False-Positive HIV-1 Test Results in a Low-Risk Screening Setting of Voluntary Blood Donation

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Context.—Persons at risk of human immunodeficiency virus 1 (HIV-1) infection have been classified incorrectly as HIV infected because of Western blot results, but the frequency of false-positive Western blot results is unknown.

Objectives.—To determine the frequency of false-positive HIV-1 Western blot results in US blood donors and to make projections to other screened populations. Secondarily, to validate an algorithm for evaluating possible false-positive cases.

Design.—A retrospective cohort study of HIV-1 enzyme immunoassay (EIA) and Western blot results from large blood donor screening programs in which donors with suspected false-positive Western blot results underwent HIV-1 RNA polymerase chain reaction (PCR) testing and follow-up HIV-1 serology.

Setting.—Five US blood centers participating in the Retrovirus Epidemiology Donor Study.

Participants.—More than 5 million allogeneic and autologous blood donors who successfully donated blood at 1 of the 5 participating centers from 1991 through 1995.

Main Outcome Measures.—Rate of false positivity by Western blot and true HIV-1 infection status as determined by HIV-1 RNA PCR and by serologic follow-up of blood donors more than 5 weeks after donation.

Results.—Of 421 donors who were positive for HIV-1 by Western blot, 39 (9.3%) met the criteria of possible false positivity because they lacked reactivity to p31. Of these, 20 (51.3%) were proven by PCR not to be infected with HIV-1. The false-positive prevalence was 4.8% of Western blot–positive donors and 0.0004% (1 in 251,000) of all donors (95% confidence interval, 1 in 173,000 to 1 in 379,000 donors).

Conclusions.—A false diagnosis of HIV-1 infection can result from the combination of EIA and Western blot testing in blood donor and other HIV-1 screening programs. Individuals with a positive Western blot result lacking the p31 band should be counseled that, although they may be HIV infected, there is uncertainty about this conclusion. These individuals should be further evaluated by RNA PCR testing (if feasible) and HIV serologic analysis on a follow-up sample.

In February 1993, the FDA made a set of recommendations for Western blot interpretation that were adopted by manufacturers of FDA-licensed HIV-1 Western blot kits. These less stringent criteria were adopted to reduce the number of HIV-1 seroindeterminate Western blot interpretations, particularly in high-risk screening settings, in which HIV-1–infected persons presenting during early seroconversion or with late-stage AIDS could be classified as seroindeterminate rather than as HIV-1 seropositive. These new criteria stated that a result would be interpreted as HIV-1 Western blot positive if antibodies existed to 2 of the 3 diagnostic HIV-1 proteins: p24, gp41, and gp120/160. Since adoption of these revised criteria, several studies have identified low-risk individuals who have been falsely classified as HIV-1 infected as a result of positive HIV-1 Western blot results because minimal criteria for interpretation of a Western blot result as positive were not standardized and at least 3 different Western blot interpretation schemes were in widespread use in varied screening and diagnostic settings.4,4 Because of concerns about possible false-positive reactions, the Food and Drug Administration (FDA) criteria used in the blood donor setting for a positive Western blot result interpretation required the detection of bands of 3 specific HIV-1 gene products: a core (Gag) protein (p24), an envelope (Env) glycoprotein (gp41 or gp120/160), and a polymerase protein (p31; endonuclease).4

THE DIAGNOSIS of human immunodeficiency virus 1 (HIV-1) infection is made based on the detection of antibodies to HIV-1. This involves a 2-stage process beginning with a screening enzyme immunoassay (EIA) followed by a supplementary test to confirm the specificity of the EIA result. The supplementary test that is most often used is the HIV-1 Western blot assay. Until the early 1990s, the
METHODS

The Retrovirus Epidemiology Donor Study (REDS) is a study of viral infection in blood donors, conducted by 5 participating blood centers (Irwin Memorial Blood Centers, San Francisco, Calif; Oklahoma Blood Institute, Oklahoma City; and the Chesapeake-Potomac [Baltimore, Md], Southeastern Michigan [Detroit], and Southern California [Los Angeles] Regions of the American Red Cross), a coordinating center (Westat Inc, Rockville, Md), and a central laboratory (SRA Technologies, Rockville). As an element of REDS, anti-HIV EIA and Western blot results, including detailed band pattern data, are forwarded from all blood centers to the coordinating center and are maintained in a centralized database. Institutional review board approval for this aspect of REDS was received from all participating blood centers.

