Viral Load Monitoring in HIV Infection

Mark Holodniy, MD, FACP

Address
AIDS Research Center, Veterans Affairs Palo Alto Health Care System, 3801 Miranda Avenue (132), Palo Alto, CA 94304, USA.

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Measurement of HIV-1 viral load is now an accepted part of clinical practice for the determination of clinical prognosis and antiretroviral effectiveness in HIV infection. Consensus guidelines have been published on the appropriate use of this testing. Furthermore, recent advances in molecular technology have improved the sensitivity and reproducibility of viral load assays, and these improved assays have provided new insight into the pathogenesis of HIV disease. This article reviews new issues affecting viral load quantification, including viral subtypes, sex, compartmental differences, and other covariables.

Introduction

A tremendous amount has been written about blood plasma–associated HIV-1 since cell-free, plasma infectious HIV-1 was first described in 1984. The polymerase chain reaction (PCR) was discovered in the mid-1980s, and the first study to use qualitative molecular techniques to detect HIV-1 RNA (viral load) in plasma was reported in 1988. In the early to mid-1990s, numerous reports described the quantification of plasma viral load and the relationship of copy number to disease stage and response to antiretroviral therapy. During the same period, three methods for quantifying viral load were being developed. This development culminated in US Food and Drug Administration approval of a PCR-based assay in 1997 and, in 1999, in an ultra-sensitive version of that assay for monitoring antiretroviral therapy.

Over the past 3 years, viral load level has been validated as an important surrogate marker of HIV-1 disease progression, and reductions in viral load have been used to identify differences in the potency of antiretroviral regimens and the durability of treatment response. Because of the potency of current antiretroviral regimens and the reduction of clinically achievable end points, viral load level is now a required surrogate marker in antiretroviral clinical efficacy studies.

Guidelines for the intended use and interpretation of viral load testing results were recently published [1,2••]. Despite the widespread use and acceptance of viral load monitoring in HIV clinical practice, many questions remain. This article reviews some of the concepts in viral load measurement that have been described in the past 2 years.

Methods and Performance

Three types of viral load assays are now commercially available: reverse transcriptase PCR–based testing (Amplicor HIV-1 Monitor, Roche Diagnostics, Branchburg, NJ); nucleic acid sequence–based amplification (NucliSens, Organon-Teknika, Durham, NC); and branched DNA testing (Quantiplex, Bayer Diagnostics, Emeryville, CA). The first two assays use different methods for template (HIV RNA) amplification, and the last is a signal amplification assay (no template amplification occurs). In general, first-generation assays had lower limits of detection of 200 to 500 copies/mL. Modifications in sample preparation and altered chemistry have resulted in second- and third-generation assays with a lower limit of detection of about 50 copies/mL (Monitor Ultra/Direct, Roche Diagnostics; Quantiplex 3.0, Bayer Diagnostics). This enhanced sensitivity has decreased the upper quantitation limit to 50,000 copies/mL with Monitor Ultra/Direct and 500,000 copies/mL with Quantiplex 3.0. Some research-associated assays have been further modified to have sensitivities of less than 10 copies/mL. However, results may be highly variable below 200 copies/mL and are certainly highly variable below 50 copies/mL because of the lack of template for efficient amplification reactions [3]. Newer assays using different technologies, such as hybrid capture assay (Digene, Beltsville, MD), reverse transcriptase PCR/microparticle immunoassay (LCx , Abbott, North Chicago, IL), or transcription-mediated amplification (Gen-Probe, San Diego, CA) are now in development [4–6]. Comparative studies continue to describe significant differences in copy numbers between assays [7,8], and practitioners should be advised not to interchange assays when monitoring patients.

Recently, HIV-1 has been divided into major (M) and outlier (O) groups. Group M is further divided into subtypes (clades) A through J. This division is based primarily on *envelope* and *gag* gene sequence analysis. Subtypes have been found to have wide geographic diversity. Subtype B is the most common in the United States and has served as the prototypical viral strain for viral load assay development. Non–subtype B viruses have now been reported in the United States and Europe. The current HIV-1 Monitor PCR-based assay has had difficulty amplifying subtype A virus and subtype E virus, and other assays have had prob-

lems in the quantification of group O viruses [9,10]. Modification in PCR primer design should eliminate this problem in the future (Monitor versions 1.0+ or 1.5) [11]. However, there may be current clinical situations in which viral load assay results are not consistent with CD4 counts or clinical impressions or are falsely negative. The clinician may need to consider a non–subtype B virus that creates such discordant results.

