Variability and Prognostic Values of Virologic and CD4 Cell Measures in Human Immunodeficiency Virus Type 1–Infected Patients with 200–500 CD4 Cells/mm^3 (ACTG 175)

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Virologic measurements are increasingly used to evaluate prognosis and treatment responses in human immunodeficiency virus (HIV) type 1 infection. Markers of HIV-1 replication, including infectious HIV-1 titer from peripheral blood mononuclear cells, serum HIV-1 p24 antigen, plasma HIV-1 RNA, CD4 cell numbers, and viral syncytium-inducing (SI) phenotype, were determined in 391 virology substudy participants in AIDS Clinical Trials Group study 175. The subjects had 200–500 CD4 cells/mm^3. All markers of viral replication significantly correlated with one another and were inversely related to CD4 cell number. Disease progression to an AIDS-defining event or death or loss of >50% of CD4 cells was associated with infectious HIV-1 titer ($P < .001$), HIV-1 RNA ($P < .001$), and HIV-1 p24 antigen ($P = .007$). In multivariate proportional hazards models, p24 antigen was never significant when HIV-1 RNA level was included. In a model containing infectious HIV-1 titer ($P = .038$), HIV-1 RNA ($P < .001$), SI phenotype ($P < .001$), and CD4 cell number ($P = .18$), only the virologic parameters remained significantly associated with progression.

Quantitative measurements of human immunodeficiency virus (HIV) in the blood, including infectious HIV-1 titers in peripheral blood mononuclear cells (PBMC), HIV-1 p24 antigen, and HIV-1 RNA levels in plasma each increase with stage of disease in HIV-1 infection [1–4]. The sensitivity and variation in these assays may differ in early stages of HIV-1 infection [5–7], and the relationships among these measures of viral replication, viral phenotype, and CD4 cell numbers have not been defined. The plasma assay for infectious virus is reliable only among patients with advanced disease, although infectious HIV-1 titers in PBMC, which measures the number of infected circulating cells, are detectable through all stages of disease if drug-naive [2, 3, 5, 8]. However, culture-based assays are lengthy, labor-intensive, and costly. ELISA tests for serum HIV-1 p24 antigen are rapid, simple, and inexpensive, and the addition of immune complex dissociation (ICD) increases the sensitivity with little added cost and effort. Quantitation of plasma HIV-1 RNA by polymerase chain reaction (PCR) is sensitive and rapid [6], and levels of plasma HIV-1 RNA are thought to reflect ongoing viral replication in lymphoid tissue [9].

In addition to quantitative viral measurements, viral phenotype has been monitored in HIV-1–positive individuals. Syncytium-inducing (SI) phenotype is determined by the ability of an HIV-1 isolate to form syncytia in MT-2 cells following infection [10]. A switch from non-SI (NSI) to SI phenotype has been associated with accelerated CD4 cell depletion, reduced CD4 cell responses, progression to AIDS or death, and resistance to zidovudine [11–14].

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The virology substudy of AIDS Clinical Trials Group (ACTG) 175 measured infectious HIV-1 titers in PBMC, ICD p24 antigen, plasma HIV-1 RNA levels, CD4 cells, and viral phenotype in 391 HIV-1–infected subjects with 200–500 CD4 cells/μL [15]. In a recent analysis, HIV-1 plasma RNA levels, CD4 cell counts, and SI phenotype were each associated with clinical outcomes after the initiation of antiretroviral drugs [16]. Here we determined the relationship of these parameters, including infectious HIV-1 titers and ICD p24 antigen, two additional measures of virus load. The current analysis examines the variation and predictive power of virologic and CD4 cell measurements using the two baseline values and estimates the variation in each of the quantitative measurements. We evaluated the association between measurements at study entry, SI viral phenotype, and disease progression. These analyses define the variation in repeated virologic measurements and the virologic and immunologic markers that predict clinical outcome.

Methods

Trial design. ACTG 175 was a multicenter trial in which 2467 subjects were enrolled to receive one of four blinded regimens of antiretroviral therapy [15]. Inclusion in the trial was based on a single CD4 cell count of 200–500 cells obtained within 30 days of randomization, absence of an active opportunistic infection or previous AIDS-defining diagnosis except minimal cutaneous Kaposi's sarcoma, and blood chemistry and hematology values at or below grade 1 toxicity as defined in the protocol. The virology subset of 391 subjects was accrued at 14 sites where proximity of the clinic and laboratory ensured that blood samples could be processed within 6 h of phlebotomy.

Samples for virologic analysis were collected from patients at two time points within 3–14 days before starting study treatment. Blood was collected in acid citrate dextrose tubes to obtain plasma and PBMC by ficoll-hypaque centrifugation. At the same time points, CD4 cells were enumerated by flow cytometry. Sera and plasma were frozen within 6 h of collection at −70°C, and PBMC were placed in quantitative dilution cell cultures [8, 17].

