ROLE OF PLASMA HIV RNA TESTS

112. In view of the lack of widespread accessibility and costs of HIV RNA testing, would you recommend routine determinations for asymptomatic, stable patients on initial therapy who have stable CD4+ counts between 300 and 500 cells/μL.

Dr Volberding: Yes. The available data support the use of serial plasma HIV RNA monitoring for evaluating response to antiretroviral therapy. Even a clinically stable patient may be experiencing antiretroviral failure from, for example, the development of resistance to the drug(s). Rather than waiting for possibly irreversible overt symptomatic progression or CD4+ count decline to occur, therapy should be changed if an initial HIV RNA benefit is lost. Plasma HIV RNA levels may, in fact, be particularly useful in the patient addressed in the question, as drug failure may otherwise be so difficult to estimate. More and more evidence is accumulating to suggest that the immunologic benefits of antiretroviral therapy applied late in the disease process are incomplete. Therefore, preserving immune function by individualized, early antiretroviral therapy is of particular current interest.

Dr Katzenstein: I would add from the ACTG 175 experience, even in a stable patient with a CD4+ count of 300 to 500 cells/μL while on stable therapy, a single determination of plasma HIV RNA levels offers information about prognosis and risk over the next 3 years. A low plasma HIV RNA level (ie, <10,000 copies/mL) will provide reassurance that there is less than a 5% risk of AIDS over the next 3 years; levels higher than that should prompt consideration of additional therapy.

113. Is HIV RNA testing cost-effective? Would you consider these assays if there is no evidence of clinical deterioration?

Dr Volberding: Plasma HIV RNA testing is very rapidly becoming available as a routine clinical laboratory test in most medical centers and the costs are decreasing. The data supporting the use of HIV RNA assays are extremely strong, particularly with regard to use as a prognostic test. Titer changes in response to antiretroviral treatment are also being shown of value in retrospective and prospective surveys.

Dr Montaner: Serial viral load determinations represent a highly cost-effective approach to optimizing antiretroviral therapy. We have therefore adopted viral load–driven antiretroviral therapy within our provincial program. Data presented at the Vancouver conference demonstrate that use of viral load makes previously used laboratory tests for monitoring (ie, p24 antigen, β_2-microglobulin, neopterin, etc) redundant. Beyond this, viral load monitoring is the only way to judge the effect of therapy so that we can minimize drug exposure while optimizing the use of the drugs (and therefore resources).

Dr Katzenstein: The cost considerations are important. In the current climate, many patients are asking if they should be on a new 3-drug regimen (including a protease inhibitor), which is an expensive undertaking. For many of the reasons previously discussed, an assessment of plasma HIV RNA levels for $100 to $200 may provide a rational basis for deciding to initiate or postpone the use of a drug regimen costing $5000 to $6000 per year. The cost of an AIDS-defining OI can be very high, with hospitalization costing as much as $50,000 to $100,000. If introduction of an expensive drug reduces the risk of a clinical event by more than 50%, then the additional cost of therapy may be warranted.

114. Regarding the recently reported data on associations of HIV RNA level in plasma and risk of disease progression, can you apply cross-sectional data to prognosis and treatment decisions?

Dr Volberding: The use of cross-sectional epidemiologic data (from the MACS cohort, for example) seems appropriate for making the decision to initiate antiretroviral therapy as these data indicate the risk of progression in the untreated state. These data are not directly applicable to the use of HIV RNA assays in estimating an individual's response to a treatment strategy. That information is becoming increasingly available from trials in which HIV RNA data are compared with clinical outcome.

Dr Katzenstein: The prognostic value of plasma HIV RNA levels extends beyond the "CD4+ horizon" by providing information about risk over the next few years that is more precise than that from CD4+ counts. If the plasma HIV RNA level is low, the intensity and frequency of CD4+ count monitoring and physician visits may be reduced (eg, every 6 months). Conversely, if the plasma HIV RNA level is elevated, even in the face of a stable CD4+ count and no clinical deterioration, it may be appropriate to consider switching or adding new antiretroviral drug(s).

115. It is my understanding that the MACS data on viral load, on which the new treat-

ment recommendations rely, may be somewhat biased toward underestimation of viral load in the following ways: 1) blood was heparinized; 2) serum was not routinely separated <6 hours after collection; and 3) samples were frozen for >10 years. All these factors would underestimate what the "true" viral loads of these samples were. May we infer from this that these thresholds may not be applicable to patients today (ie, that what the MACS results indicate as a "worrisome" level may, in fact, reflect a level that was actually perhaps 5 to 10 times higher)? If so, this could lead to initiation of therapy sooner than might really be indicated, based on the "true" MACS cohort indicated levels.

Dr Volberding: It is true that the MACS data may underestimate the HIV RNA value one would expect were the studies repeated with current specimen processing recommendations. This error factor is thought to be as much as 50%; that is, the MACS numbers should be about double for thresholds in current patients. More important than the meaning of specific numbers, however, is that the MACS data show that the more virus one has, the more rapidly that person's disease will progress. By inference, these data support the use of antiretroviral drugs to attempt to control the ongoing damage caused by the repeated cycles of replication and attendant CD4+ depletion. The extension of such thinking leads to more and more confidence in treating earlier in the course of the disease. The Panel, in considering the issues, suggested that therapy be considered at a low but detectable HIV RNA titer such as one that is above 5000 to 10,000 copies/mL and that treatment be recommended when the titer (and hence, immediate progression risk) was higher (ie, >30,000 to 50,000 copies/mL). It should be noted that the *Guidelines* intentionally use *ranges* of HIV RNA levels (ie, above 5000 to 10,000; greater than 30,000 to 50,000, etc) for possible threshold levels. As noted, there is a continuum of increased risk with increasing HIV RNA levels, and as such, no single value should be considered to represent an absolute standard.