Several published studies have described important aspects of sample handling for viral load quantification. Depending on the assay, the type of plasma (the anticoagulant used) or serum may yield small but significant differences in viral load levels. Plasma always yields a higher copy number than serum. In addition, some viral load decay has been found in whole blood samples that have not had plasma separated within 8 hours of collection and stored at -20° C or -80° C. With respect to number of copies/mL of plasma and viral load stability after time of collection, EDTA has been shown to be the anticoagulant of choice [12,13]. Thus, blood for viral load testing should always be consistently collected in tubes that contain EDTA as an anticoagulant. Plasma samples seem to have stable viral loads after one or two freeze–thaw cycles and up to 6 months of storage at -80° C [14].

Most studies have found intra-assay variability for a particular sample to be less than 0.2 $\log_{10}$ copies/mL [15]. The coefficient of variation increases for interassay variability and may depend on kit lot, assay type, and operator. Variability is also greater with lower copy numbers [16]. There seems to be no diurnal and little intrapatient variation over time in patients who are clinically stable. Average variance in pooled studies was less than 0.5 log after either multiple time points over 48 hours or two time points over 2 weeks [17,18]. Variability in viral load tended to increase with greater intervals between measurements. Finally, high concentrations of interfering substances (*eg*, antiretroviral drugs, hemoglobin, or triglycerides) do not seem to affect viral load quantification with branched DNA testing [19].

Pathogenesis and Antiviral Studies

Recent studies have shown that viral load levels are extremely high during primary HIV infection, ranging from 10^5 to more than 10^7 copies/mL. Plasma HIV RNA is detectable before HIV antibody seroconversion and has been detected within 2 weeks of infection [20]. Although not approved by the US Food and Drug Administration for the diagnosis of HIV infection, viral load testing has been used with greater frequency to rule out HIV infection before seroconversion or in patients with inconsistent serologic results. Recent reports indicate that low-level false-positive viral load assay results have been seen in patients who were found, on subsequent further diagnostic testing, not to be infected [21]. In most adult acute infection studies to date, plasma viral load was detectable by 4 weeks in all patients who were truly infected.

Viral load decreases precipitously within the first 2 to 3 months of infection. In small studies, viral load did not differ significantly in patients who had symptoms associated with the acute retroviral syndrome compared with patients who were relatively asymptomatic [22]. However, viral load levels were higher 6 to 12 months after infection in patients that had symptoms of the acute retroviral syndrome than in patients who did not have such symptoms [23]. After the acute retroviral syndrome and during the time of early chronic infection, patients usually have stable, detectable viral load levels. In the Multicenter AIDS Cohort Study (MACS) [24••], less than 2% of patients had viral loads less than 500 copies/mL. More recently, ultrasensitive assays have found even fewer patients to have viral loads of less than 50 copies/mL after seroconversion [25]. The level of viral load after seroconversion has been defined as the set point or virologic equilibrium between viral replication and immunologic containment of viral replication. This steady-state level varies greatly among patients [26]. It is believed by most that without therapy, patients maintain a constant viral load for months to years. Some authors believe that there is no true viral set point [27].

In general, MACS and other studies have found that viral load level after seroconversion is highly predictive of clinical progression and death. Patients with viral loads greater than 50,000 copies/mL have the greatest risk for clinical progression and death. Patients with viral loads less than 500 copies/mL have little risk for progression, even after 10 years of infection. Some of these patients have been found to be long-term nonprogressors with slowly replicating or defective virus, efficient immunologic containment, and little or no decline in CD4 counts over time [28].

Several studies have attempted to analyze whether correlations exist between viral load and immunologic markers. Viral load levels seem to be inversely correlated with CD4 count and cytotoxic T-cell responses. Viral load is not correlated with regulated upon activation, normal T-cell expressed/secreted (RANTES) or macrophage inhibitory protein-1α levels but is correlated with levels of other cytokines, such as tumor necrosis factor. In one study, the number of activated T cells ($CD38^+$) was a stronger predictor of disease progression than viral load [29–32].

Many studies have shown rapid and sustained declines in viral load after the initiation of highly active antiretroviral therapy (HAART). In almost all treatment-naive patients, HAART results in a reduction in viral load of 2 to 3 $\log_{10}$ copies/mL, and a viral load of less than 500 copies/mL can usually be achieved within 4 to 8 weeks of the start of therapy, although this may be contingent on the baseline viral load, the potency and number of agents used in the regimen, and the treatment experience of the patients. Reaching a viral load of less than 50 copies/mL, however, may take as long as 6 months [33]. The nadir of viral load has been found to correlate with the duration of treatment response [34]. Patients who did not achieve a viral load less than 50 copies/mL were more likely than those who did achieve such a load to have

virologic failure within the first year [35••]. However, even patients who had only modest reductions in viral load (1 to 2 $\log_{10}$ copies/mL) after 8 weeks of therapy and whose viral load remained detectable had improved clinical outcomes. Therefore, clinical benefits may still be achievable despite continued detectable viral load [36••,37]. Undetectable plasma viral load does not mean that viral replication in lymphoid tissue is nonexistent. Recent pathogenesis-based studies have described the presence of infected, replication-competent cells in blood or lymph nodes despite undetectable plasma viral loads [38]. Another study recently reported the same level of viral replication in lymphoid tissues regardless of plasma viral load level [39].