Quantitative microcultures (QMC). The assay was done as described previously [8, 17]. In brief, 10^6 PBMC and serial 5-fold dilutions (2×10^5, 4×10^4, 8×10^3, 1.6×10^3, and 3×10^2) were added in duplicate to 24-well plates containing 10^6 phytohemagglutinin (PHA)-stimulated PBMC from HIV-1–seronegative donors. Cultures were done within 1 day of blood collection (fresh) in 613 assays; cryopreserved PBMC (from 4 to 52 weeks) were used in 104 assays. In 654 assays, duplicate wells were evaluated, while in 63, single wells were cultured at each dilution of patient PBMC. The supernatant fluid from all wells was tested for the presence of HIV-1 p24 antigen by ELISA after 14 days of culture, and the infectious HIV-1 titer per 10^6 PBMC (IUPM) was calculated for each assay on the basis of expression of HIV-1 p24 antigen at each dilution [18].

ICD p24 antigen. HIV-1 p24 antigen was tested in serum using an ICD ELISA (DuPont, Boston). In brief, 90 μL of sera was incubated with glycine (pH 4) for 1 h at 37°C, then treated for 15 min with Tris base at room temperature. The treated sera were then transferred to an HIV antibody–coated plate, and a routine HIV-1 p24 antigen ELISA was done. Samples that exhibited $\geq$10 pg/mL of HIV-1 p24 antigen were confirmed as positive with neutralizing antibody.

Detection of SI virus. SI viruses were assayed by cocultivation of supernatant fluids from cell dilution cultures with MT-2 cells [10, 17]. Supernatant fluids from cell cultures containing >30 pg/mL HIV-1 p24 antigen were added to separate cultures of 5×10^5 MT-2 cells in log growth phase or 10^6 PHA-stimulated donor lymphocytes. MT-2 cultures were split twice weekly and observed for the presence of syncytia for 14 days. PBMC cultures were assayed for HIV-1 p24 antigen production to confirm growth of virus from the inoculum. Viral supernatants that produced HIV-1 p24 antigen in PBMC cultures without evidence of cytopathology in MT-2 cells were considered to be NSI isolates. Virus isolates that resulted in cytopathology in MT-2 cells within 14 days were considered to be SI isolates [10, 17].

HIV-1 RNA PCR. Plasma samples corresponding to the blood samples assayed by QMC were obtained from 348 subjects in the virology subset. The plasma samples were kept frozen at −70°C and assayed (Amplicor; Roche Molecular Systems, Branchburg, NJ) to quantitate plasma HIV-1 RNA. Each subject's paired plasma samples were assayed on the same plate with four controls (containing 0, 1500, 15,000, and 150,000 copies of HIV-1) included in each assay. Copy numbers of each plasma sample were calculated on the basis of the optical density readout of the final ELISA hybridization detection of amplicon at 6 dilutions of input RNA and then adjusted for the efficiency of extraction, reverse transcription, and amplification of an internal target control sequence. The assays were done in three laboratories (Stanford University, Palo Alto; Beth Israel Deaconess Medical Center, Boston; and Case Western Reserve, Cleveland) certified in the performance of the assay by Roche Molecular Diagnostics and by the ACTG Virology Quality Assurance Program [6, 7].

Statistical analyses. Infectious HIV-1 titer, plasma HIV-1 RNA level, and HIV-1 p24 antigen level were all analyzed after a $\log_{10}$ transformation. Plasma HIV-1 levels below the limit of quantitation (200 copies/mL) were assigned the value of 200 copies/mL. When the QMC produced all negative or positive results, the infectious titer was outside the measurable range. In these cases, a titer was imputed by assuming that the assay, extended by 1 dilution, was positive or negative [8]. Spearman correlations of preentry and entry determinations of the same parameter and of entry determinations of different parameters were calculated. We used logistic regression analysis to investigate the association between percentage of subjects having the SI viral phenotype and other virologic parameters and CD4 cell count. Kaplan-Meier estimates and Cox proportional hazards models were used to investigate the associations between time to disease progression and virologic parameters and CD4 cell count.

Results

Virologic characteristics. Numbers of subjects, assays, and median baseline values for three virologic measures and CD4 cell numbers are shown in table 1. Median baseline infectious HIV-1 titer in PBMC and HIV-1 p24 antigen concentration

Table 1. Characteristics of baseline virology and CD4 cell measurements.

	PBMC titer IUPM	p24 antigen pg/mL	RNA copies ($\times 10^3$)/mL	CD4 cells/ mm^3
Determinations				
2	340 (87%)	351 (90%)	298 (76%)	390 (100%)
1	37 (9%)	38 (10%)	68 (17%)	1 (0%)
0	14 (4%)	2 (1%)	25 (6%)	0 (0%)
Median	24.6	9.1	19.3	335
Quartiles*	(4.55, 91.3)	(5.5, 27.4)	(05.1, 55.1)	(256–415)
Log_{10} mean (SD)	1.32 (1.06)	1.09 (0.66)	4.20 (0.74)	2.51 (0.14)

NOTE. IUPM, infectious units/10^6 cells. PBMC, peripheral blood mononuclear cells.
* 25th and 75th percentiles, respectively.

were 24.6 IUPM and 9.1 pg/mL, respectively. Two hundred six subjects (53%) had at least one baseline HIV-1 p24 antigen determination ⩾10.0 pg/mL, and in this group the median HIV-1 p24 antigen level was 24.0 pg/mL. Median plasma HIV-1 RNA was 19.3×10^3 copies/mL and median CD4 cell count was 335 cells/mL.

