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FALSE-POSITIVE ELISA TEST RESULT FOR HUMAN IMMUNODEFICIENCY VIRUS IN A PATIENT DIAGNOSED WITH EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS. CASE REPORT

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Palabras clave: Anticuerpos Anticitoplasma de Neutrófilos; Reacciones Falso Positivas; Anticuerpos Anti-VIH; Ensayo de Inmunoadsorción Enzimática; Síndrome de Churg-Strauss.

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RESUMEN

Introducción. Para el tamizaje y diagnóstico del virus de inmunodeficiencia humana se ha evidenciado que el ensayo de cuarta generación es el más sensible y específico en comparación con el de tercera generación. Aunque se ha visto una reducción tanto en los falsos positivos como de los falsos negativos, existen condiciones y enfermedades como la granulomatosis eosinofílica con poliangiitis que pueden incidir en un resultado falso positivo de estas pruebas.

Presentación del caso. Hombre de 73 años que asistió a una institución de cuarto nivel de complejidad en la ciudad de Bogotá por sintomatología consistente en malestar general, pérdida de peso significativa no provocada, alteraciones motoras, aparición de lesiones mucocutáneas en cavidad oral y pérdida de fuerza en ambos miembros inferiores. Dada su sintomatología y antecedentes, se realizó un ensayo por inmunoabsorción ligado a enzimas de cuarta generación (ELISA por sus siglas en inglés) para virus de inmunodeficiencia humana con resultado positivo. La prueba confirmatoria posterior fue negativa. El perfil autoinmune fue positivo para anticuerpos anti citoplasmáticos de neutrófilos, lo cual llevó a hacer el diagnóstico de vasculitis de pequeños vasos de tipo granulomatosis eosinofílica con poliangiitis, por lo que se consideró que el resultado de la prueba ELISA fue un falso positivo.

Conclusiones. Aunque las pruebas rápidas han mejorado el diagnóstico de la infección por VIH en el mundo, resulta necesario reconocer que existen causas de falsos positivos distintas a las razones técnicas. Una de estas causas puede tener relación con la existencia de enfermedades como las vasculitis de pequeños vasos.

ABSTRACT

Introduction: For HIV screening and diagnosis, it has been demonstrated that the fourth-generation assay is more sensitive and specific compared to the third-generation assay. Although a reduction in both false positive and false negative results has been observed in the general population, there are conditions that should be studied, such as eosinophilic granulomatosis with polyangiitis, as they may lead to a false positive result of these tests.

Case presentation: 73-year-old male patient treated in a quaternary care center in the city of Bogotá, Colombia, with symptoms of general malaise, significant unprovoked weight loss, distal motor disturbances, appearance of mucocutaneous lesions in the oral cavity, and loss of strength in both lower limbs. Based on the symptoms, a fourth-generation enzyme-linked immunosorbent assay (ELISA) for human immunodeficiency virus was performed, obtaining a positive result. A subsequent confirmatory test yielded a negative result. The autoimmune profile of the patient was positive for anti-neutrophil cytoplasmic antibodies, which led to a diagnosis of small vessel vasculitis of the eosinophilic granulomatosis type with polyangiitis, so the ELISA test result was considered a false positive.

Conclusions: Although rapid tests have improved the diagnosis of HIV infection worldwide, it is necessary to recognize that there are causes of false positive results other than technical reasons. One such cause may be related to the occurrence of diseases such as small vessel vasculitis.

INTRODUCTION

Human immunodeficiency virus (HIV) detection tests are the main tool for detecting the presence of infection. Diagnostic schemes are based on serial tests that increase the efficiency in the detection of positive cases by means of different serological tests, in order to reach a definitive diagnosis (1,2). These tests are based on the formation and detection of immune complexes, being the ELISA test the main method used, with a sensitivity of 99.5% (2).

