. In consequence, the slope of
the linear regression curve for p24 was less steep than that for
RNA, and the lowest regression curve was without the distinct
terminal increase seen with RNA at very low CD4 cell counts
(figure 1C). Peak average p24 levels were achieved in patients
with CD4 cell counts of 30. Remarkably, at even lower CD4
cell counts, these levels dropped again and were negatively cor-
related with the CD4 cell count (ANOVA, P = .0308; figure
1D). Antiretroviral treatment with 1 or 2 nucleoside analogue
reverse-transcriptase inhibitors did not significantly change
these cross-sectional virus load distributions (not shown).

**Table 1. Prediction of CD4 lymphocyte depletion by viral p24 or RNA concentrations at baseline.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Slopes derived from all CD4 counts</th>
<th>Slopes derived from CD4 counts prior to PI therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>136</td>
<td>136</td>
</tr>
<tr>
<td>No. of CD4 cell counts (total/patient)</td>
<td>1258</td>
<td>929</td>
</tr>
<tr>
<td>Median (range)</td>
<td>8 (2–29)</td>
<td>6 (2–22)</td>
</tr>
<tr>
<td>Mean CD4 slope (cells/year)</td>
<td>−17.1</td>
<td>−36.6</td>
</tr>
<tr>
<td>Median slope (Q1, Q3)</td>
<td>−8 (−37, 16)</td>
<td>−22 (−61, 0)</td>
</tr>
<tr>
<td>Spearman rank correlation R (P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 slope vs. baseline CD4</td>
<td>−.074 (.39)</td>
<td>−.19 (.024)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline p24</td>
<td>−.21 (0.016)</td>
<td>−.30 (.001)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline RNA</td>
<td>−.12 (.158)</td>
<td>−.19 (.023)</td>
</tr>
<tr>
<td>Spearman R (P); baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CD4 = 200; n = 71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 slope vs. baseline CD4</td>
<td>.092 (.45)</td>
<td>−.12 (.31)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline p24</td>
<td>−.35 (.003)</td>
<td>−.36 (.002)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline RNA</td>
<td>−.27 (.020)</td>
<td>−.28 (.017)</td>
</tr>
<tr>
<td>Spearman R (P); (CD4 &gt; 200; n = 65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 slope vs. baseline CD4</td>
<td>−.18 (.15)</td>
<td>−.033 (.79)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline p24</td>
<td>−.13 (.28)</td>
<td>−.32 (.010)</td>
</tr>
<tr>
<td>CD4 slope vs. baseline RNA</td>
<td>−.10 (.42)</td>
<td>−.32 (.009)</td>
</tr>
<tr>
<td>Univariate linear regression coefficient (P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 slope vs. log CD4</td>
<td>−9.52 (.29)</td>
<td>22.2 (.023)</td>
</tr>
<tr>
<td>CD4 slope vs. log p24</td>
<td>−15 (.019)</td>
<td>−19.3 (.005)</td>
</tr>
<tr>
<td>CD4 slope vs. log RNA</td>
<td>−7.8 (.24)</td>
<td>−11.7 (.10)</td>
</tr>
<tr>
<td>Multivariate linear regression (P)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 slope vs. log p24 and log CD4</td>
<td>−16.6 (.010)</td>
<td>−22.5 (.001)</td>
</tr>
<tr>
<td>CD4 slope vs. log RNA and log CD4</td>
<td>−13.4 (.14)</td>
<td>−27.4 (.004)</td>
</tr>
<tr>
<td>and log CD4</td>
<td>−14.9 (.049)</td>
<td>−26.2 (.001)</td>
</tr>
<tr>
<td>and log CD4</td>
<td>−19.7 (.057)</td>
<td>−40 (.001)</td>
</tr>
</tbody>
</table>

*Predictive values.* To assess the predictive values of viral protein and RNA concentrations, follow-up data, including at least 1 further CD4 cell count and assessment of clinical stage, were available for 136 of the patients from the Swiss HIV Co-
hort Study database, with a median observation period of 2.7
years (range, 0.09–4.9 years). Fifty-nine patients (35%) had clin-
ic AIDS at baseline, and another 35 patients (21%) developed
AIDS during follow-up. Eighty-three patients (49%) died dur-
ing follow-up. The median number of CD4 cell counts available
for calculation of slopes, including the baseline value, was 8
per patient (range, 2–29 CD4 cell counts).

