CONCISE COMMUNICATION

Heat-Denatured Human Immunodeficiency Virus Type 1 Protein 24 Antigen: Prognostic Value in Adults with Early-Stage Disease

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$CD4^+$ lymphocyte count and human immunodeficiency virus (HIV) type 1 RNA level are useful for determining when to initiate antiretroviral therapy but are not used widely in developing countries due to the high cost. Heat-denatured protein 24 (p24) antigen is an inexpensive assay that predicts disease progression among persons with advanced disease but has not been assessed among persons with early-stage disease. Plasma levels of heat-denatured p24 antigen were quantified in baseline study-visit specimens obtained from injection drug users enrolled in a longitudinal cohort study of HIV-1 infection. Of the 494 study participants (median initial $CD4^+$ lymphocyte count, 518 lymphocytes/mm^3), 90 (18%) progressed to acquired immunodeficiency syndrome within 5 years. p24 antigen level correlated with both $CD4^+$ lymphocyte count ($r = -0.34$; $P < .0001$) and HIV-1 RNA level ($r = 0.55$; $P < .0001$). p24 antigen level >5 pg/mL predicted disease progression, comparable with that of cutoff $CD4^+$ lymphocyte count <350 lymphocytes/mm^3 and HIV-1 RNA level >30,000 copies/mL. Heat-denatured p24 antigen level predicted subsequent clinical disease progression in early-stage HIV-1 infection and correlated with both $CD4^+$ lymphocyte count and HIV-1 RNA level.

It is estimated that 36 million people are infected with human immunodeficiency virus (HIV) type 1, of whom 95% live in the developing world [1]. $CD4^+$ lymphocyte count and HIV-1 RNA level are excellent predictors of clinical disease progression and response to therapy [2] and are used to determine when to initiate therapy [3]. Although antiretroviral therapy is becoming more affordable in developing countries, $CD4^+$ lymphocyte and HIV-1 RNA monitoring remain costly. A less expensive test with which to monitor disease progression in this setting would allow for appropriate use of antiretroviral therapy.

Signal amplification–boosted HIV-1 protein 24 (p24) antigen level in heat-denatured plasma is comparable with HIV-1 RNA level in predicting $CD4^+$ lymphocyte decline and survival among persons with advanced HIV disease [4] and is effective in monitoring response to therapy [5].‘

However, the ability of heat-denatured p24 antigen level to predict clinical disease progression among adults with early-stage HIV-1 infection has not been assessed. Such a capability is necessary to determine whether the test can be used to decide when to initiate therapy. Although native p24 antigen can be detected in patients with advanced HIV disease and levels correlate with response to therapy [6], p24 antigen often is not detected in patients during early-stage disease because of antigen-antibody complexes [7]. The heat-denatured p24 antigen assay dissociates these complexes, thereby increasing the ability of the assay to detect p24 antigen in patients during early-stage disease. If the predictive power of heat-denatured p24 antigen level is comparable with that of $CD4^+$ lymphocyte count and/or HIV-1 RNA level, the test, given the low cost [8], could be of particular benefit in countries with the highest burden of HIV-1 infection.

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AIDS Linked to the Intravenous Experience cohort study was approved by the institutional review board of the Johns Hopkins Bloomberg School of Public Health; informed consent was obtained from all study participants. Human experimentation guidelines of US Department of Health and Human Services and Johns Hopkins Bloomberg School of Public Health were followed.

The authors do not have any conflicts of interest related to this work.

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Table 1. Clinical and demographic characteristics of the 494 study participants.

Characteristic	Value
Black race, %	97
Age, years	33.9 (29.8–38.2; 6.5)
$CD4^+$ lymphocyte count, lymphocytes/mm^3	518 (356–719; 308)
Log_{10}, $CD4^+1$[a]	2.72 (2.55–2.86; 0.24)
bDNA, copies/mL	7586 (1438–27,080; 41,634)
Log_{10} bDNA[a]	3.88 (3.16–4.43; 0.81)
p24, pg/mL	0.605 (0.13–4.72; 38.39)
Log_{10} p24[a]	−0.218 (−0.88 to 0.67; 1.03)

NOTE. Data are median (interquartile range; SD), except where noted.
[a] Skewness, <0.5; kurtosis <1.0, consistent with no outliers.

