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FALSE-POSITIVE FOURTH-GENERATION HIV TEST ASSOCIATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA. CASE REPORT

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Palabras clave: Ensayo de inmunoadsorción enzimática; Serodiagnóstico del sida; Anemia hemolítica.

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RESUMEN

Introducción. La prueba de tamizaje para virus de la inmunodeficiencia humana (VIH) con ELISA de cuarta generación tiene gran sensibilidad y una especificidad >99% para detectar tanto antígenos como anticuerpos. Se estima que solo el 0.5% son falsos positivos.

Presentación del caso. Paciente femenino de 61 años con cuadro clínico consistente en malestar general, fiebre no cuantificada, astenia y adinamia. En los paraclínicos se evidenció anemia, por lo que se realizó prueba de Coombs, la cual resultó positiva junto con otros hallazgos de anemia hemolítica autoinmune mixta. Se realizaron dos pruebas de tamizaje para VIH con técnica ELISA de cuarta generación que fueron positivas. Dadas las recomendaciones nacionales sobre el diagnóstico de la infección por VIH, se realizó una carga viral que resultó ser negativa, por lo que se consideró el resultado como un falso positivo.

Discusión. Factores clínicos y biológicos se relacionan con resultados falsos positivos. Existen descripciones sobre fenómenos de autoinmunidad, como el lupus eritematoso sistémico o la anemia hemolítica autoinmune, con pocos casos en adultos mayores.

Conclusiones. Las pruebas rápidas han cambiado el diagnóstico de la infección por VIH en el mundo; sin embargo, como toda prueba diagnóstica, tienen falsos positivos con diagnósticos diferenciales, incluidos la anemia hemolítica autoinmune.

ABSTRACT

Introduction: The fourth-generation ELISA human immunodeficiency virus (HIV) screening test has a high sensitivity and specificity >99% to detect both antigens and antibodies. Estimates are that only 0.5% yield false positive results.

Case description: 61-year-old female patient with a clinical picture consisting of malaise, unquantified fever, asthenia and adynamia. Laboratory tests revealed anemia, so a Coombs test was performed, obtaining a positive result along with other findings of mixed autoimmune hemolytic anemia. Two fourth-generation ELISA HIV screening tests were performed obtaining positive results. Given the national recommendations on the diagnosis of HIV infection, a viral load was performed, which turned out to be negative, so the result was considered a false positive.

Discussion: Clinical and biological factors are related to false positive results. There are descriptions about autoimmunity phenomena, such as systemic lupus erythematosus or autoimmune hemolytic anemia, with few cases in older adults.

Conclusions: Rapid tests have changed the diagnosis of HIV infection worldwide; however, like any other diagnostic test, they may yield false positive results with differential diagnoses, including autoimmune hemolytic anemia.

INTRODUCTION

The number of people living with human immunodeficiency virus (HIV) worldwide rose to 36.9 million in 2017, with 1.8 million new cases that same year. Latin America ranks fifth in prevalence, with a heterogeneous distribution among the countries of the region; Colombia is one of the countries with the highest incidence rates with 150 000 cases. (1)

Autoimmune hemolytic anemia has an annual incidence of 1 case per 80 000 inhabitants in America and Europe, and is defined as the production of erythrocyte autoantibodies that cause a decrease in the lifespan of erythrocytes due to increased hemolysis. This condition is classified depending on the temperature at which the immunoglobulins sensitize the red blood cell; the most frequent form is warm autoantibody, which reacts at temperatures >37°C, mostly mediated by IgG, while cold autoantibodies do it at temperatures <32°C by IgM. (2,3)

The fourth-generation ELISA test detects HIV-1 and HIV-2 specific antibodies in blood using the HIV-1 p24 antigen. (4) Although this test has a very high sensitivity and specificity, estimates are that there may be a 0.5% chance of obtaining a false positive result, especially in pregnant patients with hyper IgM syndrome and hematologic diseases. (4-6)

The purpose of this case report is to present an older patient who was diagnosed with autoimmune hemolytic anemia, but whose HIV testing yielded a false positive result. It should be noted that this is a rare situation considering the high specificity and sensitivity of the ELISA test.

