PLASMA HIV RNA QUANTITATION

Plasma HIV RNA quantitation was discussed at the New York course by Daniel R. Kuritzkes, MD, from the University of Colorado Health Sciences Center in Denver.

The results of recent studies on viral dynamics performed with plasma HIV RNA quantitative assays have had important implications for monitoring and treating HIV infection. Viral load is proportionate to viral production, which, in turn, is proportionate to CD4+ lymphocyte destruction, although the relationship may vary in individual patients. The importance of halting viral replication in treatment has been underscored by recent findings suggesting that there is a lifetime limit to the number of CD4+ cells that an individual can generate. Overall, data available on the correlation of viral load with disease progression suggest that suppressing viral replication as completely as possible and as early in infection as possible may be the most effective treatment strategy. At present, there are insufficient data, particularly from randomized clinical trials, to support specific treatment recommendations based on viral load levels; however, the data accumulated to date have provided a coherent picture of the implications of viral load measurements that permits general recommendations for treatment and monitoring. The potential uses of quantitative plasma HIV RNA assays in the clinical setting include assessing prognosis, monitoring drug activity following initiation of antiretroviral treatment, monitoring extent and duration of viral suppression, and assessing the effects of changes in treatment. Interim guidelines for the use of viral load measurements in clinical practice have been developed by an International AIDS Society-USA appointed panel, and have been published in the June 1996 issue of *Nature Medicine*.

Current Quantitative Plasma HIV RNA Assays

At present, there are three methods available for measuring plasma HIV RNA levels: reverse transcriptase polymerase chain reaction (RT-PCR), branched DNA (bDNA) assay, and nucleic acid sequence-based amplification (NASBA). In the RT-PCR assay (which was approved by the Food and Drug Administration [FDA] in June) HIV RNA is extracted from plasma, copied into DNA by RT, then amplified by PCR using HIV-specific primers. The amplified product is detected by hybridization and quantitated to yield the number of HIV RNA copies/mL in the original sample. In the bDNA assay, virus particles in plasma are pelleted by high-speed centrifugation; the RNA is extracted and captured by hybridization to oligonucleotides bound to a 96-well plate. The captured RNA is "decorated" with alkaline phosphatase molecules by several additional cycles of hybridization using branched enzyme-conjugated oligonucleotide probes. This complex is incubated with a chemiluminescent substrate and the liberated light signal is quantitated by a luminometer. The NASBA assay is similar in concept to the RT-PCR assay but uses somewhat different reaction components to amplify target sequence. In general, the results obtained with these three types of assays have been shown to be quite similar. Although each of these assays provides comparable results and reliability, direct comparisons of values from the different assays are difficult in the absence of a uniform standard.

Correlation of Viral Load With Disease Stage

Relatively stable plasma HIV RNA levels are maintained during the short term in the quasi–steady state; significant changes can be attributed to either therapeutic interventions or de facto increases in viral replication. The plasma HIV RNA assays have been shown to have prognostic value in several respects. Plasma HIV RNA levels correlate with HIV disease stage, inversely correlate (in general) with CD4+ cell count, to be predictive of disease progression after seroconversion, and to be independently predictive of disease progression in later disease. In the AIDS Clinical Trials Group (ACTG) protocol 116B/117,