Variability of virologic parameters. To determine the variation between two samples obtained within 3–14 days in each subject, the two baseline samples for each of the virologic parameters and CD4 cell counts were compared (table 2). The correlation between the paired baseline samples for infectious HIV-1 titer in PBMC (0.58) was weaker than that for CD4 cell counts (0.70) and much weaker than that for plasma HIV-1 RNA levels (0.91) and HIV-1 ICD p24 antigen levels (0.87).

The difference between the two baseline infectious HIV-1 titer values is shown in figure 1A. A number of subjects had one or both of the preentry and entry values outside the measurable range, giving figure 1 its diamond shape. These values were imputed (see Methods). Therefore, true differences between samples was at least as large as the magnitude shown. Given that caveat, 90% of the subjects had a difference between the two baseline values with the range ± 1.87 log_{10} IUPM (i.e., 74-fold). The difference between successive determinations of log_{10} HIV-1 p24 antigen determinations demonstrates the dependence of variability of change on the subject's average HIV-1 p24 antigen level (figure 1B). There is decreasing variability with increasing levels of HIV-1 p24 antigen and conversely, higher variability at ⩽10 pg/mL. If subjects with either or both p24 levels ⩽10 pg/mL are omitted, then 90% of the subjects had differences between the two baseline measurements within the range ± 0.50 log_{10} pg/mL (3.2-fold). In contrast, HIV-1 RNA and CD4 cell count determinations were more stable across varying levels: 90% of subjects had differences in

Table 2. Spearman correlations between virologic parameters and CD4 cell count at baseline and correlations between determinations of same parameter on different days.

	CD4 cell count	HIV-1 titer in PBMC	RNA copy no.	p24 antigen
CD4 cell count	0.70 ($n = 390$) (± 0.19)	−0.20 ($n = 377$)	−0.21 ($n = 366$)	−0.19 ($n = 389$)
HIV-1 titer in PBMC		0.58 ($n = 340$) (± 1.87)	0.52 ($n = 357$)	0.38 ($n = 375$)
RNA copy number			0.91 ($n = 298$) (± 0.41)	0.38 ($n = 364$)
p24 antigen				0.87 ($n = 351$) (± 0.34)

NOTE. Correlations along diagonal are for measurements on same parameter taken on specimens ⩾72 h apart. Correlations off diagonal are between different parameters for specimens obtained closest to (but before) start of study treatment. Bottom nos. on diagonal represent 5th and 95th percentiles for difference between preentry and entry. All correlations shown were significantly different from 0 ($P < .001$). PBMC, peripheral blood mononuclear cells.