We reviewed the Western blot band patterns of all anti-HIV EIA repeat-reactive Western blot–positive donations lacking the p31 band. Our purpose was to determine the frequency of false-positive HIV-1 Western blots in US blood donors, to validate an algorithm for selecting possible false-positive cases for further evaluation, and to determine those Western blot patterns most predictive of false-positive results.

RESULTS

A review of the 5.02 million donations in the 1991-1995 REDS donation database revealed that 4650 were anti-HIV EIA repeat-reactive and 421 were HIV-1 Western blot positive (0.008% of all donations; 0.06% of EIA repeat reactive) using the 1993 FDA interpretive criteria. Thirty-nine (9.3%) of the Western blots with positive results lacked the p31 band. We calculated 95% confidence intervals (CIs) around prevalence estimates using a binomial distribution. Statistical testing was 2-tailed.

Donations collected at Irwin Memorial Blood Centers from January 1991 through February 1992 were excluded from analysis because a different confirmatory test was used. For purposes of this analysis, we applied the 1993 revision of Western blot interpretive criteria to all donations in the 5 years of this study. We selected those positive Western blot results that completely lacked p31 reactivity (ie, absence of even +/- reactivity) for further investigation and used 2 separate methods to assess whether HIV infection was present. The first method was to review the results of any follow-up anti-HIV testing (EIA and Western blot) obtained as part of routine donor notification and counseling procedures. A nonseroconverting follow-up serology was defined as either negative anti-HIV EIA results or the lack of development of a p31 band on Western blot to conform with the pre-1993 positive interpretive criteria (p24, p31, gp41, or gp120/160) on a sample collected more than 5 weeks after the index donation. The second method was to test a frozen serum aliquot of the initial Western blot–positive donation for HIV-1 RNA by PCR assay at our central laboratory. In 2 cases, testing of follow-up rather than index donations was performed because of lack of sample availability. For PCR testing, we used the FDA-licensed Roche Amplicor HIV RNA assay (Roche Molecular Systems, Somerville, NJ) modified to yield qualitative rather than quantitative results. The modification used only the undiluted PCR-amplified product for probe hybridization and detection. Each assay included the kit internal standard spiked into each test sample prior to RNA extraction to control for RNA recovery, amplification efficiency, and assay inhibitors. To control for possible errors in PCR testing, we tested a second aliquot from the same donation (or from a subsequent donation from the same donor) in an independent PCR run for those donations in which there was either no follow-up sample for serologic testing or initial PCR results and follow-up serology suggested a conflicting interpretation of HIV infection status.

The sensitivity and specificity of our modified PCR assay were evaluated on a panel of 100 frozen blood donor samples consisting of 50 anti-HIV EIA-negative samples and 50 anti-HIV-1 Western blot–positive samples with p31 reactivity. Using this sample set, the specificity of this assay was 100% and the sensitivity was 97.4%. To calculate assay sensitivity by comparing the performance of our modified assay with that of an FDA-licensed RNA PCR assay on 30 masked samples from 6 commercially prepared HIV-1 seroconversion panels. The modified PCR assay showed equivalent sensitivity to that reported by the panel manufacturer for the unmodified FDA-licensed assay. Results were identical in 29 of 30 samples; the result for the remaining sample, collected prior to HIV-1 antibody seroconversion, was indeterminate on the modified assay and negative on the licensed assay.

Table 1.—Classification of 39 Human Immunodeficiency Virus 1 Western Blot–Positive Samples Lacking p31 Reactivity.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Follow-up Serology*</th>
<th>RNA PCR Result†</th>
<th>No. of Samples</th>
</tr>
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<tbody>
<tr>
<td>False positive</td>
<td>Non-Sc‡</td>
<td>Negative§</td>
<td>15</td>
</tr>
<tr>
<td>True positive</td>
<td>Sc</td>
<td>Positive</td>
<td>7</td>
</tr>
</tbody>
</table>

*Non-Sc indicates negative enzyme immunoassay or lack of Western blot p31 reactivity at more than 5 weeks’ follow-up; Sc indicates development of Western blot p31 reactivity (+/- other bands).
†RNA polymerase chain reaction (PCR) was performed on a frozen serum aliquot from index donation (or follow-up sample if index donation was unavaiable). Two donor developed a p17 band but no p31 band on 14-week follow-up.
‡One donor developed a p17 band but no p31 band on 5-week follow-up.
§Two donors’ results for PCR assay were initially positive but were negative on retest.
**One donor had a negative serology at 3 weeks but no sample at more than 5 weeks.