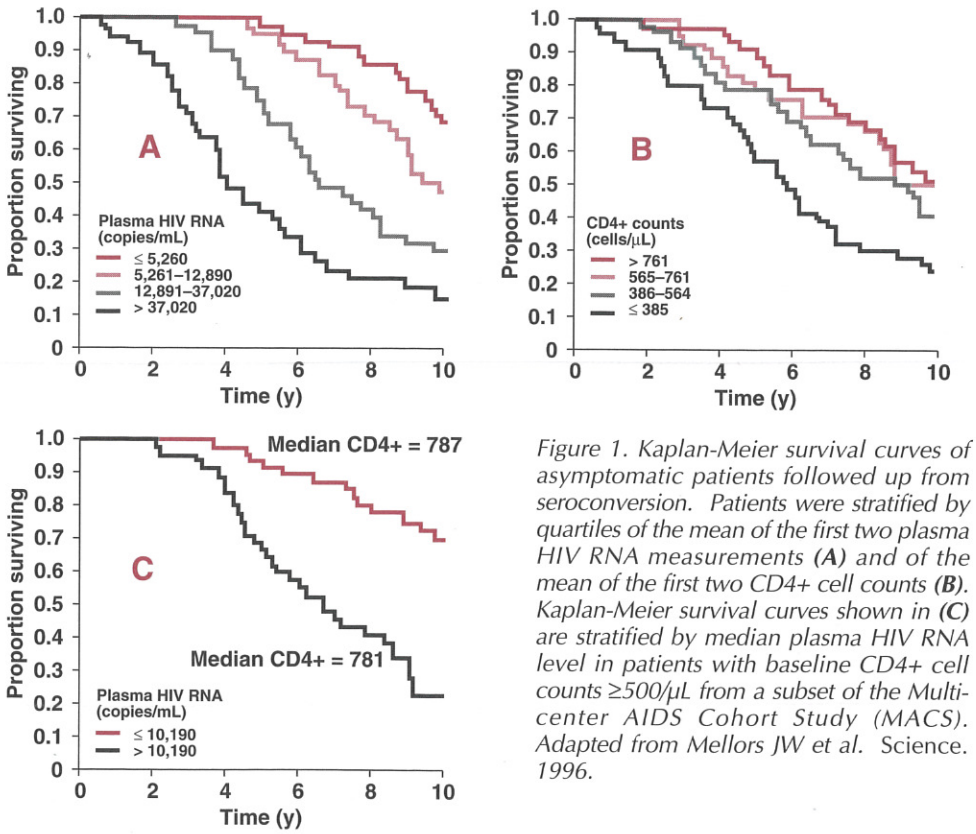

*Figure 1. Kaplan-Meier survival curves of asymptomatic patients followed up from seroconversion. Patients were stratified by quartiles of the mean of the first two plasma HIV RNA measurements **(A)** and of the mean of the first two CD4+ cell counts **(B)**. Kaplan-Meier survival curves shown in **(C)** are stratified by median plasma HIV RNA level in patients with baseline CD4+ cell counts ≥500/μL from a subset of the Multicenter AIDS Cohort Study (MACS). Adapted from Mellors JW et al.* Science. *1996.*

Table 1. Risk of Disease Progression Associated with Measured Changes in Plasma HIV RNA Levels as Reported in Major Clinical Trials.

Study/Marker effect (Reference)	No. of subjects	Median CD4+ count at entry (cells/μL)	Adjusted relative risk (95% CI)
ACTG 116B/117/ 2-fold (0.3 $\log_{10}$) decrease* (Coombs et al. *J Infect Dis.* 1996.)	100	86	0.73 (0.52, 1.02)
ACTG 116A/ 2-fold (0.3 $\log_{10}$) *increase** (Welles et al. *J Infect Dis.* 1996.)	187	139	1.45 (1.02, 2.05)
VA CS298/ 4-fold (0.6 $\log_{10}$) decrease* (O'Brien et al. *N Engl J Med.* 1996.)	270	350	0.44 (0.23–0.81)

*Decrease/increase from pretreatment value. CI, confidence interval.

there were significant differences in HIV RNA copy numbers observed in asymptomatic patients (about 35,000 copies/mL), symptomatic patients (about 70,000 copies/mL), and in patients with clinically-defined AIDS (about 140,000 copies/mL). The intraindividual correlation (r) of repeated baseline plasma HIV RNA values in patients in the ACTG immunology protocol 209 was 0.89, indicating there was little variation in individual values over the short term. In addition, although there was a general correlation between initial plasma HIV RNA levels and CD4+ cell counts in patients enrolled in ACTG 209, there was significant splay of HIV RNA copy numbers at any given CD4+ cell count. This phenomenon has been repeatedly observed in studies correlating plasma HIV RNA level and CD4+ cell count, including analysis of patients in the virology substudy of ACTG protocol 175. Overall, it has been found that there is an approximately 3-log range in viral loads for each CD4+ count. This heterogeneity is one important reason why both plasma HIV RNA and CD4+ cell count, which have been found in other studies to have independent predictive values, are useful markers and should be used together to monitor HIV disease progression.

