The clinical utility of viral load monitoring in HIV infection: strengths and limitations

Molecular techniques that quantify and detect circulating HIV RNA have led to a new understanding of the pathogenesis of HIV disease and a new surrogate marker for use in clinical management. Prior to the ability to quantify virus-associated HIV-RNA in the circulation, measurement of disease burden was by quantitative viral culture, by p24 antigen levels, or indirectly by CD4 T-cell enumeration. However, since most patients had no detectable virus or p24 antigen in the plasma during the period of clinical latency following primary infection when their CD4 T-cell count was essentially normal, the virus was thought to be “silent” or “inactive” during this period. High levels of detectable virus by culture or p24 antigen were known to coincide with the fall in CD4 T-cell count to levels below 200/mm³ and the onset of AIDS-associated symptoms. This perspective changed when the p24 antigen method was modified to break apart the antibody-antigen complexes by acid hydrolysis.¹ This method, referred to as immune-complex dissociated p24 antigen method (ICD-p24) demonstrated that viral p24 antigen was indeed present at various levels during the period of clinical latency, reflecting active viral replication. With the advent of PCR, scientists further developed a method which could quantitate either RNA or DNA copies of HIV in infected individuals, a method referred to as competitive PCR (QC-PCR).² This technique revealed that individuals in the clinically latent period could have 10⁴ to 10⁵ virions per ml of plasma at any time during the course of their infection. Although more sensitive than the ICD-p24 method, QC-PCR was laborious and not standardised for routine use in clinical laboratories. Nevertheless, development of these two assays and the demonstration that viral replication was occurring during this period of clinical latency and could be accurately monitored in response to therapy eventually led to the development of commercially available assays that could quantitate RNA in a precise and reproducible manner.

There are now three commercially available assays that quantify HIV-RNA load in plasma and that detect some level of HIV-RNA in essentially all infected individuals: bDNA (branched DNA, Chiron Corporation), RT-PCR (reverse transcription polymerase chain reaction, Roche Diagnostics) and NASBA (nucleic acid sequence based amplification, Organon Teknika). The three different methods yield highly reproducible RNA determinations on the same sample and each assay has low intra-assay variability. While CD4 T-cell count measurements have great biologic variability, viral load measurements remain remarkably stable in clinically asymptomatic patients over weeks to months, as long as antiretroviral therapy is not changed. The RT-PCR and NASBA assays can detect viral levels as low as 500 copies/ml plasma, while the original bDNA assay has a lower limit of 10 000 copies/ml. A second-generation bDNA test now available can also detect as low as 500 copies/ml.

Several studies have recently applied these new molecular techniques to sensitively measure viral dynamics during acute infection and following antiretroviral therapy. Approximately 10 billion new HIV particles are produced and destroyed daily, and as many as 10 million CD4 T-cells are destroyed daily by HIV as well.³ This extraordinarily high rate of viral replication and CD4 T-cell turnover has led to a very different concept of disease pathogenesis. Rather than the paradigm of a long “quiet” latency period in the natural history of a chronic viral infection, it is now evident that the immune system of the HIV-infected individual is waging war daily, even during early and asymptomatic disease.³ It is equally clear that if viral replication and mutation continue to occur at significant rates while on an antiretroviral regimen, strong selection pressure is exerted for the evolution of drug-resistant strains. Based upon this new concept of disease pathogenesis, the best chance for preserving the immune system and for preventing the complications of AIDS may be to intervene to shut down viral replication with antiretroviral agents in combination. Viral load measurements, as a direct reflection of the degree of viral replication, have emerged as a new surrogate marker well suited to guiding decisions in clinical management.

Viral load measurements are excellent predictors of rapid progression to AIDS and death and correlate with these clinical outcomes better than CD4 T-cell count.⁴ Mellors and colleagues have reported from the Multi-center AIDS Cohort Study that individuals with viral load measurements greater than 10⁴ copies/ml plasma within six months of seroconversion had a median survival of six years, compared to individuals without detectable virus (< 10⁴ copies/ml plasma in this study) whose median survival was over 12 years.⁷ Those with viral load measured at greater than 10⁴ copies/ml plasma were at greatest risk for death. While CD4 T-cell counts were also independent predictors of risk for disease progression and death, they were not as strongly predictive as viral load. Using the combination of viral load and CD4 T-cell count together was more predictive than either measurement alone. Similar analyses performed on stored sera from cohorts of injection drug users, hemophiliacs, and HIV-infected newborns (in whom CD4 T-cell counts are very poor surrogate markers) have confirmed that high HIV viral load is highly predictive of poor clinical outcomes in these groups.
as well. Conversely, decreasing or low viral loads are associated with improved survival.

Despite the enthusiasm for the use of viral load monitoring in routine clinical practice, few data are available from clinical studies to establish guidelines for the viral load parameters useful in instituting or switching antiretroviral therapy. When stored samples from the VA Cooperative Study of AIDS-related early and delayed zidovudine therapy were further analyzed for viral load, those individuals who derived clinical benefit from zidovudine therapy showed an early reduction (mean 0.5 log decline) in viral load upon institution of zidovudine therapy. From other available data, clinically effective nucleoside combinations achieve 1.5 log decrease in viral load, and triple combination therapies that include a protease inhibitor may be associated with as much as a 1.5-2.0 log decline in viral load. These reductions have been sustained for up to a year, and sustained reductions in viral load are associated with improved clinical outcomes. However, the level of viral load that one should aim for in clinical practice has not been established. Because the lowest viral load associated with the best clinical outcomes, and because undetectable viral loads are now achievable with combination therapies, some propose that this should be the goal of therapy. The theory that the optimum long-term clinical benefit results when combination antiretroviral therapy makes HIV undetectable in plasma is one that, while entirely consistent with what is known about the pathogenesis of HIV infection, remains untested in any large clinical endpoint trial.