*Prediction of CD4 cell slopes.* Baseline concentrations of
CD4 cells, p24, and RNA were related by various methods to
the ensuing CD4 cell slopes available from 136 patients, as
shown in table 1. For calculation of the CD4 cell slopes, either
all available CD4 cell counts after baseline or only those before
onset of treatment with a PI were included in the analyses. In
both groups and by all analyses, the baseline concentrations of
p24 were significantly correlated with the ensuing CD4 cell
slopes, except for patients with baseline CD4 cell counts >200
when analyzed by Spearman rank correlation. When the slopes
were restricted to CD4 cell counts determined during the period
before PI therapy, correlations of p24 antigen and HIV RNA
levels with the CD4 cell slope were markedly better in most
analyses, compatible with the observation that PI-containing
drug combinations are capable of reversing the depletion in
CD4 cells. The slopes before PI therapy, therefore, represent
the natural course of CD4 cell depletion better than do those
derived from all available CD4 cell counts. In theory, the period
free of any therapy would be even more suitable. However,
there were only 35 patients for this analysis, and there were no
significant correlations (not shown). Similarly, when CD4 cell
counts in a period of unchanged treatment were analyzed for
Figure 2. Kaplan-Meier analysis of quartiles of viral RNA and p24 concentrations with respect to survival and progression to clinical AIDS, stratified for baseline CD4 cell count. A–D, Fraction progressing to death among 169 patients; A and B, 83 patients with CD4 cell counts >140/µL median; C and D, 86 patients with CD4 cell counts ≤140/µL. E–H, Progression to clinical AIDS among 108 patients; E and F, 54 patients with CD4 cell counts >255/µL median; G and H, 54 patients with CD4 cell counts ≤255/µL.

75 available patients, there were no significant correlations with p24 or RNA levels (not shown).

p24 was a better predictor of CD4 cell depletion than was RNA in all Spearman analyses, except for patients with CD4 cell counts >200, for whom the predictive values of RNA and p24 antigen were about equal. p24 level prevailed over RNA level in univariate linear regression analyses. In multivariate analysis, which also adjusted for the baseline CD4 cell count, p24 level was superior to RNA level with the slopes derived from all CD4 cell counts and equal to RNA level for slopes
Table 2. Multivariate Cox proportional hazard regression of survival (169 patients, 83 events).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Risk ratio (95% CI)</th>
<th>P</th>
<th>Risk ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log CD4 at baseline</td>
<td>0.334 (0.240-0.465)</td>
<td>.001</td>
<td>0.397 (0.279-0.566)</td>
<td>.001</td>
</tr>
<tr>
<td>CDC stage C at baseline</td>
<td>2.544 (1.488-4.349)</td>
<td>.0006</td>
<td>2.675 (1.563-4.580)</td>
<td>.0003</td>
</tr>
<tr>
<td>Age, per 10 years</td>
<td>1.474 (1.187-1.830)</td>
<td>.0004</td>
<td>1.427 (1.161-1.753)</td>
<td>.0007</td>
</tr>
<tr>
<td>PI-containing therapy*</td>
<td>0.116 (0.029-0.457)</td>
<td>.032</td>
<td>0.127 (0.033-0.496)</td>
<td>.003</td>
</tr>
<tr>
<td>HIV-1 p24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.554 (0.698-3.460)</td>
<td></td>
<td></td>
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<tr>
<td>Quartile 3</td>
<td>2.013 (0.931-4.350)</td>
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<tr>
<td>Quartile 4</td>
<td>2.877 (1.351-6.130)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2</td>
<td>1.572 (0.662-3.736)</td>
<td>.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 3</td>
<td>2.231 (0.989-5.031)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 4</td>
<td>2.293 (0.976-5.385)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Model was adjusted for all variables listed. CI, confidence interval; PI, protease inhibitor; HIV-1, human immunodeficiency virus type 1.
* Entered as time-dependent variable.

from before PI treatment. In conclusion, p24 level was a significant predictor of CD4 cell decline in models adjusted for CD4 cell counts and was superior or equivalent to RNA level when all available CD4 cell values or CD4 cell values from dates before the commencement of PI therapy were used. Stratification by CD4 cell counts at baseline (≤200 vs. >200) showed that the superiority of p24 level was more pronounced at lower CD4 cell counts. The CD4 cell count at baseline was a significant covariate in some but not all groups.