Table 2. Viral Load and HIV Disease Progression in Patients Enrolled in the NUCA 3001/3002 Studies.

Marker	Relative risk (95% CI)
Asymptomatic → CDC B/AIDS	
Plasma HIV RNA ($\log_{10}$ increase)	1.75 (1.23, 2.50)
CD4+ cell count (50% decrease)	1.39 (0.93, 2.04)
CDC B → AIDS	
Plasma HIV RNA ($\log_{10}$ increase)	3.19 (1.19, 8.57)
CD4+ cell count (50% decrease)	3.12 (1.45, 8.67)

CI, confidence interval.
Data presented by Phillips AN et al at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC. Abstract 32.

Correlation of Viral Load with Progression

Plasma HIV RNA levels have been correlated with rates of HIV disease progression in a number of natural history and treatment studies. In a subset of the Multicenter AIDS Cohort Study (MACS) evaluated by Mellors and colleagues, asymptomatic subjects enrolled after seroconversion were placed in quartiles on the basis of baseline plasma HIV RNA levels, and the rate of progression to AIDS and death over subsequent intervals was analyzed. In the quartiles ranging from the lowest (<4530 copies/mL) to the highest baseline plasma HIV RNA values (>36,270 copies/mL), 8%, 26%, 49%, and 62% of patients progressed to AIDS by 5 years, respectively. The proportions of patients who died within 5 years were 5%, 10%, 25%, and 49%, respectively. Figure 1A shows a Kaplan-Meier analysis of survival by quartiles based on the mean plasma HIV RNA measurement from the first two clinic visits. These data indicate an extraordinary ability of initial plasma HIV RNA levels to discriminate risk of death very early in the course of disease. Also, the data show that even though patients with lower viral loads progress more slowly, progression still occurs. It does not appear that there is a specific threshold of plasma HIV RNA levels below which patients do not progress. A similar analysis by CD4+ cell count quartile (Figure 1B) showed that the quartile with the lowest counts could be discriminated on the basis of an increased risk, but that the remaining three quartiles could not be distinguished on this basis.

In another analysis, patients with baseline CD4+ counts greater than or equal to 500 cells/μL were grouped according to baseline viral loads above or below about 10,000 HIV RNA copies/mL, the median value for the subpopulation. The average CD4+ count was 780 cells/μL. As shown in Figure 1C, there was a significantly reduced rate of death in those patients with plasma HIV RNA levels less than about 10,000 copies/mL, with a median time to death not having been reached after 10 years, compared with a median survival time of 6.8 years in those HIV-infected patients with higher viral loads. According to Dr Kuritzkes, the exact progression rate beyond 10 years is difficult to determine reliably because of the small numbers of patients who have been followed for such a duration. Analysis of patients' plasma HIV RNA levels at given intervals before they developed AIDS showed an increase in median viral load values with a decrease in the interval of time to the development of AIDS; in contrast, patients whose disease did not progress exhibited relatively stable viral loads.

This correlation between pretreatment plasma HIV RNA levels and disease progression has also been demonstrated in large

Table 3. Use of the Plasma HIV RNA Assays in Clinical Practice: Interim Recommendations of an International AIDS Society-USA Panel.