On discontinuation of HAART, the viral load can return to the pretreatment level or can overshoot the baseline level as much as 10-fold [40,41]. Re-initiation after acute discontinuation will result in a virologic decline back to undetectable levels [42]. Other reasons for virologic rebound include development of drug resistance, poor adherence to therapy, and drug interactions that reduce the effectiveness of HAART [43]. Viral load may not decline in a predictable manner in some patients; this may be related to the transmission of drug-resistant strains in recently infected patients [44]. When clinicians are choosing the next appropriate regimen, genotypic resistance testing may have a virologic advantage over clinical impressions in previously treated patients [45]. Other discordant responses have been described. In some patients, CD4 counts have increased in the absence of viral load decline [46]. In other cases, CD4 counts have continued to decline in the absence of a detectable plasma viral load. The reasons for these discordant responses require further study.

Differences in Sex and Race

After controlling for CD4 count and other variables, several studies—including the AIDS Link to Intravenous Experiences (ALIVE) study, the Swiss HIV Cohort Study, the Italian Cohort of Naive Antiretroviral (ICONA) study, and comparisons between the Women's Interagency HIV Study and MACS—have found a statistically significant difference (0.1 to 0.25 $\log_{10}$ copies/mL) in baseline viral load between men and women [47–50]. The studies conflict over whether this difference is also significant between women who acquired infection through injection drug use and women who acquired it through heterosexual sex. Differences in viral load results between studies may also be related to the medium used (serum or plasma), the assay used, or the duration of storage of samples before analysis. The ALIVE study results also suggest that risk for clinical progression is higher in women. This conflicts with results from the Swiss HIV Cohort studies, which suggest that women may have slightly less risk for progression when viral load and CD4 count are controlled. Finally, there seem to be no differences in viral load according to race or ethnicity or virologic responses to HAART between men and women in the few studies that have addressed this specifically [51,52].

Maternal Transmission

Viral load is clearly detectable in cervicovaginal lavage (CVL) fluid and in cervical tissue. Viral load levels tend to be lower in CVL than in blood plasma, and detection is correlated with higher plasma viral loads [53]. Blood viral load is not affected by menstrual cycle phase [54], but CVL viral load seems to be lower in the follicular phase and highest during the menstrual phase [55]. Viral load in CVL has also been shown to decline significantly with initiation of or changes in therapy [56]. Pregnancy in HIV-infected women does not seem to affect plasma viral load levels [57].

Several studies have concluded that the likelihood of perinatal transmission increases with increasing maternal blood viral load levels. A CD4 count or a diagnosis of AIDS was less predictive of transmission than viral load level [58••,59]. A recent meta-analysis of published data concluded that transmission occurred but was unlikely at viral loads below 1000 copies/mL and that viral load level was a stronger predictor of transmission in untreated than in treated women in the pre-HAART era [60]. What impact HAART will have in further reducing viral load (and, therefore, transmission) requires further study.

Finally, viral load in breast milk is significantly higher in women with HIV-infected infants than in women with uninfected infants and is significantly correlated with mastitis [61]. Therefore, women with postpartum mastitis are probably more likely than women without mastitis to transmit HIV to uninfected newborns.

Pediatric Viral Load

Viral load levels in the neonatal period can differ significantly depending on whether HIV infection occurred in utero or during the peripartum period. The viral load at birth and 1 month after birth is much higher in babies infected in utero [62]. In general, infants have a rapid increase in viral load over the first 1 to 2 months followed by a slow decline over the next 2 years. Unlike adults, who experience a reduction in viral load concomitant with seroconversion and development of a cellular immune response, children usually maintain viral load levels greater than 10^5 copies/mL during the first year of life [63].

The prognostic value of viral load in infants and children seems to parallel that in adults. A high viral load level at 4 weeks of age or a high viral load level maintained to 6 months of age in the absence of therapy was highly predictive of disease progression within the first 2 years. Viral loads greater than 100,000 copies/mL correlate with the presence of syncytial-inducing strains of virus, growth retardation, encephalopathy, opportunistic infections, and increased risk for death [64–67]. Children and adults seem to have the same magnitude of viral load reduction after starting or changing HAART [68].