Subjects and Methods

Study population. Participants were injection drug users enrolled in a longitudinal cohort study who were HIV-1 seropositive at their baseline visit; they were followed-up semiannually [9]. Blood samples were obtained for $CD4^+$ lymphocytes, and plasma samples were frozen. Persons were excluded if their $CD4^+$ lymphocyte count and HIV-1 RNA level were not available at the baseline visit. AIDS-defining diagnoses and deaths occurring during the 5 years after each participant's initial visit at which blood samples were obtained were included in the analysis. Ascertainment of clinical endpoints was performed, as described elsewhere [10].

Laboratory techniques. T cell subsets were measured according to modified whole-blood staining methods and by use of flow cytometric procedures. HIV-1 RNA level in plasma was quantified by using a second-generation (version 2.0), branched-chain DNA signal amplification assay (Chiron), with a quantification limit of 500 copies/mL and a linear dynamic range of 5.0×10^2–1.5×10^6 copies/mL. Heparinized plasma specimens for the quantitation of HIV-1 RNA levels were processed within 4–6 h of collection and were stored at −70°C until testing (without thawing and refreezing). Undetectable virus loads were coded as the lower limit of detection (i.e., 500 copies/mL). HIV-1 p24 antigen levels were quantified as described elsewhere [4].

Statistical analysis. Associations among $CD4^+$ lymphocyte count, HIV-1 RNA level, and p24 antigen level were compared by using Spearman's rank correlation coefficients, with confidence intervals (CIs) that were calculated by using Fisher's Z-transformation. Log transformation was used for $CD4^+$ lymphocyte count, HIV-1 RNA level, and p24 antigen level, because it improved skewness, kurtosis, and other measures of normality. Cox proportional hazards models determined univariate and multivariate baseline visit predictors of subsequent progression to AIDS. Kaplan-Meier survival analysis of time to AIDS was performed; the significance of the difference of the curves was assessed by use of the log-rank test. To facilitate comparison of hazard ratios among $CD4^+$ lymphocyte count, HIV-1 RNA level, and p24 antigen level, we determined standardized hazard ratios, which represent the change in hazard for a 1 SD change of the log-transformed variables. The hazard ratios for a 1 SD increase in log-transformed HIV-1 RNA and p24 antigen level, as well as a 1 SD decrease in log-transformed $CD4^+$ lymphocyte count, are reported.

Results

Of the 665 HIV-1–infected cohort participants, 13 had missing baseline $CD4^+$ lymphocyte counts, and 157 did not have sufficient plasma samples available to perform HIV-1 RNA and p24 antigen analyses. One participant developed AIDS after the initial study visit (before a blood sample was drawn) and thus was excluded. Thus, 494 participants were included in the study; the clinical and demographic characteristics of these participants are listed in table 1. HIV-1 RNA was undetectable in 55 participants, and heat-denatured p24 antigen was not detected in 1 patient. During the first 5 years after the baseline visit, participants reported no antiretroviral therapy use at 85% of study visits, dual nucleoside reverse-transcriptase inhibitor therapy at 15% of visits, and highly active antiretroviral therapy at 0.1% of visits. Of the 494 participants, 90 (18%) developed AIDS within 5 years of the baseline visit; only 2 (4%) of 55 with undetectable HIV-1 RNA levels developed AIDS. There were 94 deaths, of whom 48 had AIDS, during the 5-year follow-up period.

Baseline $CD4^+$ lymphocyte count, HIV-1 RNA level, and heat-denatured p24 antigen level were statistically significantly correlated with each other. Heat-denatured p24 antigen level correlated more strongly with HIV-1 RNA level ($r = 0.55$; 95% CI, 0.50–0.62; $P < .0001$) than with $CD4^+$ lymphocyte count ($r = -0.34$; 95% CI, −0.42 to −0.26; $P < .0001$). The weakest correlation was between HIV-1 RNA level and $CD4^+$ lymphocyte count ($r = -0.21$; 95% CI, −0.29 to −0.12; $P < .0001$).

Cox proportional hazards models were constructed to assess predictors of progression to AIDS at the baseline visit (tables 2 and 3). For these analyses, 28 participants were excluded, because they had no follow-up visits. In univariate models, $CD4^+$ lymphocyte count, HIV-1 RNA level, and heat-denatured p24 antigen level were all highly predictive of subsequent disease progression. To best compare the relative ability of all 3 variables to predict disease progression, the standardized hazard ratio of these variables, which reflects the change in hazard for a 1 SD change in the variable, was used. In these standardized univariate models, a 1 SD decrease in log(CD4) or increase in log(bDNA) or log(p24) each resulted in a hazard ratio of disease progression of ~2. All 3 measures were statistically equivalent and had comparable χ^2 results.