Parameter	Recommendation
• Plasma HIV RNA level that suggests initiation of treatment	• >5,000–10,000 HIV RNA copies/mL and a CD4+ count/clinical status suggestive of progression • >30,000–50,000 HIV RNA copies/mL regardless of laboratory/clinical status
• Target level of plasma HIV RNA after initiation of treatment	• Undetectable; <5,000 HIV RNA copies/mL is an acceptable target
• Minimal decrease in plasma HIV RNA level indicative of antiretroviral activity	• >0.5-log decrease
• Change in plasma HIV RNA level that suggests drug treatment failure	• Return toward (or within 0.3–0.5 log of) pretreatment value
• Suggested frequency of plasma HIV RNA measurement	• At baseline, take two initial measurements 2 to 4 weeks apart • Repeat assessments every 3 to 4 months, in conjunction with CD4+ cell counting • Measure at shorter intervals as critical thresholds for decision points are neared • Measure 3 or 4 weeks after initiating or changing treatment
• Optimal specimen handling	• All samples should be processed and frozen within 6 hours to ensure that no significant signal degradation occurs • The anticoagulant recommended for the particular assay type should always be used and samples should always be of plasma (not serum) • The same single assay type (eg, bDNA, RT-PCR, or NASBA) should always be used for the same individual patient • Specimens for individual patients should always be handled in a consistent manner

Adapted from Saag MS et al. *Nature Med.* 1996.

clinical trials in patients with advanced disease (ACTG 116B/117) and in those with more intermediate disease (ACTG 175).

Correlation of Changes in Viral Load with Progression in Treatment Trials

Studies assessing the effect of changes in viral load on disease progression in HIV-infected patients given antiretroviral treatment have all yielded the same general conclusion: "Less virus is better for the patient and reducing viral load is good." The Veterans Affairs Cooperative Study 298 reported several years ago compared immediate with delayed zidovudine treatment in patients with minimal or no symptoms and CD4+ counts greater than 200 cells/μL. O'Brien and colleagues recently reported the virologic/immunologic analyses from this study, and detailed the effect of treatment-related changes in plasma HIV RNA levels and CD4+ cell counts on the risk of progression. Multivariate analysis of changes between pretreatment and week 8 of treatment showed that each 4-fold (75%, or 0.6-log) decrease in plasma HIV RNA levels was associated with a 50% to 55% decreased risk of disease progression ($P = .0086$); similarly, each 10% increase in CD4+ cell count was associated with a 50% reduction in the risk of progression ($P = .0069$). These two factors together accounted for virtually all of the beneficial effects of treatment noted in delaying disease progression; once the effects of these two factors had been accounted for, the timing of initiation of treatment proved to have no effect on risk of progression (relative risk for early vs delayed treatment, 1.06; $P = .76$). In ACTG protocols 116A and 116B/117, each 50% decrease in viral load in response to treatment was associated with a 33% reduction in the rate of disease progression; CD4+ cell count increases were also a strong predictor of favorable clinical response to treatment. Similarly, in ACTG protocol 175, a 1-log decrease in plasma HIV RNA was associated with a statistically significant 70% to 80% reduction in disease progression independent of the actual pretreatment plasma HIV RNA level, pretreatment CD4+ cell count, and change in CD4+ cell count. In contrast, changes in CD4+ cell counts noted up to week 8 of treatment

were not significantly associated with changes in the risk of HIV disease progression.

Table 1 summarizes the findings in three major trials that examined the effects of treatment-related changes in plasma HIV RNA on progression. Overall, the data from ACTG protocols 116A and 116B/117 (represented in the table as risk of progression in the absence of plasma HIV RNA reduction of a given magnitude) indicate a 30% reduction in the risk of clinical progression with each 2-fold (0.3-log) decrease in plasma HIV RNA levels. The data from The Veterans Affairs Cooperative Study 298 show a 55% decrease in risk of disease progression for each 0.6-log decrease in viral load. In addition to these studies, data from ACTG protocol 175 indicate an approximately 65% decrease for each 10-fold (1-log) decrease in viral load. The data from these studies demonstrate that the degree of clinical benefit can be predicted by the magnitude of reduction in plasma HIV RNA levels. The magnitudes of reduction used in analyzing these clinical trial populations generally were those that were convenient to examine statistically, particularly given the statistical power generated by having large numbers of data points from large numbers of patients. Focusing on the magnitude of decreases for which the statistical associations were reported in these study patients is not necessary in clinical practice—ie, at present, there is no reason to believe that a 50% decrease in viral load represents a specific 'threshold.' It is necessary, however, to remember that because of intraassay and biologic variation, an approximately 0.5-log decrease in plasma HIV RNA levels is taken as minimal evidence of a true antiretroviral effect.