Currently, there are four different generations of ELISAs for HIV detection, each with improved diagnostic performance over the previous one. Third-generation tests detect not only immunoglobulin G antibodies but also immunoglobulin M antibodies, which increases their performance. Likewise, fourth generation tests not only identify these antibodies, but also detect the p24 antigen, which makes these tests the most accurate diagnostic method (1-3).

Although fourth generation tests are usually very effective, there may be false-positive results resulting from four main causes: technical artifacts, chronic diseases, multiparity, and unusual circumstances (3,4). The incidence of false positive results for the fourth generation ELISA is reported to be between 0.2 and 0.5% (5-7).

Chronic conditions that can lead to false positives in ELISA include autoimmune diseases such as Sjögren's syndrome, and non-autoimmune diseases such as leprosy infection or alcoholism (3). Multiple mechanisms have been proposed to explain this phenomenon, especially in patients with systemic lupus erythematosus (SLE), such as molecular mimicry, cross-reactivity, and antibody generation (5,6).

The following is the case of a patient with active vasculitis in whom HIV infection was suspected given the clinical presentation of the symptoms, which was subsequently ruled out through confirmatory tests.

CASE PRESENTATION

A 73-year-old man from the city of Bogotá (Colombia) was admitted to the emergency department of the Fundación Cardioinfantil, a quaternary care institution located in the same city, after experiencing symptoms of lower limb weakness for three months, hand weakness that began four days before admission, unquantified fever for approximately 15 days, pain in the right knee on mobilization, soft stools for a week, weight loss (10 kg), and the appearance of erythematous lesions predominantly in the lower limbs.

He had a history of chronic obstructive pulmonary disease (COPD) without pulmonary function tests, for which he had been on oxygen therapy with salbutamol and ipratropium bromide plus beclomethasone at 2 liters/minute for 16 hours a day for a year. He had no history of smoking or intravenous drug use. His surgical history included cholecystectomy. He denied risky sexual behaviors and reported no significant family history.

On physical examination, the patient was in regular general condition with the following vital signs: heart rate of 78 bpm, respiratory rate of 17 rpm, and blood pressure of 114/78 mmHg. Moreover, there was evidence of moist mucous membranes, anicteric sclerae, whitish lesions in the oral cavity (Figure 1), soft abdomen without pain on palpation with mild distension, and positive bowel sounds. No swallowing or sphincter alterations were detected. On neurological examination, he was alert, oriented, obedient to simple commands, and there was no evidence of alterations in cranial nerves. He had muscle strength 1/5 in the lower limbs (Medical Research Council score) and 3/5 in the left upper limbs. It was not possible to evaluate gait patterns due to lower limb weakness that limited standing. The patient had muscle stretch reflexes (/++++), with no evidence of muscle fasciculations. No signs of meningitis were found.

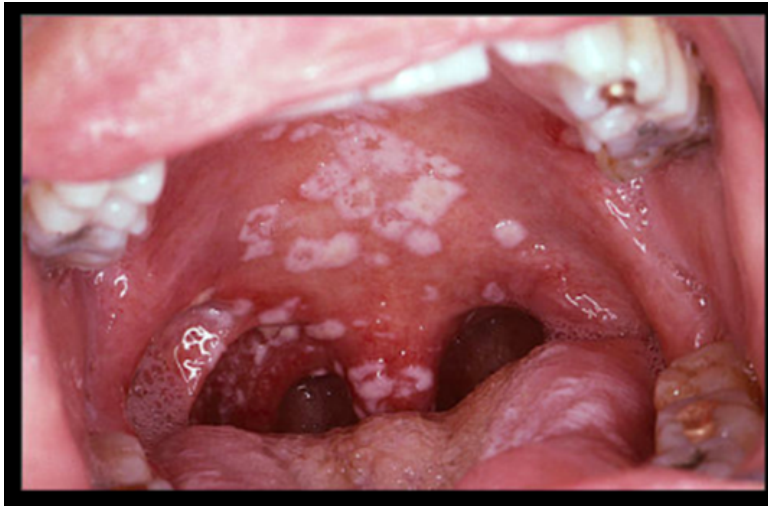


Figure 1. Whitish plaques on the palate.

