Changes in Virus Load Markers during AIDS-Associated Opportunistic Diseases in Human Immunodeficiency Virus–Infected Persons


Division of Infectious Diseases, Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan

Human immunodeficiency virus (HIV) load markers are being used increasingly to monitor disease progression and evaluate antiretroviral therapy. This study examined plasma HIV RNA and p24 antigen levels before, during, and after 15 AIDS-associated opportunistic disease events in patients with AIDS (median CD4 cell count = 65/μL). Plasma HIV RNA was detected during 13 of the 15 events (median level before an event = 21,000 copies/mL). There was an increase in the level of plasma HIV RNA with the onset of an AIDS-associated opportunistic disease during 11 of 13 events for which HIV RNA was detectable (median level during an event = 145,000 copies/mL). There was a decline in the level of HIV RNA with the recovery from disease (median level after an event = 29,700 copies/mL). In contrast, there was no consistent or significant change in p24 antigen levels or CD4 cell counts with either the onset of or recovery from an event. Clinical interpretation of plasma HIV RNA changes must take into account this reversible elevation during AIDS-associated opportunistic disease.

The amount of human immunodeficiency virus (HIV) present in the plasma of infected persons appears to be a reliable marker of disease activity and is being used with increasing frequency to monitor HIV disease progression and guide antiretroviral therapy. Indicators of plasma virus load (e.g., quantitative culture [1–3], p24 antigen [4], and HIV RNA [5–7]) generally increase as the CD4 cell count falls [8] and decrease with the initiation of antiretroviral therapy [9, 10], and, thus, they can be used as prognostic guides [11]. Moreover, a therapeutic response measured as a reduction in viral RNA appears to be associated with an improved clinical outcome [12].

There is also evidence that stimulation of the immune system with cytokines, such as interleukin-2 [13], or by vaccination [14, 15] can cause a burst in HIV viremia. These observations are significant because they suggest that events that stimulate the immune system may trigger HIV production and thereby potentially exacerbate disease progression. Furthermore, these observations suggest that interpretation of HIV virus load changes could be confounded by circumstances unrelated to viral escape or therapeutic failure. Reliance on transient changes in HIV virus markers, therefore, could lead to inappropriate or unnecessary modification of antiretroviral therapy.

The purpose of this study was to examine plasma HIV RNA to determine whether the occurrence of AIDS-associated opportunistic disease is associated with an increase in the level of HIV and whether the subsequent resolution of disease is associated with a decrease of HIV RNA in plasma.

