



Case report

Falsely positive fourth generation ADVIA Centaur® HIV Antigen/Antibody Combo assay in the presence of autoimmune hepatitis type I (AIH)



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ABSTRACT

A 51-year-old woman was admitted to the hospital with abdominal pain, jaundice, and transaminitis. The patient's laboratory results showed elevated liver enzymes, high antinuclear antibodies (ANA) titer, positive anti-smooth muscle antibody, and hypergammaglobulinemia. Given risk factors for HIV infection, an ADVIA Centaur® HIV Antigen/Antibody Combo assay was performed showing a reactive sample with a follow up HIV-1 nucleic acid test (NAT) proving to be negative. Following confirmation of autoimmune hepatitis type I via a liver biopsy, steroids were initiated and significant clinical improvement of symptoms as well as resolution in transaminitis were noted. Autoimmunity is the most likely causative factor in inducing a false positive reactive screening assay. It is important to recognize that cross-reactivity with autoimmune conditions and HIV specific proteins is a potential concern for false reactive samples.

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Introduction

According to the World Health Organization (WHO), 38 million people are living with human immunodeficiency virus (HIV) and the virus continues to pose a high burden worldwide in regard to testing and treatment [1]. Novel fourth generation HIV screening and confirmatory assays have played a pivotal role in early detection of HIV infection by combining both antigen and antibody screening, allowing for earlier detection of even very low titer anti-HIV antibodies during the seroconversion window [2]. Manufacturers have diligently worked to improve the overall sensitivity and specificity with goals to reduce detection and diagnostic delay as well as reduction in the amount of retesting performed. However, with these improvements there are recent documented studies showing varying clinical conditions that potentially cause interference in screening and confirmatory testing, resulting in an increased numbers of false positive HIV results and excessive

retesting and confirmatory testing [3–5]. Examples of instances that have shown interference include general suboptimal conditions related to ionic strength and pH, malignant neoplasms, alcoholic hepatitis, tuberculosis, history of multiple pregnancies, recent rickettsial infection, recent influenza or hepatitis B vaccinations, viral infections, renal failure, blood transfusion, liver disease, and autoimmune disorders (e.g. Sjogren's, systemic lupus erythematosus, polychondritis, rheumatoid arthritis, and autoimmune-hemolytic anemia) [2,6–13]. To date, there has been one case of autoimmune hepatitis that tested falsely positive when an HIV screening assay was administered [14]. A number of other autoimmune conditions have been recognized for causing false elevations in screening assays presumed to be secondary to human heterophilic antibody binding or molecular mimicry [15]. Given the rarity in documented cases, we present the second case of autoimmune hepatitis with a concomitant falsely positive HIV screening assay.

Case report

A 51-year-old female with a past medical history of type 2 diabetes mellitus, hypertension, depression, and gastroesophageal reflux disease was admitted to the hospital for abnormal liver enzymes. The patient reported one month of abdominal discomfort primarily located in the epigastric region with additional

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radiation to the left upper quadrant which had been progressively worsening over that same time period. Additionally, she noticed worsening jaundice and scleral icterus. Prior to this admission, she had recently visited her primary care provider (PCP) with similar complaints and was given omeprazole for suspected gastroesophageal reflux. The PCP gave orders for general laboratory studies and told the patient to follow up as needed. The patient was notified by her PCP of abnormal labs and was instructed to be admitted to the hospital for further evaluation.