Other Compartments

Much controversial material has recently been written about cerebrospinal fluid (CSF) viral load levels and their correlation with AIDS dementia complex (ADC). Whether CSF viral load reflects only diffused plasma viral load or is indicative of brain or central nervous system (CNS) production has not been resolved. Several published series show a fair correlation between plasma and CSF viral load levels, although plasma levels tend to be more than 1 $\log_{10}$ copy/mL greater than CSF levels [69]. Although significant individual patient variation was seen and not all studies agree, higher CSF viral loads seem to correlate with the presence and severity of ADC, the diagnosis of AIDS, a CD4 count less than 200 cells/mm^3, lymphocyte pleocytosis, and the presence of CNS opportunistic infections [70,71]. In some single time-point cross-sectional studies, plasma viral loads also correlated with ADC [72]. Brain tissue viral loads in autopsy studies were found to correlate only minimally with antemortem ADC and CSF viral loads and did not correlate at all with CSF viral loads in patients without ADC [73]. In a small longitudinal study, CSF viral load levels seemed to increase over time (0.5 $\log_{10}$ copies/mL over 3 years) and correlated with an increase in CSF neopterin levels; this suggests that increasing levels of immune activation were occurring to account for endogenous CNS viral production [74,75]. After initiation of HAART, there are profound reductions in CSF viral load that can mirror those seen in plasma in terms of magnitude [76].

Viral loads in semen correlate with viral loads in blood plasma but not with CD4 counts. However, viral load seems to be significantly higher in blood plasma than in semen. In a small number of patients analyzed with serial semen samples, seminal viral load also increased over time in patients who progressed to AIDS [77]. Reductions in seminal viral load are also seen after antiretroviral therapy. The magnitude of reduction seems to depend on the potency of the regimen [78].

Lastly, viral load has rarely been detected in saliva and stool and has not been reported in sweat or tears [79]. The clinical significance of this for patients or health care workers is unclear.

Covariables

Numerous small studies have attempted to evaluate the impact of comorbid conditions, procedures, vaccinations, and acute infections on HIV viral load. Limited data suggest that HIV-infected patients with sickle-cell anemia are more likely than HIV-infected patients without sickle-cell anemia to remain asymptomatic nonprogressors with lower viral burdens [80]. The converse seems to be true for hemophiliac patients [81]. In general, transfusion with leukocyte-depleted erythrocytes, hemodialysis, and ultraviolet B phototherapy showed no effects on viral load levels [82–84], whereas viral load was seen to decrease threefold or more in patients who had recently undergone splenectomy or who had received systemic steroid therapy [85,86]. Several previous studies reported that immunization with influenza and other vaccines or treatment with exogenous interleukin-2 produce transient increases in viral load [87,88]. These reports are primarily in patients who were untreated or suboptimally treated with antiretroviral agents and were not receiving HAART. In addition, viral load has been shown to increase by 0.5 $\log_{10}$ copies/mL after tuberculin skin testing, primarily in drug-naive patients in whom the skin-test results were positive [89]. More recent studies in both adults and children indicate that vaccination with influenza or diphtheria-pertussis-tetanus vaccines, or the administration of interleukin-2 in the presence of HAART, does not produce significant increases in viral load [90–92].

Several co-infections have been studied for their impact on or association with HIV-1 viral load. Human T-cell lymphotropic virus-I and -II co-infections have not been found to have any significant effect on HIV-1 viral load [93,94]. Co-infection with oncogenic strains of cervical human papillomavirus, oral *Candida* species, and Kaposi's sarcoma–associated herpesvirus DNA in peripheral blood mononuclear cells with or without Kaposi's sarcoma correlate with higher plasma HIV viral loads [95–98]. Acute infections with *Pneumocystis carinii* pneumonia, cytomegalovirus, *Mycobacterium avium* complex, and *Plasmodium falciparum* malaria have shown significant acute increases in HIV viral load that, on initiation of effective therapy for the acute infection, returned to baseline levels [99–102]. This phenomenon seems to occur consistently in both adults and children with HIV infection. Viral load level was also found to be predictive of opportunistic infections in the MACS cohort [103]. The relationship between hepatitis C virus (HCV) and HIV viral load is less clear. The HCV viral load is higher in patients co-infected with HIV than in patients without HIV infection. It is not clear whether the converse is true. The HCV viral load is not affected by HAART [104,105].

Conclusions

Much has been learned about HIV disease pathogenesis and treatment response through the use of HIV viral load measurement. Viral load monitoring has become the standard of care in clinical practice to assess risk for disease progression and antiretroviral response. The assays available today are more sensitive, robust, and reproducible than those used in the past. Furthermore, we now better understand the limitations of these assays and the clinical and sample factors associated with viral load variability.

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