Increases in viral load during treatment have been associated with an increased risk of disease progression. In an analysis of patients from NUCA 3001/3002, the North American trials that evaluated zidovudine and lamivudine monotherapy and combination therapy, the effects of increases in plasma HIV RNA levels from the lowest levels observed after treatment initiation on clinical progression were examined (Table 2). Progression from asymptomatic disease to the Centers for Disease Control and Prevention (CDC) stage B HIV disease and from CDC stage B HIV disease to AIDS was assessed at 24 to 52 weeks following the treatment-associated plasma HIV RNA nadir value. It was found that there was a 3-fold increased risk of progression for each 1-log increase in plasma HIV RNA from the nadir value.

Plasma HIV RNA Assays in Clinical Practice

Issues that require more detailed investigation include: (1) Is there a threshold plasma HIV RNA level for starting treatment? (2) Is there a threshold reduction for treatment benefit? (3) Is there a threshold loss of suppression for withdrawing treatment—ie, in late-stage patients in whom no antiretroviral regimen appears to reduce viral load?

Despite the need for increasingly refined guidelines, sufficient data are now available to suggest that plasma HIV RNA assays should be performed routinely as part of the initial evaluation of HIV-infected patients to aid in assessing their stage of disease and risk of progression, and may prove useful in assessing response to treatment in individual patients (see Table 3). The data available support the contention that treatment should be considered in patients with viral loads greater than 5000 to 10,000 HIV RNA copies/mL of plasma and CD4+ counts/clinical status suggestive of progression. Therapy should be initiated in patients with greater than 30,000 to 50,000 HIV RNA copies/mL, regardless of clinical or laboratory status. According to Dr Kuritzkes, the important issue in deciding on treatment goals with assay use is not determining the minimal effect necessary for benefit, but to strive to achieve the maximal level of viral suppression (ie, viral loads below the limits of assay detection).

Daniel R. Kuritzkes is Associate Professor of Medicine and Microbiology at the University of Colorado Health Sciences Center in Denver.

Suggested Readings

Cao Y, Ho DD, Todd J, et al. Clinical evaluation of branched DNA signal amplification for quantifying HIV type 1 in human plasma. *AIDS Res Hum Retroviruses.* 1995;11:353–361.

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. (In press.)

Embreton J, Zupancic M, Ribas JL, et al. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature.* 1993;362:359–362.

Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science.* 1993;262:1011–1018.

Hammer SM, Katzenstein DA, Hughes MD, et al. Virologic markers and outcome in ACTG 175. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.

Havlir DV, Richman, DD. Viral dynamics of HIV: implications for drug development and therapeutic strategies. *Ann Intern Med.* 1996;124:984–994.

Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med.* 1989;321:1621–1625.

Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature.* 1995;373:123–126.

Holodniy M, Katzenstein DA, Sengupta S, et al. Detection and quantification of human immunodeficiency virus RNA in patient serum by use of the polymerase chain reaction. *J Infect Dis.* 1991;163:862–866.

Holodniy M, Mole L, Winters M, Merigan TC. Diurnal and short-term stability of HIV virus load as measured by gene amplification. *J Acquir Immune Defic Syndr.1994;7:363–368.*

Kappes JC, Saag MD, Shaw GM, et al. Assessment of antiretroviral therapy by plasma viral load testing: standard and ICD HIV-1 p24 antigen and viral RNA (QC-PCR) assays compared. *Acquir Immune Defic Syndr Hum Retrovir.* 1995;10:139-149.

Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* 1996;272:1167–1170.

(continued)