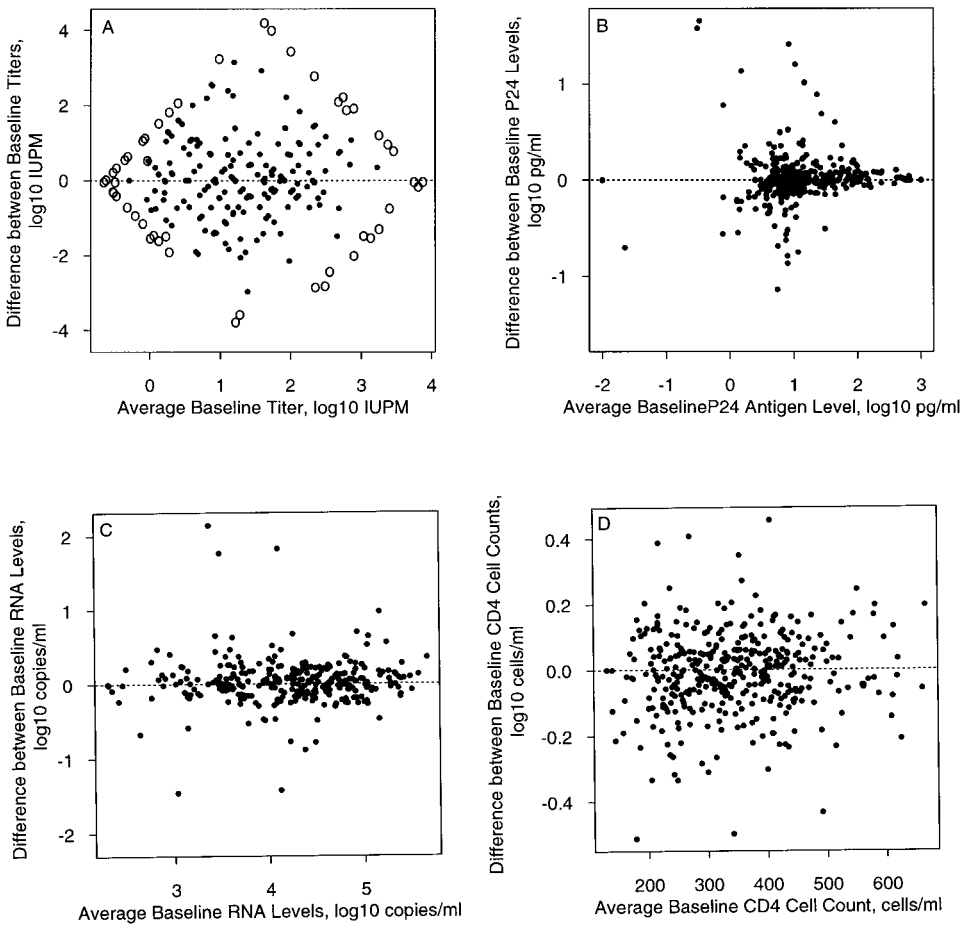

Figure 1. Differences between entry and preentry determinations vs. mean of 2 determinations (baseline level). Infectious HIV-1 titer (**A**) (O = imputed value), immune complex–dissociated p24 antigen (**B**), plasma HIV-1 RNA (**C**), and CD4 cell counts (**D**). IUPM, infectious units/10^6 cells.

plasma HIV-1 RNA within the range ±0.41 $\log_{10}$ copies/mL (2.6-fold) and differences in CD4 cell counts within the range ±0.19 $\log_{10}$ cells/mm^3 (1.5-fold) (figure 1).

Interrelationship among virologic parameters and association with CD4 cell count. The correlations between virologic parameters and with CD4 cell count are shown in table 2. The correlations between each virologic parameter and CD4 cell count were very similar (from −0.19 to −0.21), reflecting significant inverse correlation despite the large variability in levels of each virologic parameter for any specified CD4 cell count. For example, for any CD4 cell count between 200 and 500 cells/mm^3, there were subjects with HIV-1 titers in PBMC from below the detectable limit, 0.4 IUPM, to above the detectable limit of the assay, 2503.2 IUPM (data not shown).

Significant correlations were also observed between the virologic parameters. The correlations between HIV-1 p24 antigen level and plasma HIV-1 RNA level and infectious HIV-1 titer in PBMC (table 2) were low, 0.38, but significant ($P < .001$). The correlation of infectious HIV-1 titer in PBMC and plasma HIV-1 RNA level was greater (0.52; $P < .001$) (table 2). Because the correlation between the two baseline IUPM values was only 0.58, the correlation between any variable and IUPM is not expected to be higher than that value. Thus, the correlation between plasma HIV-1 RNA and infectious HIV-1 titer in PBMC was limited only by the variability in repeated measures of IUPM.

There were significantly higher percentages of subjects with the SI viral phenotype among subjects with higher infectious HIV-1 titers in PBMC, higher plasma HIV-1 RNA levels, and lower CD4 cell counts (table 3). However, there was no association between the percentage of subjects having the SI viral phenotype and HIV-1 p24 antigen level. In a multivariate logistic regression model, plasma HIV-1 RNA levels and CD4 cell counts were significantly associated with SI phenotype ($P = .006$ and $P = .003$, respectively). However, when infectious HIV-1 titer in PBMC was included with plasma HIV-1 RNA levels and CD4 cell counts, the CD4 cell count and infectious HIV-1 titer were significantly associated with the SI phenotype ($P = .006$ and $P = .018$, respectively), but the plasma HIV-1 RNA level was not ($P = .24$).

Associations between baseline measurements and disease progression. Kaplan-Meier curves for the time to progression to a study end point (≥50% CD4 cell decline, AIDS, or death) are shown in figure 2. The Kaplan-Meier curves for time to AIDS or death and to death had similar patterns (data not shown). For each quantitative measure, subjects were catego-

Table 3. Percentage of subjects with syncytium-inducing (SI) virus by baseline levels of virologic parameters.

Range of parameter	% with SI virus (no./total)	*P**
HIV-1 titer in PBMC (log_{10} IUPM)		
<0	8 (2/24)	<.001
0–0.99	11 (9/81)	
1–1.99	16 (23/140)	
2–2.99	27 (17/64)	
⩾3	38 (8/21)	
Plasma HIV-1 RNA (log_{10} copies/mL)		
<3	11 (2/18)	<.001
3–3.99	14 (11/81)	
4–4.99	16 (27/174)	
⩾5	46 (19/41)	
p24 antigen (log_{10} pg/mL)		
<0.50	19 (6/31)	.90
0.50–0.99	18 (27/148)	
1.00–1.49	17 (13/76)	
1.50–1.99	17 (8/46)	
⩾2	16 (5/31)	
Baseline CD4 count (cells/mm^3)		
<200	33 (7/21)	<.001
200–299	26 (27/102)	
300–399	14 (15/109)	
400–499	12 (9/73)	
⩾500	4 (1/27)	

NOTE. IUPM, infectious units/10^6 cells.
* Association with virologic parameter as continuous variable.

rized into quartiles for each measure. This enabled the gradient of risk across the population to be compared.

There were highly significant associations between both baseline plasma HIV-1 RNA copy number ($P < .001$) and baseline infectious HIV-1 titer in PBMC ($P < .001$) and progression to a study end point (⩾50% CD4 cell decline, AIDS, or death), to AIDS or death, and to death. The gradient of risk was steeper for the plasma HIV-1 RNA copy number than for the infectious HIV-1 titer in PBMC. However, the associations for each of these two measures was stronger than the association seen for HIV-1 p24 antigen, although the latter was significant ($P = .004$ for study end point, $P = .007$ for AIDS or death, and $P = .05$ for death). For all measures, subjects had disease progression in all quartiles, suggesting there may not be a threshold below which the risk of disease progression in the ensuing 2–3 years is zero. There was also a highly significant increase in risk of progression ($P < .001$) with SI phenotype compared with NSI phenotype.

