

Report of a False-Positive HIV Test Result and the Potential Use of Additional Tests in Establishing HIV Serostatus

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Considering the lifelong implications of a positive human immunodeficiency virus (HIV) test result, physicians should be aware of the limitations of tests for HIV. A 43-year-old man had a reactive enzyme-linked immunosorbent assay and an indeterminate result on Western blot analysis. The results of subsequent enzyme-linked immunosorbent assay and Western blot tests were interpreted as positive, and the patient was informed that he had HIV infection. Persistently undetectable plasma HIV-1 RNA, combined with normal physical examination findings, CD4⁺ cell count, and CD4/CD8 ratio, prompted further testing, which revealed that the patient was not infected with HIV. False-positive HIV test results are uncommon, but they can occur. In the appropriate clinical setting, follow-up and the use of other laboratory tests, such as determination of plasma viral load, may help identify such cases. *Arch Intern Med.* 2000;160:2386-2388

Frequent testing for human immunodeficiency virus (HIV) infection in high-risk populations is encouraged both so that effective therapy can be offered and so that efforts can be made to prevent transmission. Considering the lifelong implications of a positive HIV test result,¹ physicians should be aware of the limitations of tests for HIV. The aim of our report is to describe a case of a probable false-positive HIV test result and discuss how, in the appropriate clinical setting, prolonged follow-up, along with the use of other laboratory tests, such as determination of plasma viral load (PVL), may help identify such cases.

(WB) analysis were indeterminate, with reactivity at the p17 region. The patient was referred for evaluation. He reported a possible previous exposure to HIV-1 when a condom broke during a sexual encounter with a woman of unknown HIV status 6 weeks before the indeterminate WB result. He denied any recent symptoms or any other predisposing risk factor for HIV. He had never received a hepatitis B or influenza vaccination, and he did not have renal failure, systemic lupus erythematosus, positive rheumatoid factor, cystic fibrosis, hepatitis, or a sexually transmitted disease. The results of his physical examination were unremarkable, and his absolute CD4⁺ cell count and CD4/CD8 ratio were in the normal range. Subsequent HIV testing revealed a reactive ELISA (HIVAB HIV-1 WIA; Abbott Laboratories, Abbott Park, Ill), while the results of WB (HIV-1 Western Blot Kit; Cambridge Biotech Corp, Rockville, Md) were positive, with bands at gp160 and gp41.

The patient was informed that he likely had HIV infection. A concurrent sample of plasma was sent for HIV RNA analysis (using the Quantiplex b-DNA assay [detection limit 400 copies per cubic millimeter]; Chiron Corp, Emeryville, Calif), with an undetectable result. Because HIV-1 RNA was undetectable, the

REPORT OF A CASE

A 43-year-old man underwent his first serological assay for HIV-1 as part of a routine testing program implemented on incarcerated individuals. The enzyme-linked immunosorbent assay (ELISA) was reactive, and the results of Western blot

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ELISA, WB, and PVL were performed again. The ELISA was weakly reactive and the result was reported as positive, the WB result was positive, with the same (bands at gp160 and gp41) pattern, while plasma HIV-1 RNA was again undetectable. Further tests performed at the National Center for Infectious Diseases, Centers for Diseases Control and Prevention, Atlanta, Ga (Diagnostic Serology Section, HIV Laboratory Investigations Branch), were negative for HIV-1 and HIV-2 antibodies using synthetic peptide dot blots and negative for HIV-1 subtypes using synthetic peptide ELISAs (A through F and O). A serum sample was also negative for human T-cell leukemia and lymphoma virus I/II and syphilis. One month after his first HIV-1 test, the patient was diagnosed as having type 2 diabetes mellitus. Over the following 30 months, a sample of his plasma was sent for PVL determination twice more; both times, the PVL was again undetectable at a sensitivity of 400 copies per cubic millimeter. Antibody studies were again performed 24 and 26 months after the patient's initial presentation. His ELISA result was positive and his WB result was indeterminate (Novapath; Bio Rad Diagnostic Group, Hercules, Calif), with reactivity to the p17 matrix this time. A sample of his peripheral blood lymphocytes was lectin stimulated and cultivated in an interleukin 2-containing medium after the potentially inhibitory CD8⁺ cells had been deleted, and the culture supernatants were monitored over a 2-week period (days 4, 7, 11, 14, and 18) for both HIV-1 p24 antigen (which would detect HIV-1 replication) and the reverse transcriptase enzyme (which would detect any acquired immunodeficiency syndrome virus replication). No p24 antigen or reverse transcriptase activity was detected.² He remains healthy, on an oral regimen for his diabetes, and has been told that he is not infected with HIV.