Laboratory evaluation on admission included the following results: AST 1273 U/L with a repeat AST 1505 U/L and an ALT 1800 U/L with a repeat ALT 2032 U/L. Her total bilirubin was 9.6 mg/dL with alkaline phosphatase 137 U/L (NR 42–98 U/L). Her acute hepatitis panel was negative. Additional outpatient labs showed a detectable Epstein-Barr viral capsid antigen (EBV VCA) immunoglobulin G (IgG) and Epstein-Barr nuclear antigen (EBV NA) IgG indicating antibodies to a prior Epstein-Barr virus (EBV) infection with no evidence of an acute EBV infection. An ultrasound of the liver was performed showing known surgical absence of her gallbladder as well as a mildly enlarged liver suggestive of fatty liver infiltration. After further questioning, she admitted that her sister was diagnosed with autoimmune hepatitis. Given her elevated liver enzymes, scleral icterus, jaundice, family history of autoimmune hepatitis, and abdominal pain, gastroenterology was consulted for further recommendations. Gastroenterology requested that the patient be worked up for intrinsic causes including autoimmune hepatitis, primary biliary cholangitis, Wilson's disease, hemochromatosis, and alpha-1-antitrypsin deficiency.

The initial workup consisted of ANA, IgG, anti-smooth muscle antibody, anti-LKM-1 antibody, alpha-1-antitrypsin, ceruloplasmin, and anti-mitochondrial antibody. Initial laboratory results can be seen in Table 1.

In the initial workup of her elevated liver enzymes, there was suspicion for possible HIV infection (e.g. history of IV drug use and spousal infidelity). An ADVIA Centaur[®] HIV Antigen/Antibody Combo assay screening assay was performed and was found to be reactive. The sample was retested and remained positive. Given that our laboratory did not have access to a differentiation assay it was decided to pursue an HIV-1 NAT, ultimately providing a negative result.

For a definitive diagnosis of autoimmune hepatitis, gastroenterology recommended a liver biopsy which showed acute hepatitis most consistent with autoimmune hepatitis with portal, periportal, and septal fibrosis, with moderate to severe

portal and periportal inflammation with piecemeal necrosis. A consultative opinion from an outside specialty facility was obtained and reported predominant lobular hepatitis with histologic findings consistent with autoimmune hepatitis without evidence of malignancy or cirrhosis. The patient was started on empiric prednisone 40 mg daily with a noticeable downward trend in her liver enzymes as well as symptomatic relief of abdominal pain and jaundice. After significant improvement she was discharged home with plans to follow up with gastroenterology as an outpatient for long term management for autoimmune hepatitis.

Discussion

Among several novel HIV screening assays, the assay utilized in this case was an ADVIA Centaur[®] HIV Ag/Ab Combo (CHIV) assay. The CHIV assay is a magnetic microparticle-based chemiluminometric immunoassay that detects antibodies against the HIV-1 (group M and O), HIV-2, and p24 antigens. This assay utilizes recombinant antigens that include HIV-1 envelope protein (gp41/120), HIV-2 envelope protein (gp36) and three monoclonal antibodies specific to HIV p24 antigen. A synthetic peptide is added to detect HIV-1 O antibodies. A reactive sample is determined by a signal-to-cut off (S/O) ratio (established by the manufacturer) [2]. Per the CHIV manufacturer product manual, the S/O ratio is set at 1.0. If the index is greater than 1.0, the serum sample must be retested in duplicate after centrifugation to confirm reactivity. If the sample remains reactive, per CDC recommendations the patient should be followed up with a FDA approved supplemental antibody immunoassay (differentiation assay) that differentiates between HIV-1 and HIV-2 antibodies. All samples that are initially reactive on the initial Ag/Ab immunoassay as well as non-reactive or indeterminate on the differentiation assay should be tested with a FDA approved HIV-1 nucleic acid test to definitively rule out HIV infection [16].

The CHIV assay has been tested against a number of interferences to evaluate potential cross-reactivity that would produce reactive samples. Among the reported list, alcoholic hepatitis was the only disease process that was reactive and confirmed reactive using a FDA-approved differentiation assay [17]. Examples of interferences that were applicable to this case report included antinuclear antibody, diabetes mellitus, EBV IgG and IgM, and elevated human immunoglobulin G, all of which showed no interference or any cross-reactivity with the fourth-generation assay (per manufacturer report). Additionally the report discusses other situations such as sample hemolysis, lipemia and hyperbilirubinemia, hypergammaglobulinemia, or hyperproteinemia that above a certain threshold could show possible interference and produce a false positive result [17].

