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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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, 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, SK11, 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...
did not reach statistical significance, most likely due to the limited size of the study population.

When WB patterns in the 72 subjects with a reactive HTLV-1/2 EIA were compared, those with more than 500 CD4 cells/µl showed a higher mean number of bands than subjects with CD4 cell counts below 200 cells/µl (4.1 versus 3.1; \( P = 0.006 \)). However, the critical bands used for HTLV-2 diagnosis tended to be present in most instances regardless of CD4 cell count and are as follows: rgp46-II (84.6% versus 75.8%), p24 (87.2% versus 81.8%), and rgp21 (100% versus 97%) in subjects with CD4 cells counts of >500 versus <200 cells/µl, respectively.

These results suggest that although the serological reactivity to HTLV-2 may be lower in subjects with severe HIV-related immunosuppression, it does not substantially affect the reliability of the serological diagnosis of HTLV-2 infection. In fact, we did not find any relationship between the CD4 cell count and the rate of EIA reactivity (\( P = 0.972 \)) or WB positivity (\( P = 0.524 \)) within the group of patients with CD4 cell counts below 200 cells/µl. Since all 175 individuals in this group were tested for HTLV-1/2 by PCR, we could exclude seronegative infections in all 142 subjects lacking EIA reactivity. However, HTLV-2 infection was demonstrated in all 21 subjects with positive WB results as well as in most individuals (10 out 12) with EIA reactivity but indeterminate WB results. These results contrast with prior reports of a relatively high rate of false-negative HTLV-1/2 EIA results in HIV-positive as well as HIV-negative populations (11, 12). However, our data are in agreement with more recent reports in which no cases of "occult" HTLV-1/2 infections have been found in HTLV-1/2 EIA-nonreactive individuals (3, 4, 7).

Although we could not test HTLV-1/2 PCR in immunocompetent subjects with indeterminate HTLV WB results, results obtained in the immunosuppressed group are consistent with 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.

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