Prediction of survival. Because baseline CD4 cell counts were likely to influence the progression rate, Kaplan-Meier curves were stratified for CD4 cell counts (figure 2). Among 83 patients with CD4 cell counts above the median of 140 μL, the curves were similar for p24 (figure 2A) and RNA levels (figure 2B). For p24 levels, the courses of quartile 1 compared with quartile 2 and of quartile 3 compared with quartile 4 were similar, whereas for RNA levels, quartile 1 and quartile 4 moved separately (although not differently from quartile 1 or, respectively, quartile 4 of p24), but quartile 2 ran together with quartile 3. Among the 86 patients with CD4 cell counts ≤140 μL, both markers showed little discrimination regarding progression, and all quartile curves rose steeply and in 1 group during at least the first year of follow-up. For further analysis, p24 was compared with RNA level in separate multivariate Cox proportional hazard models, which were adjusted for CD4 cell count, disease stage C, age, and starting a PI-containing regimen (table 2). Transmission category and sex did not reach statistical significance in univariate and multivariate analyses and therefore were dropped from the final model. The results of this analysis indicated that viral protein level, but not viral RNA level, was a significant independent prognostic factor of survival (P = .032 vs. P = .19).

Prediction of progression to clinical AIDS. Among the 54 patients with baseline CD4 cell counts above the median of 255 cells/μL, the Kaplan-Meier curves of p24 and RNA levels were again similar (figure 2E and 2F). Quartile 4 of RNA was better separated from the other quartiles than was quartile 4 of p24, which was not different from quartile 3. In the 54 patients with CD4 cell counts ≤255/μL, p24 level again showed the pattern of quartile 1 moving with quartile 2 and quartile 4 moving with quartile 3; on the other hand, for RNA level, there was a very clear separation of quartile 1 and quartile 4 from each other and, for both of them, from quartile 2 and quartile 3 (figure 2G and 2H). Log-rank testing (not stratified for CD4 cell count) clearly showed RNA level to be superior to p24 level (table 3). Multivariate Cox proportional hazard regression analyses indicated that clinical progression correlated highly significantly with baseline RNA levels but only weakly with p24 levels. Despite an overall significant correlation (P = .043), none of the individual p24 quartiles had risk ratios that excluded the value 1. In agreement with figure 2, there also appeared to be no increase in the risk ratios of quartiles 3 and 4 of p24.

Discussion

There is ample evidence that the concentration of viral RNA in serum or plasma is predictive of disease progression. A correlation of baseline RNA levels with clinical progression and/or survival was found during the first 6–12 months after seroconversion [9, 24, 25] among both patients who were asymptptomatically infected [26] and patients with or without symptoms who had relatively well-preserved numbers of CD4 cells [10, 27, 28]. In more advanced disease, the predictive value of RNA was less evident, but the importance of CD4 cell count increased [29–32]. Some of these studies also assessed the predictive value of p24 antigen [28, 32–34]. In all, p24 was less frequently detectable than was viral RNA. In 2 studies, p24 was never significant when included together with RNA into multivariate proportional hazards models [28, 32]. However, in studies investigating patients early after seroconversion, the detectability of p24 antigen was found to be a stronger predictor of AIDS than was RNA concentration [34], even when analyzed by multivariate Cox proportional hazard models [33].