In Cox multivariate models with 2 variables, the combination of HIV-1 RNA level and $CD4^+$ lymphocyte count was the strongest predictor of disease progression (full-model χ^2, 75.48);

Table 2. Cox proportional hazards univariate models of hazard ratio (HR) and standardized HR (SHR) of progression to AIDS within 5 years of baseline visit.

Model	Variable	HR (95% CI)	SHR (95% CI)	Wald χ^2	*P*
A	Log(CD4 + 1)	0.05 (0.022–0.127)	2.04 (1.64–2.50)	43.80	<.0001
B	Log(bDNA)	2.65 (1.96–3.57)	2.18 (1.71–2.77)	40.36	<.0001
C	Log(p24)	2.05 (1.66–2.53)	2.09 (1.69–2.60)	45.47	<.0001

NOTE. CI, confidence interval.

Table 3. Cox proportional hazards multivariate models of hazard ratio (HR) and standardized HR (SHR) of progression to AIDS within 5 years of baseline visit.

Model, variable	HR (95% CI)	SHR (95% CI)	Partial Wald $\chi^{2,a}$	*P*	Full-model LHR $\chi^{2,b}$
A					
Log(p24)	1.72 (1.38–2.14)	1.75 (1.39, 2.19)	23.68	<.0001	65.55
Log(CD4 + 1)	0.12 (0.048–0.31)	1.67 (1.33–2.08)	19.93	<.0001	
B					
Log (p24)	1.65 (1.30–2.09)	1.67 (1.31–2.14)	17.21	<.0001	62.90
Log(bDNA)	2.00 (1.43–2.80)	1.74 (1.33–2.28)	16.30	<.0001	
C					
Log (CD4 + 1)	0.093 (0.040–0.22)	1.77 (1.43–2.17)	30.77	<.0001	75.48
Log(bDNA)	2.41 (1.76–3.30)	2.02 (1.57–2.60)	30.20	<.0001	
D					
Log(p24)	1.37 (1.07–1.75)	1.38 (1.07–1.78)	6.33	.01	81.90
Log (CD4 + 1)	0.134 (0.055–0.33)	1.61 (1.30–2.00)	19.45	<.0001	
Log(bDNA)	2.01 (1.42–2.85)	1.75 (1.32–2.31)	15.55	<.0001	

NOTE. CI, confidence interval; LHR, likelihood ratio. HR is per 1 log increase in CD4, bDNA, and p24; SHR is per 1 SD decrease in log(CD4 + 1) and per 1 SD increase in log(bDNA) and log(p24). When $\log_{10}$ was used for p24, a value of 0 was treated as 0.001 (i.e., log10 [0.001]). All $CD4^+$ lymphocyte, bDNA, and p24 levels were from the initial study visit.

[a] Based on maximum LHR.

[b] Based on differences in −2 log LHR for the full model and a model without covariates. Full-model χ^2 exceeds the sum of partial χ^2, because the partial χ^2 exclude predictive associations shared by multiple components.

however, the combination of heat-denatured p24 antigen level and $CD4^+$ lymphocyte count also was a strong predictor of subsequent disease progression (table 3). The standardized hazard ratios of disease progression were similar for each variable included in the models.

The ability of the 3 variables to predict clinical disease progression, according to a specific cutoff, was assessed. Cutoffs for $CD4^+$ lymphocyte count and HIV-1 RNA level in HIV treatment guidelines ($CD4^+$ lymphocyte count, <350 $CD4^+$ lymphocytes/mm^3, and HIV-1 RNA level >30,000 copies/mL, respectively, for the version 2.0 b-DNA assay) were assessed [3], because these cutoffs represented approximately the first and third quartile, respectively, of the distribution of these variables among our study population. The third quartile of heat-denatured p24 antigen level also was assessed (>5 pg/mL). In this cohort, 77% had a $CD4^+$ lymphocyte count >350 lymphocytes/mm^3, 77% had HIV-1 RNA level ⩽30,000 copies/mL, and 76% had p24 antigen level ⩽5 pg/mL. Each of these 3 cutoffs equally predicted progression to AIDS during the 5 years of follow-up, with very similar survival curves for the low-risk and high-risk groups (figure 1).