Methods

Fourteen subjects who fulfilled entry criteria were selected from a longitudinal cohort of 50 HIV-infected patients followed at Henry Ford Hospital. Persons were eligible for inclusion if they had had an AIDS-associated opportunistic disease, had appropriately collected blood samples, and had been maintained on stable antiretroviral therapy throughout the sampling period. The subjects had a mean age of 37.3 years (range, 23–53). There were 9 men and 5 women; 10 were black and 4 were white. Eight subjects acquired HIV infection through homosexual activity, 4 through injection drug use, and 2 through heterosexual activity.
Table 1. Plasma HIV RNA response during AIDS-associated opportunistic disease (OD) events.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>CD4 cell count before OD</th>
<th>Plasma HIV RNA (copies/mL)</th>
<th>% value before OD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before OD</td>
<td>During OD</td>
</tr>
<tr>
<td>1</td>
<td>CMV enteritis + KS</td>
<td>6</td>
<td>18,200</td>
<td>145,000</td>
</tr>
<tr>
<td>2</td>
<td>PCP</td>
<td>16</td>
<td>16,000</td>
<td>36,760</td>
</tr>
<tr>
<td>3</td>
<td>Bacterial pneumonia</td>
<td>18</td>
<td>265,928</td>
<td>444,486</td>
</tr>
<tr>
<td>4</td>
<td>MAC</td>
<td>25</td>
<td>30,000</td>
<td>180,000</td>
</tr>
<tr>
<td>5</td>
<td>Cryptococcal meningitis</td>
<td>28</td>
<td>39,000</td>
<td>30,800</td>
</tr>
<tr>
<td>6</td>
<td>PCP + bacterial pneumonia</td>
<td>47</td>
<td>130,562</td>
<td>397,679</td>
</tr>
<tr>
<td>7</td>
<td>Candida esophagitis</td>
<td>62</td>
<td>31,600</td>
<td>32,050</td>
</tr>
<tr>
<td>8</td>
<td>Intestinal pneumonia</td>
<td>65</td>
<td>13,000</td>
<td>152,000</td>
</tr>
<tr>
<td>9</td>
<td>Candida esophagitis</td>
<td>113</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>Candida esophagitis</td>
<td>144</td>
<td>14,000</td>
<td>50,000</td>
</tr>
<tr>
<td>11</td>
<td>PCP</td>
<td>154</td>
<td>24,000</td>
<td>38,000</td>
</tr>
<tr>
<td>12</td>
<td>PCP + bacterial pneumonia</td>
<td>181</td>
<td>ND</td>
<td>245,000</td>
</tr>
<tr>
<td>13a</td>
<td>OHL + Candida esophagitis</td>
<td>233</td>
<td>40,000</td>
<td>263,000</td>
</tr>
<tr>
<td>14</td>
<td>KS</td>
<td>248</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>13b</td>
<td>OHL + bacterial pneumonia</td>
<td>323</td>
<td>21,000</td>
<td>165,000</td>
</tr>
</tbody>
</table>

NOTE. CMV, cytomegalovirus; KS, Kaposi's sarcoma; PCP, Pneumocystis carinii pneumonia; MAC, disseminated Mycobacterium avium complex; ND, not detected (below detection limit of assay [10,000 RNA copies/mL]); OHL, oral hairy leukoplakia.
* Calculation based on denominator of 10,000 HIV RNA copies/mL.

Overall, 15 clinical events, some involving multiple processes diagnosed simultaneously, occurred in these 14 patients: These events, which form the basis of this report, are shown in table 1. Serial blood samples were obtained from each patient, with EDTA used as an anticoagulant. The plasma was separated by centrifugation at 400 g for 10 min and stored frozen at -70°C until assayed. Specimens from each subject were assayed in parallel and done in duplicate for the plasma HIV RNA assay and the plasma HIV p24 assay.

Plasma HIV RNA was measured in parallel samples by use of a branched chain signal amplification assay (Quantiplex HIV-RNA assay kit; Chiron, Emeryville, CA). The test has a limit of sensitivity of 10,000 copies/mL. HIV p24 antigen levels were measured by use of an HIV-1 p24 antigen assay with a kinetic standard (Coulter, Hialeah, FL) after dissociation of immune complexes (ICD-Prep kit, Coulter). Results were obtained as colorimetric optical density changes per unit of time and analyzed using computer software (SOFTmax, version 2.01; Molecular Devices, Menlo Park, CA). The amount of p24 antigen was determined from a four-parameter curve of known amounts of purified p24 antigen prepared in normal human serum and diluted with 200 µL of neutral buffer (equal parts of glycine and TRIS buffer) as recommended by the manufacturer.

Results

Plasma samples were obtained prior to diagnosis, during the acute stage of illness, and after recovery from disease for all 15 events. The median interval between the prediagnosis sampling and that done during the acute stage was 2.2 months (range, 1–10). The median interval between the acute-stage sampling and the postdiagnosis sampling was 2.0 months (range, 1–6).

For the most part, the subjects had relatively advanced HIV disease, with a median initial CD4 cell count of 65 cells/µL (table 1). There was no consistent or significant change in CD4 cell count with either the onset or recovery from an AIDS-associated opportunistic disease (data not shown).