Source: Image obtained during the study.

Marked hypotrophy was found in the lower limbs, so a capillary filling test was performed (2 sec). In addition, multiple skin lesions (some crusted) with necrotic centers and other ulcerated lesions were observed (Figure 2).

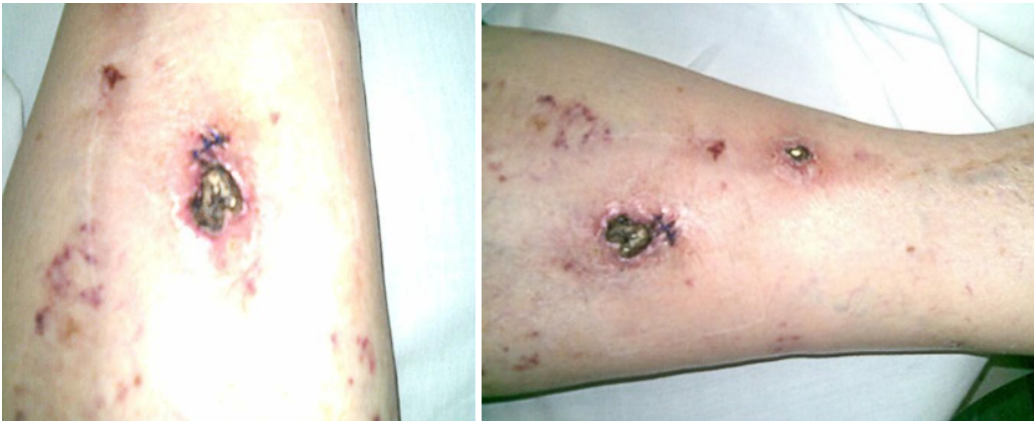


Figure 2. Multiple skin lesions on both lower limbs, some crusted with necrotic center, others ulcerated.

Source: Images obtained during the study.

During the first day of hospital stay, a complete blood count was performed, which showed eosinophilia and, in the context of a patient with skin lesions, it was decided to perform a fourth generation ELISA test for HIV, which yielded a positive result. In view of these findings, a viral load test was requested on day 2 of hospitalization, which was negative. Additionally, chest X-ray and high-resolution chest CT scan were requested, with no pathological findings (Figures 3 and 4).



Figure 3. Chest X-ray.

Source: Image obtained during the study.

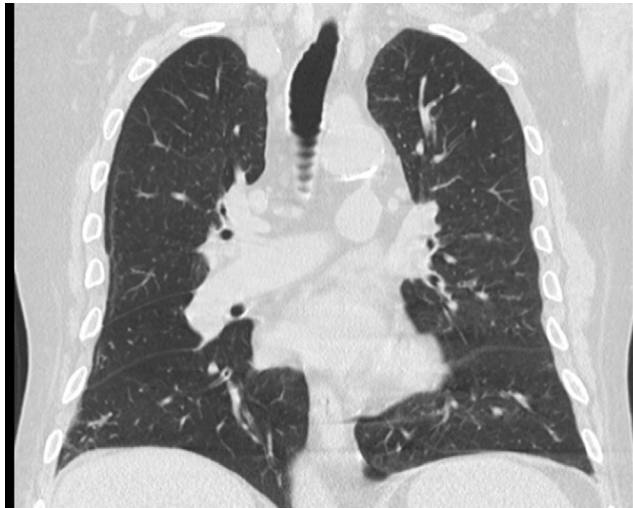


Figure 4. High resolution computed axial tomography.

Source: Image obtained during the study.

On days 2 and 3 of hospitalization, a fecal occult blood test was performed with positive results (Table 1), so a colonoscopy was requested, which revealed ulcerative proctosigmoiditis (ulcerative colitis) with a polyp in the sigmoid colon. One week after the patient's admission, immunological tests were performed, and positive anti-myeloperoxidase antibodies (MPO) and perinuclear pattern antibodies (p-ANCA) were found (Table 2).