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for 15 donors were identified as false positive based on a negative PCR assay in association with absence of progressive seroconversion on follow-up serology; in 5, the follow-up sample showed negative EIA results, and in 10, the Western blot showed absence of p31 reactivity. When compared with the index Western blot, the follow-up Western blot showed loss of reactivity to at least 1 HIV-1 antigen in 7 cases and no change in 2 cases. In a donor with an initial Western blot band pattern of gp41 plus gp120/160, an additional p17 band was reported in a 14-week follow-up sample, but no p31 band or additional bands were detectable on follow-up and the PCR result was negative. Two of these 15 cases were initially considered to have positive PCR results; however, the discrepancy with follow-up serology prompted repeat PCR testing. In 1 case, the PCR result was positive on repeat testing of the index donation but negative for the follow-up donation; in the second case, the PCR result was negative on repeat testing of the index donation and for the follow-up donation. Sample mix-up was excluded by performing HLA class II DR typing for 19 alleles using PCR on initial and follow-up repository samples in both cases; these results established donor identity in each case.

Results for 19 donations were classified as HIV-1 Western blot true positive (ie, donor infected with HIV-1); 7 based on a positive PCR result alone and 12 based on a positive PCR result and positive follow-up serology. In 7 of the 12 donors with follow-up serology, samples were available at an interval of 2 to 4 weeks after index donation; 6 of these 7 early follow-up samples showed p31 reactivity.

Table 2 relates the false-positive or true-positive Western blot classification of the 39 donors to the Western blot band pattern seen with the index donation. In addition to reactivity to Env antigens (gp41, gp120/160), reactivity to a maximum of 5 additional viral bands was possible (p17, p24, p51, p55, and p66) at the time of index donation. Results for the 5 samples with index donation patterns of Env bands only were all classified as Western blot false positive. Results for 13 of 15 samples with Env plus 1 additional viral band (9 with p24 reactivity, 3 with p66 reactivity, and 1 with p17 reactivity) were also false positive. In contrast, there were 2 false-positive results among 7 samples that contained Env plus 2 additional viral bands and no false-positive results in 2 samples that contained 3 additional bands or among the 10 samples that contained 4 or 5 additional bands.

The rate of false-positive Western blot results documented in this study was 0.00041% of all donations tested (95% CI, 0.00026%-0.00058%) and 4.8% of donations with results classified as Western blot positive. Prevalence was 5.5 times higher in donations from first-time allogeneic donors (8/836,382 [0.00096%]) than in donations from repeat allogeneic donors (11/3,983,824 [0.00028%]). The prevalence rate of false-positive Western blot results in first-time vs repeat donations was 3.46 (95% CI, 1.39-8.61). Of the 19 HIV-1 false-positive classifications in allogeneic donors, 6 occurred in donations originally screened with the Abbott anti–HIV-1 EIA (prior to March 1992) and 13 in donations screened with the Abbott anti–HIV-1/HIV-2 assay. The rate of false-positive Western blot results using the anti–HIV-1 screening assay (0.00057%) was not statistically different from that using the anti–HIV-1/HIV-2 assay (0.00034%). The prevalence rate of screened anti–HIV-1 vs anti–HIV-1/HIV-2 Western blot false-positive donations was 1.65 (95% CI, 0.63-4.35).

The results for 5 of 6 possible false-positive donations from autologous donors were found to be true positive (ie, donors were HIV infected). In 2 of these cases, the positive autologous donation was preceded by an autologous unit given 1 week previously. In 1 case, the autologous donor had given a previous donation that tested HIV EIA negative and in the second case, the preceding autologous unit was HIV-1 EIA repeat reactive and Western blot indeterminate. The predictive value of a positive Western blot pattern lacking p31 for predicting HIV infection was 83% in autologous donors (95% CI, 42%-99%) but only 42% in allogeneic donors (95% CI, 28%-58%; 14 of 33 were HIV infected). These results were not statistically different (P = 0.9) by the Fisher 2-tailed exact test.