COMMENT

The patient underwent a routine screening implemented on incarcerated individuals and was referred to

our HIV clinic owing to an indeterminate HIV WB test result. In patients with an indeterminate HIV WB result in this setting, the likelihood of subsequent seroconversion is 74%.³ The results of his second HIV test were reported as positive based on Centers for Disease Control and Prevention and Association of State and Territorial Public Health Laboratory Directors guidelines,^{4,5} and the patient was incorrectly informed that he had HIV infection. His normal physical examination findings, combined with an undetectable PVL, a normal CD4⁺ cell count and percentage, and a normal CD4/CD8 ratio prompted further evaluation with more than 30 months of follow-up that ultimately revealed that he was not infected with HIV. In retrospect, the weakly reactive ELISA and the minimal number of bands on WB might have been clues to the false-positive nature of the result.

A recent retrospective cohort study evaluated the ELISA and WB results from more than 5 million allogeneic and autologous blood donors. More than 50% of the blood donors with positive WB results lacking p31 reactivity who were enrolled in that study were not infected with HIV, while all donors who showed reactivity only to *env*, as was the case in our patient, were not infected with HIV-1. The study concluded that individuals with a positive WB result lacking the p31 band should be told that although they may be HIV infected, there is uncertainty about this conclusion and that they should be further evaluated by PVL testing and HIV serological analysis on a follow-up sample.⁶

Our patient did not have a condition associated with false-positive HIV-1 ELISA results, such as autoimmune disease, renal failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver diseases, parenteral substance abuse, hemodialysis, or vaccinations for hepatitis B, rabies, or influenza.^{7,8}

The WB test detects antibodies to specific denatured HIV-1 proteins, such as core (p17, p24, and p55), polymerase (p31, p51, and p66), and *env* (gp41, gp120, and gp160).⁹ The absence of all bands is considered a negative test result. A

WB test is positive if reactivity is detected to gp41 and gp120/160 *env* bands or to either of these *env* bands plus the p24 *gag* band.^{4,5} Causes of indeterminate WB results include testing during the window period, infection with HIV-2, loss of core antibodies late in HIV-1 infection, and nonspecific antibody reactions (eg, due to lymphoma, multiple sclerosis, injection drug use, liver disease, or autoimmune disorders).^{7,8} Also, there appear to be healthy individuals with antibodies that cross-react with specific HIV-1 peptides or recombinant antigens.¹⁰ From other parts of the world, there have been reports of different subtypes or HIV-related strains that are not as readily detectable by assays based primarily on the HIV-1 subtype B, which is found in the United States.¹¹

The patient described herein is noteworthy because he belongs to a group with a high pretest probability for HIV-1 infection and did not have conditions associated with false-positive serological test results, but who nonetheless was found to have a false-positive test result. As seen in this case, even individuals with a high pretest likelihood of HIV infection who do not belong to one of the established groups with a high incidence of false-positive ELISA results and indeterminate or false-positive results on WB can still have a false-positive HIV test result when the current standard diagnostic protocol is used. Lack of detectable viral RNA in plasma samples, along with normal physical examination findings, CD4⁺ cell count, and CD4/CD8 ratio, can help physicians identify such cases. These laboratory tests are standard in cases of newly diagnosed HIV infection. However, clinicians should exercise caution when using HIV-1 RNA assays to detect HIV infection. Plasma viral load assays are designed to monitor the effectiveness of antiretroviral therapies and to quantitatively measure the rate of viral replication in patients who present with confirmed HIV infection. A previous report described 3 patients with false-positive HIV-1 RNA results, including a 20-year-old woman who had PVL testing performed to further analyze an indeterminate result on HIV-1 WB as-

say.¹² In the patient described herein, however, PVL testing made the physician suspicious of a false-positive result and led to further workup.

In conclusion, false-positive HIV-1 test results on both ELISA and WB are extremely rare, but they do occur. The Association of Public Health Laboratories now recommends that patients who have minimal positive results on WB, eg, p24 and gp160 only, or gp41 and gp160 only, be told that these patterns have been seen in persons who are not infected with HIV and that follow-up testing is required to determine actual infective status. The clinician must judge the test results within the context of other epidemiological and clinical information. In the appropriate clinical setting, positive ELISA and WB test results in patients with a normal CD4⁺ count and CD4/CD8 ratio and undetectable HIV-1 RNA should be questioned, repeated, or confirmed with supplemented testing. A false-positive serological test result may be supported by normal CD4⁺ count and CD4/CD8 ratio and undetectable HIV-1 RNA, but is ultimately established by subsequent serological testing and, especially, close follow-up.

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