To investigate the relative importance of the viral measures with CD4 cell counts for predicting disease progression, multivariate proportional hazards models were fitted. In undertaking the modeling, all possible models involving subsets of two, three, four, and five variables were fitted using data from the 310 subjects with data available on all five measures. The HIV-1 p24 antigen level was never significant when included in any model with plasma HIV-1 RNA level, and it was not further investigated (data not shown).

Table 4 shows the multivariate model containing HIV-1 RNA, HIV-1 infectious titer, viral phenotype, and CD4 cell counts. Lower HIV-1 RNA levels were most strongly associated with decreased risk of disease progression with hazard ratios (HR) of 0.31 per 1 log_{10} copies/mL lower RNA level for a study end point, 0.19 for AIDS or death, and 0.21 for death. Presence of the NSI phenotype was also significantly associated with decreased risk of progressing to a study end point, AIDS or death, but not death; however, HR for all three types of end points were similar among categories (HR = 0.34, 0.36, and 0.42, respectively). Decreased HIV-1 titer in PBMC was less predictive of progression than was HIV-1 RNA and phenotype. Infectious HIV-1 titer remained a significant predictor of study end point only. However, again there was little change in hazard among the three categories of end points (HR = 0.69, 0.82, 0.74/1 log_{10} of IUPM reduction). It is likely that the loss of statistical significance for SI phenotype and infectious HIV-1 titer reflects lower numbers of events for AIDS or death and death and not a change in risk of progression. In this model, after adjusting for all three viral parameters, CD4 cell count was not significant.

Discussion

Understanding variation among virologic measurements and CD4 cells and their contribution to prognosis is particularly important as these surrogate end points assume a greater role in the evaluation of antiretroviral drug activity. Here we describe intra- and interassay variation for infectious HIV-1 titer in PBMC, HIV-1 ICD p24 antigen, HIV-1 plasma RNA, and CD4 cell counts and the relationship of each of these, along with viral phenotype, to predict disease progression in a largely asymptomatic HIV-1–infected population.

Of the virologic assays, infectious HIV-1 titer in PBMC had the greatest variability using the 5th and 95th percentiles as determinants (±1.87 log_{10} IUPM or 74-fold). This was not totally unexpected since, as a biologic assay, the titer of infectivity is dependent on viral replication in PBMC from different donor sources in real time on different days. Interassay variation in addition to biologic variation within the subject may contribute to the variability of infectious HIV-1 titer in PBMC. An analysis of data from the ACTG Virology Quality Assurance Program based on interassay variation using identical samples between laboratories demonstrated that any difference <30-fold would not be clinically significant [19]. Similar variation in repeated infectious titer measurements has been observed in the baseline analysis of infectious HIV-1 titer in other ACTG trials (ACTG 241 [20] and ACTG 143 [8]). In contrast, HIV-1 ICD p24 antigen and HIV-1 plasma RNA are serologic and biochemical tests, respectively, and were done as batch