Plasma HIV RNA levels before, during, and after AIDS-associated opportunistic disease are shown in table 1. Prior to opportunistic disease, the median level was 21,000 RNA copies/mL. There was increase in the level with the onset of an opportunistic disease during 11 of the 13 events for which HIV RNA was detectable (median level during event, 145,000 copies/mL; P = .002, Wilcoxon signed rank test of values before and during opportunistic disease). The median increase in plasma HIV RNA was 360%. Patients with higher baseline levels tended to have higher absolute increases (r = .37, P = .16) but lower percent increases (r = -.61, P = .03) in plasma HIV RNA.

For 12 of the 13 disease events for which HIV RNA was detectable, there was a decline in the plasma level of RNA after recovery from disease. The median level after the AIDS-associated opportunistic disease events was 29,700 RNA copies/mL (P = .002, Wilcoxon signed rank test of values during and after opportunistic disease). There was no significant change in HIV RNA levels between the onset and recovery phases (P = .3, Wilcoxon signed rank test of values before and after opportunistic disease).

In contrast to plasma HIV RNA levels, there was no consistent or significant change in p24 antigen levels with either the onset or recovery from opportunistic disease (data not shown).
Discussion

Markers of the progression of HIV disease and the therapeutic efficacy of antiretroviral therapy in HIV-infected patients are urgently needed. Plasma HIV RNA levels have recently been shown to be predictive of clinical response to therapy [12], and a clinical trial is underway to determine whether the use of HIV RNA as a marker can contribute to improved clinical care. However, the results of the current study show that caution must be used when interpreting plasma HIV RNA levels.

Among the 13 clinical events with detectable plasma HIV RNA, 8 (62%) were associated with at least a 3-fold rise in viral RNA concentrations during the acute period of illness. A more modest relative increase (1.5—2.5 times the baseline level) was detected in 3 (23%) of the 13 subjects. The plasma HIV RNA levels in 2 patients (15%) were stable or slightly decreased, and in 2 others they were below the detection limit (10,000 RNA copies/mL) of the branch chain DNA assay. In addition, plasma HIV RNA levels returned to or near baseline levels after the opportunistic diseases resolved.

These observations support the concept that the burst of plasma HIV RNA did not directly reflect AIDS progression or therapeutic failure but, rather, was a transient condition resulting from the opportunistic disease, possibly by increasing the numbers of activated CD4 lymphocytes capable of supporting productive HIV infection. This temporary burst of plasma HIV RNA, however, could confuse the clinical interpretation of AIDS progression and of therapeutic failure if changes in plasma HIV RNA virus load are the only markers considered, especially if only one assessment was made or if measurements were made too close together.

Too few measurements were done to establish the height, duration, or kinetics of the plasma HIV viremia burst in this study; however, values for most samples assayed at 1 and 2 months after resolution of disease had returned to baseline. The other markers tested, immune complex—dissociated p24 antigen and CD4 cell count, essentially remained stable during the periods studied. In addition, the sample size in this study was too small to allow any pathogen-specific associations to be made; however, no striking trends were observed.

Since all patients in this study received uninterrupted antiretroviral therapy, it is not possible to assess the impact of their treatment on plasma HIV RNA levels. However, it is worth emphasizing that increased amounts of plasma HIV RNA occurred despite antiretroviral therapy. This could reflect the induction of HIV provirus in latently infected cells that is unaffected by the reverse transcriptase inhibitors. Alternatively, this finding is consistent with the idea that the number of activated cells capable of supporting the productive infection of HIV is an important rate-limiting factor affecting the amount of HIV RNA found in the plasma. Since similar responses in plasma HIV RNA have been observed with other types of stimuli, such as interleukin-2 therapy [13], a common pathway may be involved (e.g., cytokine induction).

Last, the pathogenetic and clinical significances of these observations need further work. It is possible that multiple bursts in HIV production caused by events that stimulate the immune system may enhance HIV disease progression and thereby exacerbate immune decline. If this is true, it is feasible that suppression or avoidance of these bursts could have an important clinical impact on the health of HIV-infected patients and the rate of disease progression. Alternatively, rises in plasma HIV RNA production may justify increased vigilance for an impending opportunistic disease.

References