Interpretation of HIV Serologic Testing Results

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CASE

A 33-year-old male patient visited the outpatient clinic at Brigham and Women's Hospital for a routine follow-up for obesity, obstructive sleep apnea, allergic rhinitis, and depression. He was maintained on a nocturnal continuous positive airway pressure device, loratadine, duloxetine, and fluticasone nasal spray. He was a resident of Boston and had not traveled outside the country. He denied intravenous drug use or high-risk sexual behavior, and he had not received any blood products. He had received his most recent influenza vaccine about 6 months earlier. He was screened for type 2 diabetes and hyperlipidemia. As a part of routine clinical care, he was also offered HIV screening in accordance with the current CDC recommendations (1).

The HIV assay [HIV 1/O/2 Enhanced (EHIV)], which was performed on the ADVIA Centaur analyzer (Siemens Healthcare Diagnostics), yielded a reactive result. As per the assay protocol developed by the manufacturer, the initially reactive sample was retested in duplicate after centrifugation; both results were reactive. The positive screen was followed up with a confirmatory western blot (WB) analysis, which yielded an indeterminate result. The presence of an isolated p24 band in the WB (GS Western HIV-1; Bio-Rad Laboratories) was of concern regarding possible early HIV seroconversion.

DISCUSSION

The Siemens EHIV screen performed in this case is a double antigen-bridging microparticle chemiluminescent immunoassay that detects antibodies against p24, gp41, gp120 (from HIV-1), gp36 (from HIV-2), and a synthetic peptide from group O HIV-1 (Fig. 1). A positive result indicates the presence of antibodies that recognize any of these antigens, regardless of their isotype or subclass. Although such third-generation HIV immunoassays have greatly improved analytical sensitivity and specificity, false-positive results have not been eliminated completely. A common cause of false-positive serologic screens for HIV is recent influenza vaccination or an incidental viral infection (2, 3). In addition to flu vaccination and viral infections, false-positive HIV-1 immunoassay results have been reported in a variety of other conditions, such as autoimmune disease, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parental substance abuse, hemodialysis, and vaccinations against hepatitis B and rabies (4). Thus, a positive result in an HIV screening test must be followed up with a more specific confirmatory test.

WB is routinely used to confirm a reactive HIV serologic screening result. These assays, which contain either viral lysate or recombinant HIV proteins, allow the determination of the antigenic specificity of the antibodies in the patient’s serum. The predominant type of HIV in the US is HIV-1. A confirmatory test for HIV-1 infection was recommended because this patient had not traveled to any part of the world with a high prevalence of HIV-2, such as West Africa. The major antibody specificities detected in HIV-1 WB analysis include gp160, gp120, p65, p55, gp41, p40, p31, and p24. To be reported as positive, the WB assay requires reactivity against the gp41 and gp120/160 bands encoded by the env (gp160, envelope glycoprotein) gene or against either one of these env bands plus the p24 band encoded by gag [Pr55(Gag)]. Such a result is highly specific for the presence of HIV infection (5). A negative result implies the absence of any of the above bands. The result is called indeterminate when the band profile does not meet the criteria for a positive

QUESTIONS TO CONSIDER

1. What factors are known to cause false-positive HIV serologic test results?
2. What factors are known to cause an indeterminate WB result?
3. What further testing or clinical history would be of help in evaluating a patient with an indeterminate WB result?

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Nonstandard abbreviations: WB, western blot; NAT, nucleic acid testing.

4 Genes: env, gp160, envelope glycoprotein [HIV-1 gene]; gag, Pr55(Gag) [HIV-1 gene].
result. The patient’s WB yielded an indeterminate result. In this case, the result was reported as indeterminate because a sharp p24 band and a weak p40 band were observed (Fig. 1).

After exposure to HIV, it usually takes an individual at least 3 weeks to build up an antibody titer sufficient to be detectable by a third-generation HIV immunoassay. This period is called the “seroconversion window.” Because antibodies to p24 develop early during the course of infection, an indeterminate WB pattern seen during this window is often associated with an isolated p24 band (6). Qualitative reverse-transcription PCR analysis for HIV is used to screen for or confirm the presence of HIV infection during the seroconversion window, and the screen can become positive as early as 10 days after exposure (7). Nucleic acid testing (NAT) for HIV is also used when a rare HIV genotype is suspected; such testing plays a critical role in neonatal HIV screening, owing to interference from maternal antibodies. Besides early seroconversion, other causes of indeterminate HIV-1 WB results in the setting of HIV infection include infection with HIV-2 and advanced AIDS (6). An indeterminate WB result can also arise from antibodies that are cross-reactive to HIV antigens, such as those associated with HTLV infection; with vaccination against influenza, hepatitis, or rabies; or with animal handlers exposed to unusual viruses. Nonspecific antibody binding to nonviral cellular components in the WB HIV antigen preparation can also produce an indeterminate WB result. Such results may be associated with frequent transfusions, injection drug use, liver disease, multiple pregnancies, rheumatoid factor, lymphoma, multiple sclerosis, various autoimmune disorders, a positive result in the rapid plasma reagin test, and chronic hemodialysis (6).

