Can the Level of Immunosuppression in Human Immunodeficiency Virus-Infected Patients Affect the Reliability of Human T-Cell Lymphotropic Virus Type 2 Serological Diagnosis?

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Received 14 July 2005/Returned for modification 20 September 2005/Accepted 21 October 2005

A total of 175 human immunodeficiency virus (HIV)-positive intravenous drug users (IDU) with CD4 cell counts of <200 cells/μl were matched with 175 HIV-positive IDU with CD4 cell counts of >500 cells/μl. Enzyme immunoassay (EIA) reactivity and human T-cell lymphotropic virus type 2 (HTLV-2) Western blot (WB) positivity were more frequently observed in subjects with CD4 cell counts of >500 cells/μl. Most of the subjects with low CD4 cell counts and EIA reactivity carried HTLV-2 infection (WB positive and/or PCR positive). No subjects with low CD4 cell counts and a lack of reactive EIA were PCR positive for HTLV-2. Therefore, a negative EIA result can confidently discharge HTLV-2 infection in HIV-infected patients with severe immunosuppression, whereas PCR should be performed for subjects with a reactive HTLV EIA which is not further confirmed by WB.

As with other retroviruses, the diagnosis of human T-cell lymphotropic virus type 1 (HTLV-1) or type 2 infections is generally made using serological assays that assess the presence of specific HTLV-1/2 antibodies. The recommended algorithm advises to first perform a screening enzyme immunoassay (EIA) to detect both viruses followed by Western blot (WB) testing of reactive samples to confirm infection and discriminate between HTLV-1 and HTLV-2. This approach is often accompanied by a high proportion of indeterminate WB results when low-risk populations, such as blood donors (1, 10), are tested, although subsequent PCR analyses discharge the presence of HTLV-1/2 infection in almost all cases. In contrast, in high-risk populations, such as intravenous drug users (IDU), indeterminate HTLV WB patterns or even nonreactive EIA results may be seen in persons with true HTLV-2 infection (6, 11). Since IDU are often coinfected with human immunodeficiency virus type 1 (HIV-1), it has been suggested that immunosuppression could explain the inability to mount and/or maintain an appropriate level of HTLV-2 antibodies, particularly in advanced stages of HIV disease (6). A similar poor antibody response has been described for hepatitis C virus in HIV-positive individuals, causing “occult” infections (2). Herein, we assess the impact of HIV-related immunosuppression on the performance of current serological tests used for HTLV-2 diagnosis.

A large group of former IDU known to be HIV positive and on regular follow-up at our institution was analyzed. We have previously reported a high prevalence of HTLV-2 infection in this population (8). A total of 175 IDU had severe immunosuppression, with a CD4 cell count below 200 cells/μl. They were matched by age, gender, and place of residence with 175 HIV-positive IDU with a preserved immune status and CD4 cell counts above 500 cells/μl. Serum specimens were tested for antibodies to HTLV-1/2 using a commercial EIA (Murex HTLV I+II; Abbott, Barcelona, Spain). Samples with repeated EIA reactivity were tested using a commercial WB (Bioblot HTLV; Genelabs, Singapore). These WB strips contain HTLV-1 viral lysate and three recombinant envelope proteins: the transmembranous HTLV gp21 (rgp21), the surface gp46 from HTLV-2 (rgp46-II), and the surface gp46 from HTLV-1 (rgp46-I). The HTLV European Research Network criteria (5) were used for interpreting WB patterns. Briefly, HTLV-2 positivity was considered when reactivity to at least two recombinant envelope bands (rgp21 and rgp46-II) and the gag band p24 was seen. HTLV-2 infection was considered negative when no bands appeared in the WB. Other WB patterns were interpreted as HTLV indeterminate.

A specific HTLV-1/2 PCR was performed in all subjects with CD4 counts below 200 cells/μl, as previously described elsewhere (9). Briefly, the HTLV-1/2 PCR was performed using nested primers (12P1, SK111, 12P5, 1P1, and 2P3) directed against the pol region that permits typing of HTLV-1 and HTLV-2. This assay has shown a high specificity, and the sensitivity is approximately 10 copies of HTLV-1 and/or HTLV-2 per PCR. Statistical analyses were performed using the t test for continuous variables and the chi-square test and one-way analysis of variance for categorical variables.

A total of 72 (24%) subjects were EIA reactive, and HTLV-2 infection was confirmed by WB in 52 of the subjects (overall prevalence, 17.3%). No cases of HTLV-1 infection were recognized in this population. All remaining 20 EIA-reactive samples showed indeterminate HTLV WB patterns. Table 1 summarizes the main results in the two study populations. Both HTLV-1/2 EIA reactivity and HTLV-2 WB positivity were more frequently seen in patients with CD4 cell counts above 500 cells/μl, while indeterminate WB patterns were more frequently recognized in subjects with CD4 cell counts below 200 cells/μl than in patients with CD4 counts of >500 cells/μl (36.4% versus 20.5%). However, this difference...
TABLE 1. Mean features of the study population and HTLV serological results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Immunosuppressed group (n = 175)</th>
<th>Immuno competent group (n = 175)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CD4 count (cells/μl)</td>
<td>131.5</td>
<td>771.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean plasma HIV RNA</td>
<td>3.2 log</td>
<td>2.7 log</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of reactive HTLV EIA (%)</td>
<td>33 (18.9)</td>
<td>39 (22.3)</td>
<td>0.428</td>
</tr>
<tr>
<td>No. of positive HTLV-2 WB (%)</td>
<td>21 (63.6)</td>
<td>31 (79.5)</td>
<td>0.137</td>
</tr>
<tr>
<td>No. of indeterminate HTLV WB (%)</td>
<td>12 (36.4)</td>
<td>8 (20.5)</td>
<td>0.137</td>
</tr>
</tbody>
</table>

a Percentage obtained in HTLV-1/2 EIA-reactive samples.
b CD4 count of <200 cells/μl.
c CD4 count of >500 cells/μl.

previous reports that have shown that PCR for HTLV-1/2 is the most sensitive and specific assay (7). Taken together, HTLV-1/2 PCR is strongly recommended for HIV-infected patients with indeterminate HTLV WB results.

In summary, a high prevalence of HTLV-2 infection in IDU was detected in our cohort. Lack of EIA reactivity can exclude HTLV-2 infection confidently even in subjects with HIV-1 advanced immunosuppression. However, HTLV-1/2 WB-positive patterns could change and revert to indeterminate WB in HIV-1-infected subjects with severe immunosuppression.

This work was supported in part by grants from FIPSE (grant no. 3035/99), Fundación IES (Investigación y Educación en Sida), and RIS (Red de Investigación en Sida) (grant no. G03/173).

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