In the present study, we compared viral RNA and p24 concentrations, as measured by a method combining heat-mediated immune complex dissociation with signal amplification–boosted ELISA, in a single plasma sample taken in 1993–1994, before the availability of highly active antiretroviral treatment, from patients at various stages of chronic HIV infection. The advantages of this antigen detection consist in its high analytical...
sensitivity and broad quantification range (0.5–6400 pg/mL) and its irreversibly releasing all antigen from immune complexes. This sets the procedure apart from acid- or base-mediated immune complex dissociation, in which the pH neutralization that has to be achieved before the actual antigen test can be done may lead to a partial recomplexation of the antigen, resulting in individually unpredictable underdetection [13].

An important advantage of the procedure compared with quantification of viral RNA is its simplicity and its considerably lower cost. In Switzerland, p24 quantification costs only 18% of the price of viral RNA quantification. Although samples from both early and advanced disease stages were investigated in the present study, there were few patients with CD4 cell counts <200 (table 1), and the study cohort was representative of the severely status of immunodeficiency, which renders the patient highly vulnerable to AIDS-defining illnesses. The fact that the virus load, as measured by both p24 and RNA levels, appears to be of no importance during the last 1–2 years before death (i.e., in the very period during which AIDS is most likely to occur), as shown by the Kaplan-Meier analyses of figure 2C and 2D, further supports the interpretation that it is not a higher production of virus particles that is responsible for rapid clinical progression in this advanced stage.

The increasing RNA concentration in advanced disease could thus reflect that not only the CD4 cells but also the lymph node structure is destroyed and therefore could indicate a particularly severe status of immunodeficiency, which renders the patient highly vulnerable to AIDS-defining illnesses. The fact that the virus load, as measured by both p24 and RNA levels, appears to be of no importance during the last 1–2 years before death (i.e., in the very period during which AIDS is most likely to occur), as shown by the Kaplan-Meier analyses of figure 2C and 2D, further supports the interpretation that it is not a higher production of virus particles that is responsible for rapid clinical progression in this advanced stage.

Taken together, the present study provides preliminary evidence that HIV p24 concentrations, as detected by this significantly improved procedure, may be a valuable predictive marker for certain aspects of disease progression. Larger studies provide compelling evidence that p24 level is a better predictor of clinical progression and survival. We are currently unable to explain conclusively why p24 level should be a better predictor of survival and RNA level a better predictor of clinical progression. It is particularly puzzling that viral RNA level, which is markedly inferior to p24 level in prediction of the CD4 cell slope in patients with CD4 cell counts <200 (table 1), should in this very group be superior to p24 level in predicting progression to AIDS, as suggested by the Kaplan-Meier analysis (figure 2G and 2H).

This discrepancy suggests that—indeed of CD4 cell depletion—there could be an additional mechanism that would account for both a high viral RNA (but not p24) load in plasma and an increased progression to AIDS. Histopathologic analysis has shown an almost complete destruction of the lymph node architecture and fine structure in advanced HIV infection, and it is assumed that this loss of functional structure contributes significantly to the immunodeficiency [35]. One particular feature of this destruction is the loss of the follicular dendritic cell network, which is known to serve as a kind of filter that retains the particles produced in the lymphatics [1, 2, 35, 36]. Recent studies have indicated a virus half-time of only a few minutes [37]. A considerable fraction of particles should thus be eliminated while they are entangled within the follicular dendritic cell network. When the network is destroyed, significantly more particles would reach the bloodstream, thus increasing the viral RNA load in plasma. In contrast, p24 antigen should pass freely through the network, owing to its small size and its higher resistance to degradation. The destruction of the follicular dendritic cell network would thus not be mirrored by a concomitant increase in plasma antigenemia. A discrepancy of p24 and viral RNA concentrations in patients with very low CD4 cell counts is indeed seen in figure 1 and is particularly manifest when only the untreated patients are compared (not shown).
with more homogenous patient populations at defined stages of CD4 cell deficiency are now needed to resolve the inconsistencies discussed above. If these results can be confirmed and perhaps even improved, this simple, inexpensive, and easily automatable procedure, which does not require cumbersome sample transport and pretreatment procedures, might contribute to improvements in the monitoring and treatment of HIV infections worldwide.

Acknowledgments

We thank Soksimon Kaing for excellent technical assistance and Matthias Egger for statistical advice.

References