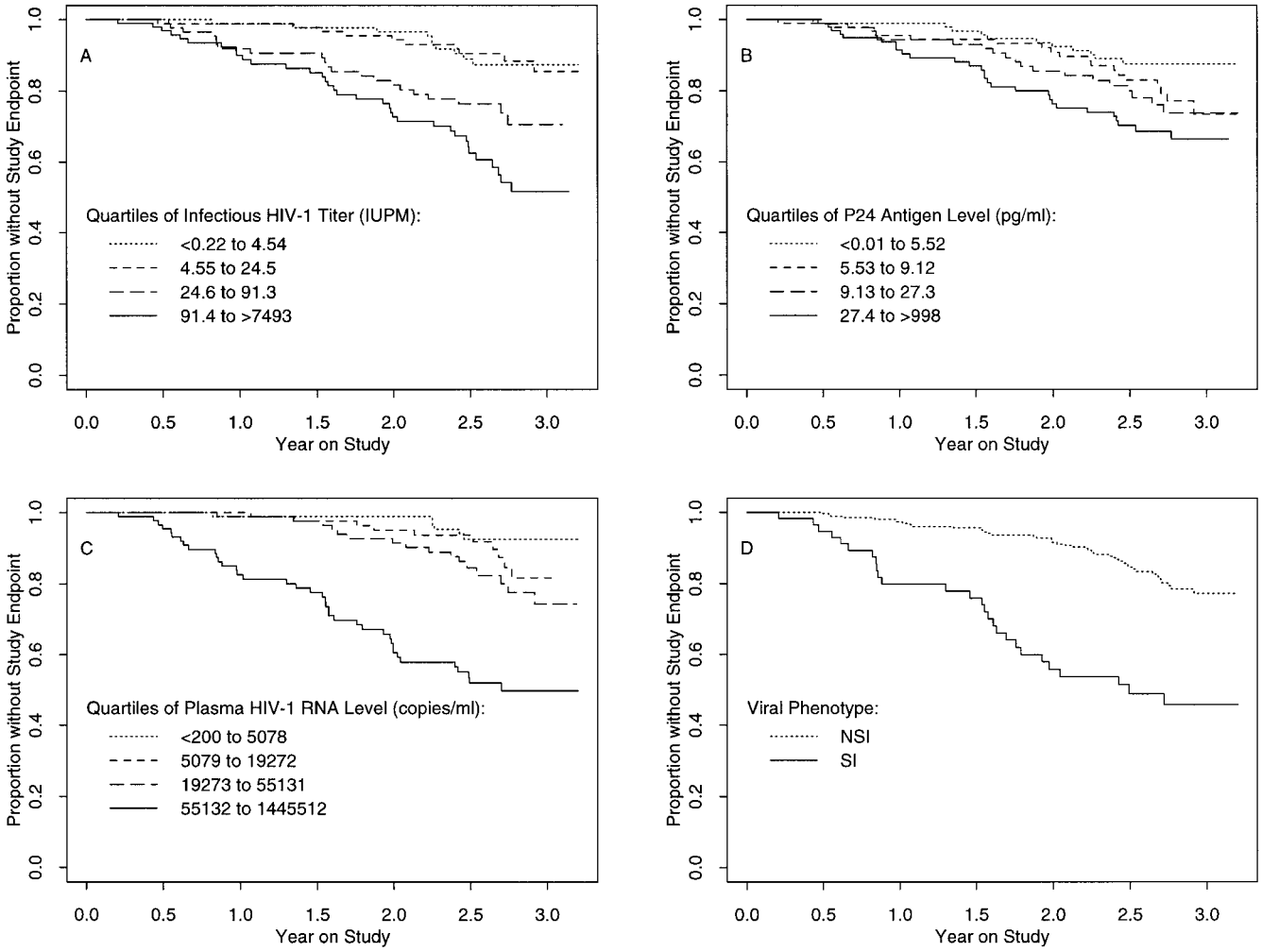

Figure 2. Kaplan-Meier curves show proportion of subjects by quartiles that did not reach study end point, defined as 50% drop in CD4 cell count, AIDS, or death. Infectious HIV-1 titer (**A**), immune complex–dissociated p24 antigen (**B**), plasma HIV-1 RNA (**C**), and viral phenotype (**D**). SI, syncytium-inducing; NSI, non-SI.

assays. Thus, there was no interassay variation; differences primarily represented measurement error and biologic variation between samples. The intrabaseline variability for HIV-1 RNA was consistent over the range of values detected. However, the variability in HIV-1 ICD p24 values was dependent upon HIV-1 p24 antigen concentration, with increased variability for values $\leqslant$10 pg/mL. Therefore, the reliability of HIV-1 ICD p24 antigen measurements $\leqslant$10 pg/mL is greatly reduced and should not be considered in clinical evaluations.

Although virologic measurements were all significantly correlated with CD4 cell count, all of the virologic measurements were more highly correlated with one another than with CD4 cell number. A similar correlation between HIV-1 RNA and CD4 cell counts has been observed in other recent studies (ACTG 241 [20], the Multicenter AIDS Cohort Study (MACS) [21]), the North American trials of lamivudine [22], and ACTG 143 [8]. Differences were also observed in the correlations among the virology measurements. Surprisingly, the correlation between infectious HIV-1 titer and HIV-1 RNA level (0.52) was nearly the same as the correlation between the two baseline infectious HIV-1 titer values (0.58). This suggests the association between infectious HIV-1 titer and HIV-1 RNA levels may be affected by the assay variability of titer measurements and is actually greater than suggested by the correlation coefficient. In support of this, a 1 $\log_{10}$ difference between HIV-1 RNA levels corresponded to a mean 0.96 $\log_{10}$ IUPM difference in infectious HIV-1 titer, implying a close relationship (data not shown).

In addition to quantitative virologic measurements, detection of SI virus appears to have a role in defining risk for clinical progression. The presence of SI virus was associated with increasing levels of infectious HIV-1 titer and plasma HIV-1 RNA and with declining CD4 cell count. These relationships are similar to observations in ACTG 241 [20] but differ from

Table 4. Hazard ratios (95% confidence intervals) from multivariate proportional hazards models for predicting risk of progression to study end point, to AIDS/death, and to death.

	Hazard ratio		
	Study end point	AIDS/death	Death
No. of events	65	41	24
HIV-1 RNA	0.31 ($P < .001$)	0.19 ($P < .001$)	0.21 ($P = .001$)
Per 1 $\log_{10}$ copies/mL decrease	(0.18, 0.54)	(0.09, 0.41)	(0.08, 0.54)
CD4 cell count	0.82 ($P = .18$)	0.80 ($P = .25$)	0.89 ($P = .65$)
Per 100 cells/mm^3 increase	(0.60, 1.10)	(0.54, 1.18)	(0.53, 1.48)
SI/NSI phenotype	0.34 ($P < .001$)	0.36 ($P = .004$)	0.42 ($P = .073$)
NSI vs. SI	(0.20, 0.59)	(0.18, 0.73)	(0.16, 1.08)
HIV-1 titer	0.69 ($P = .038$)	0.82 ($P = .40$)	0.74 ($P = .33$)
Per 1 $\log_{10}$ IUPM decrease	(0.49, 0.98)	(0.52, 1.30)	(0.40, 1.36)