Incidence of HIV infection in other medical or public health HIV screening settings would be expected to be higher than that in allogeneic blood donors. Thus, the frequency of HIV infection presenting with a positive Western blot result lacking p31 should also be higher in such settings. Our data illustrate this by documenting a 9-fold higher frequency of HIV infection presenting with a Western blot lacking p31 in autologous donors (who are not subjected to predonation HIV risk factor screening) than in allogeneic donors (who are questioned about HIV risk factors) (0.00253% vs 0.00029%, respectively). Our data also show a difference in the positive predictive value (PPV) of a positive Western blot result lacking p31 reactivity for predicting HIV infection in these 2 donor populations (83% in autologous donors vs 42% in allogeneic donors); this difference is clinically relevant, although it does not achieve statistical significance, probably because of the small sample size of only 6 Western blot–positive autologous donors.

Table 3 illustrates how the PPV of Western blot patterns lacking p31 can be estimated if incidence of new HIV infection is known. In the table, we assume that the rate of false-positive results, which is a consequence of assay performance characteristics, will be similar in first-time blood donors and in higher-risk populations. An illustrative example from Table 3 shows that if incidence of HIV-1 infection in a particular screening setting is greater than 1% (as in anonymous testing clinics in San Francisco or in gay men presenting to sexually transmitted disease clinics), the PPV of a positive Western blot result lacking p31 will be greater than 99%. It has recently been estimated that the incidence of HIV infection in heterosexuals in the United States is 0.5%, which would result in a PPV of 98% in this population. A documented HIV infection incidence of less than 0.1% has been reported in heterosexuals presenting to sexually transmitted disease clinics in some communities; in these settings, the PPV drops to less than 91%. The PPV of 48.7% shown in Table 3 for a high-risk population is close to the 42% seen in our study.

Information on HIV risk factors was obtained during the course of postdonation counseling from some of the donors.
Nine of the 14 HIV-infected donors for whom risk factor information was available reported probable or definite HIV exposure. One of 7 donors with false-positive results for whom risk factor information was available reported HIV infection risk behavior. Two donors had received an experimental HIV vaccine as part of a research protocol and showed a pattern of reactivity to HIV glycoprotein antigens consistent with an antibody response to an Env subunit vaccine.

COMMENT

Individuals with new HIV-1 infection may donate blood or present at a screening clinic at a time when their antibody response to HIV-1 is in the early stages and is limited to Env-only or Env plus p24 reactivity on Western blot. It has been well established that persons undergoing HIV-1 seroconversion will rapidly develop reactivity to most, if not all, of the 9 HIV-1 antigens present on the Western blot strip. In this study, in a few cases for which early follow-up samples were available, we found that p31 reactivity could be seen as early as 2 weeks following results showing a Gag plus Env pattern. In a larger study, it has been estimated that the mean interval from detectability of a positive Western blot result by current FDA criteria to detectability using previous FDA criteria (ie, presence of the p31 band, which is usually the most delayed finding) is 35 days (range, 23-47 days). During HIV-1 seroconversion, high serum or plasma levels of HIV-1 RNA are present and the PCR assay used in this study has previously been shown to be sensitive enough to detect HIV-1 RNA when p31 reactivity is absent on Western blot.

We used a combination of these 2 proven methods (follow-up serology and RNA PCR) to establish HIV infection status in 39 blood donors with possible false-positive Western blot results. All 27 donors who were assessed by evolution of Western blot patterns and by RNA PCR had concordant results (after PCR was repeated in 2 donors), strengthening our confidence in their classification. To ensure accurate classification of donors who lacked follow-up serologic data, PCR testing was carried out in 2 independent runs on 2 separate frozen aliquots in 8 of 11 such cases for which sufficient samples were available. In all 8 cases, the results were concordant with those of the original PCR assay. The initially positive PCR findings in 2 donors determined to be HIV uninfected on follow-up are most likely explained by contamination of the frozen aliquot subsequent to serologic testing but prior to PCR testing in one case and by a false-positive PCR assay result due to the presence of other PCR-positive samples in the same run in the second case.