The patient was contacted for follow-up of his HIV test results and possible NAT. Upon further questioning, however, he recalled that he had received an experimental HIV vaccine >5 years earlier. HIV vaccines may include either gag- or env-encoded proteins or both. Vaccines designed to induce cell-mediated immunity can also elicit a humoral response and produce vaccine-induced seropositivity. A majority of gag vac-
Vaccine recipients have p24, p40, and/or p55 bands in their WB (8). env vaccine recipients can have gp41, gp120, and gp160 bands. Such WB bands are often reported as indeterminate, but some HIV vaccine recipients can meet the criteria for a positive HIV WB result. These patients can pose a true diagnostic challenge.

The results of HIV testing of vaccine recipients can be easily misinterpreted and can have a negative social impact (9). Because of the blinding procedures of many vaccine trial designs, neither the patients nor the researchers may know whether a placebo or an experimental vaccine was administered. Vaccine-induced seropositivity can potentially lead to unblinding of the study participants as well as researchers, with a risk of compromising the study data. Therefore, HIV testing of vaccine trial participants is usually performed in designated laboratories with appropriate anonymization protocols that can provide interpretation of results without the risk of unblinding. The results of HIV testing in vaccine recipients need to be confirmed with NAT.

Vaccine trial participants are counseled to undergo HIV testing exclusively with the vaccine research group. Follow-up periods in such trials extend for decades, however, and patients may not recall all of the details. Therefore, the National Institute of Allergy and Infectious Diseases has provided participants in NIH-supported HIV vaccine trials with both a toll-free number for assistance and identification cards that document study participation (9). A large number of experimental HIV vaccine trials have been undertaken over the last 2 decades, and there is a steadily increasing number of HIV vaccine recipients who present for HIV screening. This trend is likely to continue, especially considering the encouraging results of the recent HIV vaccine trial in Thailand (10). Misinterpretation of the results of off-site HIV tests in vaccine trial volunteers may best be avoided through better communication between HIV vaccine researchers and local providers of diagnostic tests.

The decision for confirmatory NAT was deferred, and the vaccine research group was notified for appropriate interpretation, follow-up, and counseling regarding the patient’s HIV screening result, in accordance with the study protocol. This procedure ensured that both the patient and the researchers remained blinded to whether the patient received a placebo or a test dose of the experimental vaccine.

**Points to Remember**

- False-positive HIV serologic screens can be caused by recent influenza vaccination, incidental viral infections, autoimmune disease, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parenteral substance abuse, hemodialysis, or vaccinations against hepatitis B and rabies.

- An indeterminate WB result can be caused by a weak titer of anti–HIV-1 antibodies (as seen in early seroconversion), advanced AIDS, infection with an unusual HIV type, or recipients of experimental HIV vaccines. It can also be caused by the presence of antibodies cross-reactive against HIV antigens ( incidental viral infection; vaccination against influenza, hepatitis, or rabies; or HTLV infection) or reactivity to the nonviral components of the WB (various autoimmune disorders, multiple pregnancies, and recipients of multiple blood transfusions).

- An indeterminate WB result should be followed up with qualitative NAT if early seroconversion is suspected, with a repeat immunoassay and WB analysis performed in 2–4 weeks. Although the US Food and Drug Administration has not cleared the use of quantitative viral load for HIV diagnosis, the viral load is unlikely to be <5000 copies/mL during acute HIV infection. Persistent reactivity of the antibody screening assay with a simultaneous lack of any change in the WB pattern suggests the absence of HIV infection.

- An increasing number of recipients of experimental HIV vaccines, which can cause false-positive results in HIV serologic tests, are being offered HIV screening. Whenever possible, testing for HIV in such patients is best performed in consultation with the vaccine research group responsible for the trial. This procedure will ensure proper interpretation of test results without compromising the study data.

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This case report adds HIV vaccination to the list of well-known causes of false-positive results in HIV antibody-screening tests and illustrates the problems often associated with the interpretation of WBs. Timely and effective means of confirming HIV screening tests have become increasingly important as more centers integrate HIV screening into routine clinical care as recommended by the CDC.

In many laboratory settings, NATs for HIV-1 RNA are more widely available and less costly than WB and are not subject to indeterminate results. Although quantitative HIV-1 NATs have not been cleared by the US Food and Drug Administration (FDA) for diagnosis, they have been used for years for evaluating patients thought to be acutely infected. These patients typically have high viral loads ranging from $10^5$ to $10^6$ copies/mL, and the results present no problems with interpretation. The reports of false-positive results in viral load tests all occurred with a single method (Versant bDNA; Siemens Healthcare Diagnostics): results were <$10^4$ copies/mL.

The APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe), currently the only NAT that has been FDA-cleared for diagnosis of infection, can be used to diagnose neonatal and acute infections, confirm positive results in antibody-screening tests, and resolve indeterminate WB results. As the authors point out, a NAT was the next step in the diagnostic work-up, but it was deferred when the vaccination history was obtained.

The rare individuals who are infected with HIV-1 but who progress to AIDS either very slowly or not at all pose another diagnostic dilemma. In these long-term nonprogressors, HIV-1 antibody is easily demonstrated, but these individuals show low or undetectable HIV-1 RNA loads in the assays available to clinical laboratories. Viremic controllers have low but readily measurable virus loads. Elite controllers suppress HIV to extremely low concentrations, which are measurable only with the most analytically sensitive laboratory techniques.