NOTE. Proportional hazards models were stratified by prior antiretroviral experience and study treatment. HIV-1 RNA level, CD4 cell count, and HIV-1 titer were included as continuous variables. Total subjects in analysis, $n = 312$. SI, syncytium-inducing; NSI, non-SI; IUPM, infectious units/10^6 cells.

the results reported for ACTG 116B/117 [23] where viral phenotype was associated only with CD4 cell numbers. In a multiple regression model containing infectious HIV-1 titer, HIV-1 RNA, and CD4 cell count, increasing infectious HIV-1 titer and decreasing CD4 cells remained independent significant predictors of the SI phenotype. Although increasing levels of HIV-1 RNA were predictive of SI phenotype in a univariate model, the association of increasing HIV-1 plasma RNA with SI virus was not an independent predictor and could be explained by the other two variables, infectious HIV-1 titer and CD4 cell count. The loss of association of HIV-1 RNA levels with viral phenotype in a multivariate model was also observed in subjects with lower CD4 cell numbers in ACTG 241 [20]. This suggests that the SI phenotype is more directly related to the number of circulating CD4 cells and HIV-1–infected PBMC than to total virus level in the plasma as represented by plasma HIV-1 RNA levels.

Each virologic measurement was a univariate predictor of clinical progression, defined as reaching a study end point (50% CD4 cell decline, AIDS or death, or death). HIV-1 ICD p24 antigen, however, was not an independent predictor when multivariate hazard models included levels of plasma HIV-1 RNA. Plasma HIV-1 RNA, phenotype, and infectious HIV-1 titer, but not CD4 cell count, were independently associated with risk of disease progression. This suggests that among subjects with 200–500 CD4 cells, CD4 cell counts are less important than virologic measures in predicting clinical progression.

Of the quantitative virologic markers, plasma levels of HIV-1 RNA demonstrated the best prognostic ability. Even with this marker, there was not an observable threshold below which no disease progression was observed during follow-up, except in subjects with no measurable HIV-1 plasma RNA (i.e., with <200 copies/mL, 0/6 progressed; data not shown). Among 20 subjects with baseline plasma HIV-1 RNA of 201–1000 copies/mL, 4 progressed as did 7 of 65 subjects with baseline plasma HIV-1 RNA levels of 1001–5078 copies/mL (data not shown). Thus, although there were progressors in the lowest quartile, the risk of progression was very small in persons with plasma HIV-1 RNA levels <5078 copies/mL.

A number of recent studies have examined the relative importance of CD4 cell numbers and HIV-1 RNA levels in predicting clinical progression and survival. Mellors et al. [21] and Galetto-Lacour et al. [24] reported reduced predictive ability for clinical progression of CD4 measurements when evaluated with plasma levels of HIV-1. Conversely, other studies that included subjects with lower CD4 cell numbers and more advanced disease (Welles et al. [25], Coombs et al. [23], Hughes et al. [20], and Phillips et al. [22]) demonstrated a predictive value for CD4 cell counts even after adjustment for HIV-1 RNA levels. Thus, the prognostic value of CD4 cell measurements may vary depending upon the population studied and the length of the study, increasing in importance as CD4 cell numbers decline.

Here we assessed infectious HIV-1 titer in PBMC as an independent predictor of clinical and immunologic progression. Levels of infectious HIV-1 titer were less predictive of disease progression than were HIV-1 RNA levels and NSI as compared with SI phenotype. A different pattern was observed in ACTG 241 [20], which included patients with lower CD4 cell counts (1–443) at entry. In that study, neither infectious titer in PBMC nor viral phenotype was predictive of AIDS-defining illness or death when HIV-1 RNA or CD4 cell counts were included in the analysis. It would appear that all three viral parameters predict risk for clinical progression and that any one cannot totally represent the effect of the other two or alone explain clinical disease progression. The contribution of CD4, however, depends on the study population and possibly the level of the CD4 cell count itself.

In this study, we examined virologic and CD4 cell measurements in mostly asymptomatic HIV-1–positive subjects with

200–500 CD4 cells/mm^3. The intrabaseline variability as determined from the 5th and 95th percentiles can be applied to the assessment of virologic measurements following treatment during a clinical trial. On the basis of these evaluations, any significant changes from baseline representing a treatment effect would have to be >1.87 $\log_{10}$ or 74-fold for infectious HIV-1 titer in PBMC, 0.41 $\log_{10}$ or 2.6-fold for HIV-1 plasma RNA, and 0.50 $\log_{10}$ or 3.2-fold for serum HIV-1 ICD p24 antigen. In addition, plasma HIV-1 RNA levels, viral phenotype, and infectious HIV-1 titer in PBMC, although to a lesser extent, demonstrated prognostic value for disease progression. CD4 cell counts and HIV-1 ICD p24 antigen levels in serum did not add any power for predicting disease progression when plasma HIV-1 RNA level was included. Infectious HIV-1 titer in PBMC, because of its inherent variability, may require multiple baseline measurements, making it a less practical marker. Thus, in an asymptomatic population with 200–500 CD4 cells/mm^3, HIV-1 plasma RNA and viral phenotype provide significant prognostic information. For assessing patients in clinical practice, where culture of virus is not always possible, combining plasma HIV-1 RNA quantitation with CD4 cell count determinations is the most practical approach.