CASE PRESENTATION

Female patient, 61 years old, from Bucaramanga, housewife and white, who was treated in a tertiary care hospital from Bucaramanga, Colombia, in March 2017 due to a clinical picture of approximately 3 months of evolution. It was characterized by general malaise, unquantified fever, asthenia, adynamia and, for several weeks, edema of the lower limbs. The woman did not report hair loss, skin lesions, photo sensitivity, or mucosal lesions.

She had a history of diabetes mellitus type 2 without pharmacological treatment, primary pulmonary hypertension and chronic kidney disease. She had also been hospitalized several times for anemia, requiring transfusion support since November of the previous year. The physical examination performed on admission showed generalized mucocutaneous pallor and grade 3 edema in the lower limbs; her vital signs were: blood pressure: 220/110 mmHg, heart rate: 110/min, respiratory rate: 18/min, temperature: 38.3°C and oxygen saturation on air: 95%. Different tests were done, including serial hemograms that showed severe anemia since her admission in March 2017 until May of the same year (Table 1).

Table 1. List of hemograms.

Variable / Date	Hgb	HCT	MCV	MCH	RDW	Leucocytes	Platelets
4/03/17	5.5	15.4	89	33	18	9 800	425 000
20/04/17	7.7	23	87	33	16	19 700	400 000
22/04/17	3.8	10	86	97	18	13 000	307 000
23/04/17	3.7	9.5	86	97	18	13 200	327 000

Continues.

Variable / Date	Hgb	HCT	MCV	MCH	RDW	Leucocytes	Platelets
23/04/17	6.3	12	86	97	18	18 600	331 000
25/04/17	8.2	23	83	35	16	28 300	390 000
27/04/17	7.6	19	88	36	18	11.7	298 000
28/04/17	6.1	19	88	36	18	6 700	267 000
2/05/17	6.2	18	88	36	18	7 400	267 000
3/05/17	7.7	19	88	36	18	9 200	397 000
18/05/17	7.9	20	95	36	18	5 000	333 000

Hgb: hemoglobin; HCT: hematocrit; VCM: mean corpuscular volume; MCH: mean corpuscular hemoglobin; RDW: red blood cell distribution width.

Source: Own elaboration.

Due to the presence of anemia, different complementary tests were done, including the Coombs test, which was positive for IgG3+ / C3d 4+ on several occasions. Considering the diagnosis of anemia with possible autoimmune origin, studies were expanded to rule out the etiology with complement C4 levels at 8 mg/dL and C3 at 77 mg/dL, which were below the reference value. In addition, antinuclear antibodies tests were made, yielding a moderately positive value of 34.37 international units (U). An anti-double stranded DNA test was also done, obtaining negative results (66.02 U/mL; reference value: 60-200).

The other studies were a partial urine test that revealed significant proteinuria of 500 mg/dL, for which a test for protein in urine was performed 24 hours later, revealing nephrotic-range proteinuria of 13101.24gr. A routine fourth-generation HIV ELISA was performed, with a positive result of 0.34 (normal value: <0.25). Due to this result, the recommendations of the national guidelines for the diagnosis of HIV infection (7) were followed and a second test was performed, which was also positive with a value of 0.29. Considering these results and following again the recommendations of the national guidelines (7), a viral load test was performed, obtaining negative results (number of copies <5 000).

DISCUSSION

This is the case of an older patient with autoimmune hemolytic anemia, with suspected systemic lupus erythematosus (SLE) by nephrotic syndrome and weakly positive antinuclear antibodies. However, despite the presence of these signs, symptoms and findings, lupus was ruled out through a lab test because the patient only met 3 of the 17 criteria established by the Systemic Lupus International Collaborating Clinics for classification of the disease (6), and did not have clinical symptoms such as hair loss, skin manifestations or photosensitivity.

The presence of nephritis was not demonstrated since kidney biopsy could not be performed due to the unavailability of that service in the institution where the patient was treated. After two positive fourth-generation ELISA tests, in accordance with the national guidelines, a negative viral load test (7) was carried out, ruling out the disease and considering the tests as false positives.