The same Western blot patterns that are detectable in early seroconversion can also be seen in persons with false-positive results due to cross-reactivity with an epitope on gp41, which can give rise to multiple Western blot Env bands and a positive Western blot interpretation. Cross-reactivity to Env glycoprotein may be accompanied by a second nonspecific cross-reaction to p24 or other HIV proteins. Although most false-positive findings in our study are attributable to this mechanism, we also identified 2 donors in whom Western blot reactivity to HIV Env glycoproteins was probably a result of a true biological antibody response to receipt of an experimental Env subunit HIV vaccine. This mechanism of HIV false positivity may become increasingly significant if experimental HIV vaccination programs become more widespread. If HIV vaccination programs become routine, it will be important to develop HIV donor screening strategies that discriminate between HIV-infected persons and HIV-vaccinated, uninfected persons. Until such assay development occurs, it is important that HIV vaccine recipients be advised not to donate blood. In this study, 20 (4.8%) of 421 EIA repeat-reactive, Western blot–positive donations occurred in donors who were not infected with HIV-1. Infection with other HIV variants (eg, HIV-2 and HIV-1 group O) is extremely rare in the United States and is highly unlikely in these donors, since none were born in African countries in which these variants are endemic. Also, the routine blood bank confirmatory testing algorithm in place since March 1992 has ruled out HIV-2 infection in the 14 donors with false-positive results detected by the anti–HIV-1/HIV-2 EIA (data not shown). Twenty (5.3%) of 39 donors and 19 (57.6%) of 33 allogeneic donors with positive Western blot results lacking p31 reactivity were not infected with HIV. When these 20 false-positive results were divided by the more than 5 million donations in our database, we calculated that 0.0004% or 1 in 251,000 donations were classified as HIV-1 infected based on false-positive Western blot results (95% CI, 1 in 175,000 to 1 in 579,000). When the prevalence of false positivity was calculated for first-time donors (who, to our knowledge, were not previously screened for HIV antibody), the rate increased to 0.00096% (1 in 104,547; 95% CI, 1 in 58,000 to 1 in 210,000). When multiplied by the 12 to 14 million annual whole blood donations in the United States, our data suggest that from 48 to 56 blood donors annually are misclassified as HIV-1 infected based on a combination of false-positive EIA and Western blot results.

In this study, all 5 donors who showed reactivity only to Env were not infected with HIV-1. However, a pattern of Env plus 1 other viral band reactivity was more frequently associated with false-positive results than was reactivity to...

Table 3.—Projected PPV of a Positive HIV-1 Western Blot Result Lacking p31 as a Function of HIV Incidence

<table>
<thead>
<tr>
<th>Incidence of HIV Infection, %</th>
<th>Positive HIV-1 Western Blot Result Lacking p31, Rate per Million</th>
<th>Expected Rate of False Positivity (HIV Uninfected)†</th>
<th>PPV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>1438</td>
<td>9.6</td>
<td>99.3</td>
</tr>
<tr>
<td>0.1%</td>
<td>96</td>
<td>9.6</td>
<td>90.9</td>
</tr>
<tr>
<td>0.0%</td>
<td>9.6</td>
<td>9.6</td>
<td>50.0</td>
</tr>
<tr>
<td>0.004**</td>
<td>3.8</td>
<td>4.0</td>
<td>48.7</td>
</tr>
<tr>
<td>0.0001†</td>
<td>0.96</td>
<td>9.6</td>
<td>9.1</td>
</tr>
</tbody>
</table>

*PPV indicates positive predictive value (number of true-positive results /[number of false-positive results + true-positive results] × 100); HIV, human immunodeficiency virus.
†The rate of true-positive Western blot results lacking p31 in a screening setting has been calculated by multiplying the annual incidence of infection in that setting by 9.589% (the estimated 35-day interval in which a positively interpreted Western blot result would lack p31 divided by 365 days); this assumes that HIV incident cases are as likely to present for testing in the immediate postseroconversion phase as they are thereafter.‡The rate of false-positive Western blot results lacking p31 was measured in this study as 9.6 per million in the previously unscreened (first-time) allogeneic donor population. This rate is assumed to be representative for other cross-sectional screening settings.
§Data correspond to incidence in San Francisco, Calif, anonymous test sites (1.3%) and estimated national incidence in injection drug users and is less than the incidence in men with male sexual partners presenting to sexually transmitted disease clinics.©1998 American Medical Association. All rights reserved.
HIV infection incidence.