Discussion

In this large study among persons with early HIV-1 disease (median baseline $CD4^+$ lymphocyte count, 518 lymphocytes/mL^3), baseline heat-denatured p24 antigen level was a strong predictor of subsequent progression to AIDS and was statistically equivalent (as indicated by standardized hazard ratios and model χ^2) to $CD4^+$ lymphocyte count and HIV-1 RNA level. This was demonstrated in Cox univariate proportional hazards models of disease progression, as well as in Cox multivariate models that adjusted for $CD4^+$ lymphocyte count and HIV-1 RNA level. Heat-denatured p24 antigen level also correlated with $CD4^+$ lymphocyte count and HIV-1 RNA level, both of which are strong predictors of disease progression [2]. The p24 antigen threshold (>5 pg/mL) was prognostically equivalent to that of $CD4^+$ lymphocyte count (<350 lymphocytes/mm^3) and HIV-1 RNA level (>30,000 copies/mL; b-DNA assay version 2.0), which are cutoffs used in guidelines for the initiation of antiretroviral therapy [3].

There were differences in the variability of the tests for $CD4^+$ lymphocyte count, HIV-1 RNA level, and heat-denatured p24 antigen level in this cohort, as demonstrated by differences in the interquartile ranges of the log-transformed data (table 1). The hazard ratios in the nonstandardized Cox proportional hazards models were greatly influenced by these differences in variability. However, in Cox univariate and multivariate proportional hazards models, in which the relative hazard of the 3 log-transformed variables was standardized to the SD, $CD4^+$ lymphocyte count, HIV-1 RNA level, and heat-denatured p24 antigen level were all very comparable in predicting clinical disease progression; a 1 SD change in each variable resulted in a 2-fold change in hazard (tables 2 and 3).

If only 1 test were available, this study would suggest that heat-denatured p24 antigen level would be comparable with that of HIV-1 RNA level or $CD4^+$ lymphocyte count in predicting disease progression. In addition, p24 antigen level predicted disease progression independent of HIV-1 RNA level and $CD4^+$ lymphocyte count. Thus, p24 antigen level could be used either alone or in combination with one or both of the other tests. If used in combination, use with $CD4^+$ lymphocyte count appears to be most beneficial.

Currently, the total cost (including indirect costs, equipment, personnel, etc.) of conducting 1 $CD4^+$ lymphocyte count is $88, and the cost of measuring 1 HIV-1 RNA level is $152 (Johns