A similar case occurred in Japan in 2011, where a patient with autoimmune hemolytic

anemia and angioimmunoblastic T-cell lymphoma had a false positive result for HIV in an enzyme immunoassay test. (5)

According to the literature, there is a correlation between multiple autoimmune and non-autoimmune diseases with false positive results in ELISA tests due to the production of immunoglobulins that generate cross-reactions with antibodies against the HIV virus. (8-11)

According to Barthel & Wallace (11), the first reports of false positive results were described in the 1980s with studies by Perentice *et al.* in 1985 and Calabrese *et al.* in 1986, in which 41 SLE patients had HIV-positive ELISA test results. The researchers considered that this was caused by antibodies against altered H9 nuclear antigens. (11)

In fact, these two processes can be immunologically exclusive in cases of HIV infection and SLE. (10) On the one hand, SLE may prevent HIV infection as a result of polyclonal antibodies and stimulation and, on the other hand, it may not develop in a low CD4 environment, as in the cell depletion process seen in HIV.

Descriptions for autoimmune hemolytic anemia with pathophysiological explanations are scarce in the reported cases (5); in this scenario, the high production of CXCL13 and IL-21 may be associated with the function of T follicular helper cells (TFH) for immunopathogenesis in SLE. (5) These cells were identified as the origin of autoimmune hemolytic anemia and their cytokines CXCL13 and IL-21 play a key role in the activation and expansion of plasmacytic B cells, differentiation and hypergammaglobulinemia. (5) Immune aberrations may produce the same autoimmune response observed in hemolytic anemia in SLE patients.

All the mechanisms described above are ways of trying to explain why these tests may yield false positive results, especially with autoimmune diseases, since no single way of producing these cross-reactions between antibodies has been established. In 2003, Muta & Yamano (12) considered that the reactivity mechanism with HIV P24 antigen was an antigenic mimesis among autoimmune epitopes, such as small ribonucleoproteins (also known as Sm) or retroviral antigens. Other researchers have considered that autoimmune hemolytic anemia has numerous subtypes of polyclonal gamma globulins that coincidentally react with HIV P24 antigen. (11,12)

A study conducted in sub-Saharan Africa found that there is a correlation between the presence of schistosomiasis and false-positive results in the same test, especially in infection by *Schistosoma mansoni* and *Schistosoma haematobium*. (13) This is the consequence of a cross-relationship between HIV-1 peptides and antibodies against these HIV-1 *Schistosoma* species. (14)

SLE is one of the autoimmune diseases related to false positive results for HIV, of which there are several cases reported so far. (11-15) One such case occurred in China in 2015, where a SLE patient tested positive for HIV by ELISA, result that was later considered to be a false positive by Western blot. (11-15) Other cases have reported patients with HIV infection who later develop SLE that has been difficult to diagnose due to overlapping symptoms and positive antibodies. (10)

All this evidences the cross-reaction leading to such results, and confirms the need to perform a different post-ELISA test to confirm or rule out the disease, in accordance with the international and national recommendations of the clinical practice guidelines. (7)

Considering the above and based on a bibliographic review, this may be the first case in Colombia to report cross reaction of antibodies associated with autoimmune hemolytic anemia and false positive results for HIV, which allows

stressing the importance of making a proper diagnosis by implementing appropriate tests. It should also be borne in mind that despite their high sensitivity and specificity, any diagnostic test can yield false positive results.

There is evidence in the literature of a higher frequency of false positive ELISA test results in young Hispanic women and pregnant women (13-16), which contrasts with the case presented here as the patient was an older adult. However, autoimmune conditions, kidney failure, cystic fibrosis, multiple pregnancies, blood transfusions, liver disorders, lymphomas, intravenous drug abuse, hemodialysis, and recent vaccines for hepatitis B, rabies, or influenza have also been described in young women with false-positive HIV tests. (13-16)

CONCLUSIONS

Although this is a single case, this report shows that autoimmune diseases, including SLE and conditions that lead to generalized immune stimulation with increased production of anti-HLA-DR or other antibodies —such as those produced in autoimmune hemolytic anemia— may cross-react with HIV antigens from the ELISA assay.

It is not yet known how a test as sensitive as the fourth-generation ELISA can generate false positive results, especially in relation to autoimmune conditions; however, this case allows providing the literature with a rare case to further investigate the physiopathology of autoimmune diseases and to be cautious when performing antibody-based diagnostic tests with the implications that a positive result must have, especially when dealing with HIV infection.

ETHICAL CONSIDERATIONS

The study was conducted with the patient's verbal and written informed consent to use her medical history and photographs that did not reveal her identity. The provisions of Resolution 8430 of 1993 of the Ministry of Health of Colombia (17) and the Declaration of Helsinki (18), which determine the guidelines for research on human beings, were taken into account.

CONFLICT OF INTEREST

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