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References

1. O'Brien WA, Hartigan PH, Martin D, et al. Changes in plasma HIV-1 RNA and CD4$^+$ lymphocyte counts and the risk of progression. N Engl J Med **1996**;334:426–31.
2. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med **1989**;321:1621–5.
3. Coombs RW, Collier AC, Allain JP, et al. Plasma viremia in human immunodeficiency virus infection. N Engl J Med **1989**;321:1621–5.
4. Piatak M, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science **1993**;259:1749–54.
5. Hammer S, Crumpacker C, D'Aquila R, et al. Use of virologic assays for detection of human immunodeficiency virus in clinical trials: recommendations of the AIDS Clinical Trials Group virology committee. J Clin Microbiol **1993**;31:2557–64.
6. Lin HJ, Myers LE, Yen-Lieberman B, et al. Multicenter evaluation of quantitative methods for plasma human immunodeficiency virus type 1 RNA. J Infect Dis **1994**;170:553–62.
7. Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus 1 RNA in plasma by the AIDS Clinical Trials Group virology laboratories. J Clin Microbiol **1996**;34:2695–701.
8. Fiscus SA, DeGruttola V, Gupta P, et al. Human immunodeficiency virus type 1 quantitative cell microculture as a measure of antiviral efficacy in a multicenter clinical trial. J Infect Dis **1995**;17:305–11.
9. Cohen OJ, Pantaleo G, Holodniy M, et al. Decreased human immunodeficiency virus type 1 plasma viremia during antiretroviral therapy reflects downregulation of viral replication in lymphoid tissue. Proc Natl Acad Sci USA **1995**;92:6017–21.
10. Japour A, Fiscus S, Arduino JM, et al. Standardized microtiter assay for determination of syncytium-inducing phenotypes of clinical human immunodeficiency virus type 1 isolates. J Clin Microbiol **1994**;32:2291–4.
11. Koot M, Keet IPM, Vos AHV, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4$^+$ cell depletion and progression to AIDS. Ann Intern Med **1993**;118:681–8.
12. Tersmette M, Lange JMA, de Goode REY, et al. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet **1989**;1:983–5.
13. Richman DD, Bozzette SA. The impact of syncytium-inducing phenotype of human immunodeficiency virus on disease progression. J Infect Dis **1994**;169:968–74.
14. Kozal MJ, Shafer RW, Winters MA, et al. HIV-1 syncytium-inducing phenotype, virus burden, codon 215 reverse transcriptase mutation and CD4 cell decline in zidovudine-treated patients. J Acquir Immune Defic Syndr Hum Retrovirol **1994**;7:832–8.
15. Hammer SM, Katzenstein DA, Hughes MD, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. N Engl J Med **1996**;335:1081–90.
16. Katzenstein DA, Hammer SM, Hughes MD, et al. The relationship of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. N Engl J Med **1996**;335:1091–8.
17. US Department of Health and Human Services. ACTG virology manual for HIV laboratories. Bethesda, MD: NIH, **1994**;SIA-1-3; publication 94-3828.
18. Myers LE, McQuay LJ, Hollinger FB. Dilution assay statistics. J Clin Microbiol **1994**;32:732–9.
19. Brambilla DJ, Bremer JW, Staes B, Michels C, Reichelderfer P, DAIDS-sponsored Virology Laboratories. Intra- and inter-assay variation in estimates of viral titers from quantitative microcultures. In: Program and abstracts: XI International Conference on AIDS (Vancouver, Canada). Vancouver: XI International Conference on AIDS Society, **1996**;11:330.
20. Hughes MD, Johnson VA, Hirsch MS, et al. Monitoring HIV-1 RNA levels in plasma in addition to CD4$^+$ lymphocyte count improves assessment of antiretroviral therapeutic response. Ann Intern Med **1997**;126:929–38.
21. Mellors JW, Rinaldo CR, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **1996**;272:1167–70.
22. Phillips AN, Eron JJ, Bartlett JA, et al. HIV RNA levels and the development of clinical disease. AIDS **1996**;10:859–65.
23. Coombs RW, Welles SL, Hooper C, et al. Association of plasma human immunodeficiency virus type 1 RNA level with risk of clinical progression in patients with advanced infection. J Infect Dis **1996**;174:704–12.
24. Galetto-Lacour A, Yerly S, Perneger TV, et al. Prognostic value of viremia in patients with long-standing human immunodeficiency virus infection. J Infect Dis **1995**;173:1388–93.
25. Welles SL, Jackson AJ, Yen-Lieberman B, et al. Prognostic value of plasma human immunodeficiency virus type-1 (HIV-1) RNA levels in patients with advanced HIV-1 disease and with little or no prior zidovudine therapy. J Infect Dis **1996**;174:696–703.