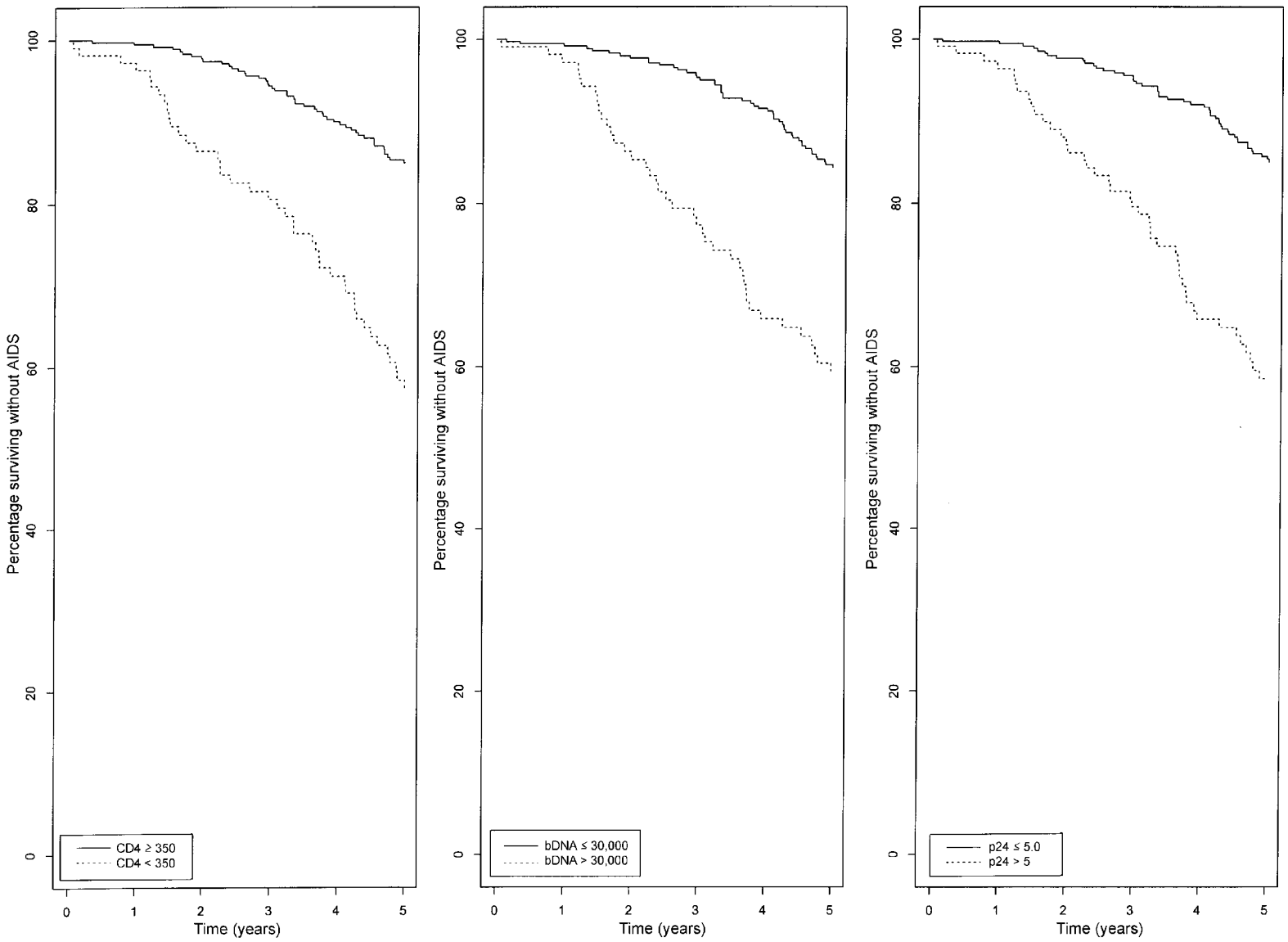

Figure 1. Kaplan-Meier curves of progression to AIDS during 5 years of follow-up, according to baseline threshold levels of $CD4^+$ lymphocyte count, human immunodeficiency virus (HIV) type 1 RNA level, and heat-denatured protein 24 (p24) antigen level. Progression to AIDS according to the following cutoffs: 350 $CD4^+$ lymphocytes/mm^3 (log-rank χ^2, 39.7; $P < .0001$), 30,000 HIV-1 RNA (b-DNA) copies/mL (log-rank χ^2: 35.8; $P < .0001$), and 5 pg/mL of p24 antigen (log-rank χ^2, 39.8; $P < .0001$).

Hopkins Hospital list prices). In contrast, the total cost for the p24 antigen test is ~$20 when used qualitatively [8] and $30 when used quantitatively. Because of the frequency with which monitoring must be performed to assess clinical disease progression and response to therapy [3], the heat-denatured p24 antigen test would be much more affordable than currently available tests. In addition, because of the stability of p24 antigen, sample transport and storage is less demanding than that required for HIV-1 RNA; the test is also easy to perform and can be readily automated.

There are at least 3 limitations of this study. First, although $CD4^+$ lymphocyte testing was conducted shortly after the participant's study visit, testing of HIV-1 RNA and p24 antigen levels was performed on plasma specimens that had been frozen and stored for several years. Previous studies have noted decreased levels of HIV-1 RNA [11] and p24 antigen [12] in stored samples. If deterioration occurred in the specimens used in this study and was not equivalent for both HIV-1 RNA and p24 antigen, this could affect the ability of each to predict disease progression, as well as the comparison of this ability between HIV-1 RNA and p24 antigen levels. Of note, a significant decrease in heat-denatured p24 antigen level over time has not been observed (J.S., unpublished data). Second, the participants in this cohort were predominantly US black persons, which could limit generalization. There are conflicting data on whether HIV-1 RNA levels differ by race; it is unknown whether p24 antigen levels differ by race. Third, most of the study participants probably were infected with HIV-1 subtype B. Although a previous study demonstrated that heat-denatured p24 antigen was more sensitive than bDNA among persons with non-B subtypes [13], the predictive value of heat-denatured p24 antigen among persons with non-B subtype HIV-1 infection needs to be assessed.

Other p24 antigen assays have been limited by decreased sensitivity in African versus North American patient populations, probably because of immune complexing of p24 antigen in African patients [14]. Of note, however, the heat-denatured p24 antigen assay is 99% sensitive and 100% specific among HIV-infected infants in Africa [15]. Nonetheless, additional studies of this assay in African adults are needed.

This study is important for several reasons. First, it establishes heat-denatured p24 antigen as an excellent predictor of clinical disease progression among persons with early-stage HIV-1 infection, which correlates closely with $CD4^+$ lymphocyte count and HIV-1 RNA level. Thus, it will be very useful for decisions regarding the initiation of antiretroviral therapy. Second, although heat-denatured p24 antigen level can be used in combination with HIV-1 RNA level and/or $CD4^+$ lymphocyte count for predicting clinical disease progression, it also could be used alone. This, together with its low cost, make it a very attractive test, particularly in resource-poor settings.

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