Our patient's lab results were not above the upper limits defined by the manufacturer. The manufacturer does make mention of a phenomenon that has been known to occur in newer fourth generation assays that could provide some insight on why our patient's initial screening assay was positive. This phenomenon is known as human heterophilic antibody (Hab) binding. These antibodies are human antibodies that bind to a component of the immunoassay (capture antibody) and form a bridge with the detection antibodies resulting in false elevations of the signal-to-cutoff ratio thus creating a falsely reactive sample [15]. This may be a plausible theory that could explain why our patient had a false positive HIV screening test. There were several Habs including the anti-smooth muscle and antinuclear antibodies as well as EBC IgM/IgG and hypergammaglobulinemia that may have interfered with the assay. Previous studies make mention of autoimmune conditions where investigators speculate

Table 1
Initial laboratory tests and results.

Laboratory Test	Patients Value	Reference range
Alpha-1-antitrypsin	179 mg/dL	83–199 mg/dL
Ceruloplasmin	34 mg/dL	18–53 mg/dL
Anti-mitochondrial Ab	Negative	–
Hepatitis A IgM	Negative	–
Hepatitis B IgM	Negative	–
Hepatitis C IgM	Negative	–
Human Immunoglobulin G	3029 mg/dL	600–1640 mg/dL
Anti-LKM-1 Ab	< 20.0	≤ 20.0 - Negative 20.1–24.9 - Equivocal ≥ 25.0 - Positive
ANA	1: 1280 (Homogenous pattern)	< 1:40 - Negative 1:40–1:80 - Low Level > 1:80 - Elevated
Anti-Smooth Muscle Ab	131	< 20 - Negative ≥ 20 - Positive
Ferritin	> 1562	9.0–587.0 nm/mL

mechanisms of cross-reactivity with HIV p24 likely secondary to antigenic mimicry between p24 and autoantibodies [8–10]. The autoantibodies produced in autoimmune hepatitis could have similar antigenic mimicry.

Although it may be difficult to speculate the exact reason for this false positive result, recent studies provide insight on how to reduce the overall false positive rates in patients tested with fourth generation CHIV assays. Demey et al performed a retrospective analysis on 56,682 samples tested by a CHIV assay. Of those tested, 449 samples tested intermediate or reactive and 38 % of the 449 were found to be falsely positive. However, the overall false positives consisted of 30 % of the total number samples, similar to the total percentage of false positive rate reported by the manufacturer. It was found that by increasing the S/O index above 1.0 there was a significant reduction in the number of false positives by 76 % as well as an improvement in the positive predictive value [18]. With these results, it is speculated that by increasing the S/O ratio, a larger number of false positive results can be reduced. Even though this does not define a causative reason for false positives it may aid in reducing the need for retesting and confirmatory testing required in the future.

Conclusion

In summary, HIV test results should be interpreted in the context of multiple factors, including HIV prevalence, pre-test probability, and an individual's overall risk for developing HIV. AIH is a chronic liver disease with progressive liver parenchymal inflammation caused by T-cell dysfunction with associated hypergammaglobulinemia and circulating autoantibodies. A number of case reports have presented chronic liver disease in the setting of falsely positive HIV assays, speculating that cross-reactivity between autoantibodies and HIV antigens secondary to antigenic mimicry may be the cause of falsely positive samples versus assay specific interferences [19,20]. Regardless of the mechanism, it is important for clinicians to recognize potential associations between false-positive HIV testing and liver disease. In this case report, we highlight a unique presentation of AIH with concurrent falsely positive HIV testing to bring awareness of additional disease processes that cause false positive HIV results. Our goal of this report is to continue to reduce the number of retests performed in low prevalence HIV areas as well as propose ways to improve the overall sensitivity of the fourth-generation HIV assays.

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Consent

Written informed consent was obtained from the patient for publication of this case report.

Declaration of Competing Interest

There are no declarations of interest.

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