Saag et al have proposed interim guidelines for the application of viral load testing to clinical management and decisions to initiate or switch antiretroviral therapy. In any individual not on antiretrovirals or on a stable regimen, an HIV-RNA level greater than 50 000-50 000 copies/ml plasma identifies a patient who, based upon our current understanding of pathogenesis, is likely to benefit from starting or changing antiretroviral therapy. A viral load in this range predicts disease progression independently of CD4 T-cell count. A patient with a relatively low viral load measurement (5000-10 000 copies/ml plasma) and a CD4 T-cell count less than 500/mm³ or HIV-related symptoms is also likely to benefit from antiretroviral agents.

After the initiation of therapy, a reduction of 0.5 log (3-fold) or more indicates effectiveness. Changes less than that in magnitude are not biologically meaningful. Though a reduction of 0.5 log documents significant antiretroviral effect, detectable virus levels of greater than 10 000 copies/ml plasma suggest ongoing viral replication. Though data from clinical trials to support this strategy are still forthcoming, many experts suggest that an undetectable viral load should be the goal of therapy if possible. Follow-up measurements every three to four months in conjunction with CD4 T-cell counts will provide useful information on the ongoing effectiveness of prescribed agents. A return in viral load to baseline measurements suggests a failing regimen and would provide a reason for instituting therapy change. Though some experts have proposed that two baseline viral measurements be obtained two to four weeks apart prior to starting antiretroviral therapy, the added expense of an extra test may be prohibitive in many settings. If a single measurement is obtained, certain clinical situations should be avoided. Transient increases in viral load measurements can occur following immune stimulation, such as with vaccinations or with new infections. Also, the viral load is known to be several logs higher during and immediately following seroconversion, and a baseline set point may not be attained for six to twelve months. Consequently, "baseline" viral loads should not be obtained within the first six months of seroconversion unless treatment is instituted and the measurement is for monitoring. If a patient is clinically stable and has not seroconverted within the past year, a single viral load measurement should provide a firm basis for decision making.

Viral load monitoring may also be applied to the management of the pregnant HIV-infected woman. Pregnant women with markedly elevated viral loads appear to be more efficient transmitters of HIV to their offspring. However, women with low viral loads may still transmit HIV and a minimum threshold predictive of transmission has not yet been identified. The efficiency of perinatal transmission is a complex issue that is dependent on a wide variety of factors other than viral load. Previous studies have demonstrated that premature rupture of membranes, low CD4 T-cell count, high CD8 T-cell count, elevated p24 antigen, breast feeding and advanced stage of HIV infection have been associated with an increased risk of transmission, and that intervention with antiretrovirals may decrease HIV perinatal transmission. Additional features of viral load and factors related to pregnancy and antiretroviral therapy may present additional challenges. These reductions have not yet been taken to learn more about the interaction of host immunologic and virologic factors on mother-to-infant transmission. Furthermore, little is known regarding the natural history and prognostic significance of HIV RNA viral load in newborn infants and during childhood. Several preliminary studies have demonstrated that HIV viral load increases dramatically shortly after birth in infected infants similar to that observed in adults during the acute retroviral infection, but frequently the levels remain extremely high in the infants instead of declining as observed in adults. This may reflect an immature immune system with an inability to control viral replication, or a viral load resulting in a more rapid progression of HIV in these infants. Definitive data are also not available on the effect of antiretroviral therapy during infancy on HIV RNA viral levels, an important clinical issue.

Viral load monitoring is unlikely to supplant CD4 T-cell count as a surrogate marker in clinical management. Viral load values do not necessarily reflect the degree to which the immune system has been weakened by HIV or define a point in the disease course at which a patient should begin prophylaxis for opportunistic infections. The two laboratory tests are best utilised together. The cost of an HIV viral load test in the United States varies from $105 to $210. Therefore, the addition of viral load monitoring to the standard outpatient management of HIV will increase the cost of HIV primary care, stretching the resources of publicly-funded programs in industrialised countries and widening even further the gap between the standards for care in the developed world and those in the developing world. However, as information from viral load monitoring enables the practitioner to use antiretrovirals more effectively, to identify therapies that are not working and to stop them, the cost associated with useless antiretroviral therapy will be minimised. If competition can drive the market price of these applied technologies lower, viral load monitoring may actually reduce the annual cost of caring for an HIV-infected individual.

A few additional caveats remain in the application of viral load measurements. Because the assay is based upon nucleic acid copy number and not infectivity, copies of both competent and defective virus are detected equally well; thus high viral loads do not necessarily reflect high viral fitness. Viral load measurements by themselves do not give an indication of viral resistance to drugs, nor do they give a direct measure of host immune status. Measurement of CD4 T-cells still provide the benchmarks
for risk of opportunistic infection and need for chemopro-
phylaxis. Nonetheless, viral load monitoring of HIV repre-
sents a great advance in our understanding of this chronic
disease and will soon become an indispensable tool in
clinical management.

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