116. Please comment on the rapidity of the increase in viral load after antiretroviral drugs are stopped. In particular, comment on the importance of determining what drugs the patient is actually taking (not just the prescribed regimen) and how this could influence the interpretation of the viral load test. If the patient "ran out" of drugs a week ago, how much might that influence the test?

Dr Saag: HIV RNA levels *rapidly* return toward the original pretreatment value after antiretroviral therapy is discontinued (usually within 2 to 4 days). Therefore, it is crucial that the clinician know precisely what medications the patient was taking at the time the blood was drawn for viral load testing. If the patient had discontinued therapy, even as recently as 1 to 2 days prior to coming to clinic, there will be a profound effect on viral load level measured at that visit. Thus, an *accurate* assessment of the drugs a patient is actually taking at the time of visit is critical to interpretation of HIV RNA values.

117. The recommendations frequently refer to dropping viral load and rising CD4+ counts as therapeutic monitoring parameters. In patients with very low CD4+ counts (<100/µL), the viral load drops but CD4+ cells don't seem to rise. Is viral load drop sufficient enough to indicate therapeutic success, irrespective of a lack of CD4+ increase?

Dr Saag: The level of circulating virus in plasma is a direct reflection of ongoing replication in the host. The CD4+ lymphocyte, being a *target* of HIV replication, is an *indirect* marker of antiretroviral therapy. The CD4+ count itself is a measurement of the relative production and destruction of the CD4+ cell population. For example, if there is a production problem, either due to nutritional, toxicity, or other factors, the CD4+ count may not rise proportionately to the decrease in viral load. Thus, the change in viral load is measuring precisely the effect of antiretroviral therapy and, therefore, is our best marker of how well drugs are working or not as antiretroviral drugs.

118. How variable are the results of the plasma HIV RNA assays? Is it like CD4+ counting? Do two or three need to be done to verify rate?

Dr Saag: In general, the tests themselves vary by 0.1 to 0.2 log, and the biologic variability ranges from 0.2 to 0.3 log. Therefore, any change >0.5 log (3-fold) in viral load represents a real change in the level of viral replication within the host. It is important to note that the "minimum" of a 0.5 log change is meant to be used as a value to indicate when a drug is *not* active. It is not meant to suggest an appropriate goal for the activity of a treatment.

An important caveat, and perhaps the most important source of variability in the clinical setting, is how the specimen is processed. Plasma needs to be separated and

frozen within 4 to 6 hours after the blood is obtained from the patient. If the blood is allowed to sit at room temperature for an extended period of time (>8 to 12 hours), a significant loss of signal will occur and lead to marked variability in the results. In general, it is always a good idea to get two viral load measurements to establish the baseline value and before making a decision to change the therapeutic regimens.

119. Are there differences in the values that are obtained (ie, numbers of copies) with different plasma HIV RNA assays (bDNA, NASBA, RT-PCR, etc)? In clinical practice, we often see sudden unexplained rises or drops in viral load. Advice? Would you repeat all unexplained rises in viral loads? What, besides acute illness or vaccines, might account for "bouncing" viral loads in patients on therapy?

Dr Saag: Generally, there are differences in the absolute values of HIV RNA obtained with the different techniques. The values obtained by bDNA tend to be a bit higher than those with quantitative RT-PCR, although variability in this has been observed. In individual cases, this difference in absolute value between bDNA and quantitative RT-PCR testing can be quite substantial; however, overall, the results are usually quite comparable between the two tests (0.3-log variation). Nonetheless, most investigators recommend choosing one viral load assay and continuing with it to follow the same patient. If a change is to be made from one assay to another (eg, due to availability or cost considerations), careful assessment should be made at the time the first result returns from the new assay and serial determinations with the new assay should be sought prior to making any changes in therapeutic regimens.

Unexplained rises or falls in viral load should be confirmed with a repeat test. Acute illnesses, vaccine administration, poor specimen handling, and inconsistency of patients taking medication (noncompliance) can be associated with bouncing viral load results in patients on therapy.

120. How often should we draw viral loads on patients after they show an initial response to a regimen? Every 3 months? Every 6 months? Then if it changes/rises, do we repeat it to be sure it's real before changing therapy?

Dr Saag: Ideally, viral load determination should be repeated when the initial baseline determination is made, and then repeated within 3 to 4 weeks after initiating or making the change in antiretroviral therapy. Once an individual has demonstrated a satisfactory response, the patient should generally be followed every 3 to 4 months with repeat viral load determinations. Any significant change from the previous value should be repeated within 2 to 4 weeks to assure reliability of the new measurement before a therapeutic change is made.

121. What evidence is there that chemotherapy induced decrease in viral load has the same survival outlook as a "naturally" low viral load? Perhaps a "naturally" low viral load is simply an indication of a more intact immune system.

Dr Saag: There are very little data that indicate whether a viral load obtained as a result of chemotherapy (eg, 500 copies/mL) portends the same prognostic value as the same viral load level achieved without the use of antiretroviral therapy. However, some data are beginning to accumulate. There does appear to be a correlation between those individuals with "naturally" low viral load levels and a more efficient immune system response that seems to translate into significantly improved long-term clinical outcome. In order to equate the same level of viral load induced by chemotherapy versus the viral load value obtained "naturally," it would have to be assumed that the chemotherapy-induced viral load could be sustained for an extended period time of time with very little host toxicity. Therefore, although complete evidence is pending, it seems better for an individual to have a naturally-occurring, low viral load than a drug-induced low viral load. However, it is better to have a drug-induced low viral load than a naturally-occurring high viral load.