It should be noted that at the time of the study, biopsy of multiple skin lesions on the lower limbs was not available. The results of the laboratory tests taken at another institution are also included in Table 1.

Table 1. Laboratory tests

Test	Result
Blood count	External: leukocytes 11,000/mm ³ ; neutrophils 33%; lymphocytes 10%. Admission: leukocytes 15,000/mm ³ ; neutrophils: 60%; lymphocytes: 11%. Eosinophiles: 29%. Hemoglobin: 10.9 gr/dL; hematocrit: 30.5%; mean corpuscular volume: 90 fL; platelets: 120.000/mm ³ .
Liver function	ALT: 44U/L. AST: 38U/L.
Electrolyte analysis	Na+: 139 mEq/L; K+: 3,6 mEq/L.
Kidney function	BUN: 11 mg/dL. Creatinine: 0.9 mg/dL.
TSH test	TSH: 4.5 µU/L.
Stool test	Occult blood (+).
Immunological tests	External: C3, C4, ANAS, ANCAS, ANTI-RNP: negative.

ALT: alanine aminotransferase; AST: aspartate aminotransferase;

Na: sodium; K: potassium; BUN: urea nitrogen; TSH: thyroid-stimulating hormone; C3: complement C3; C4: complement C4; ANAS: antinuclear antibodies; ANCAS: anti-neutrophil cytoplasmic antibodies; ANTI-RNP: ribonucleoprotein antibodies.

Source: Own elaboration based on clinical laboratory report (Fundación Cardioinfantil).

Table 2. Complementary tests

Test	Result
Infectious disease serology	RPR Syphilis: nonreactive; HIV ELISA: 1.57 (<0.1); HbsAg: negative; Anti-HCV: negative; HIV viral load: negative.
Metabolic panel	HbA1c: 6%; vitamin B12: 400 µU/L.
Colonoscopy	Ulcerated proctosigmoiditis with scant bleeding, polyp in sigmoid colon (biopsied), and mild uncomplicated diverticular disease.
Autoantibody test	Anti-MPO: 74.4U/L (<5.0); p-ANCA: 1/80; c-ANCA: negative.

RPR: rapid plasma reagin; HbsAg: hepatitis B virus surface antigen; Anti-HVC: hepatitis C antibodies; HbA1c: glycosylated hemoglobin; Anti-MPO: antibodies against myeloperoxidase.

Source: Own elaboration based on clinical laboratory report (Fundación Cardioinfantil).

On the sixth day of hospital stay, an electromyography was requested to study the weakness syndrome in which acute sensory-motor polyneuropathy in progression was evidenced, with predominance of distal involvement in the lower limbs. At the same time, a cerebrospinal fluid study was requested, which was reported to be normal (Table 3).

Table 3. Cerebrospinal fluid analysis

Lumbar puncture	
Parameter	Result
Leucocytes	3 cell/mm ³
Proteins	50mg/dL
Glucose	60 (mg/dL)
Serologic test for syphilis (VDRL)	Negative
Potassium hydroxide test	Negative
Opening pressure	12 cmH ₂ O

Source: Own elaboration based on clinical laboratory report (Fundación Cardioinfantil).

Taking into account the results of p-ANCA and myeloperoxidase positivity (Table 2), a diagnosis of ANCA-associated vasculitis was made. Therefore, 2 weeks after admission, initial treatment with methylprednisolone (500 mg intravenous) was started for 3 days, which resulted in a decrease in the neutrophil count and recovery of strength in all four limbs. The patient was discharged on the 14th day of hospitalization and continued immunomodulatory treatment with prednisolone 0.5 mg/kg/day. To date, the patient is being monitored by the Rheumatology and Internal Medicine departments and has shown recovery of strength in all 4 limbs, as well as resolution of fever and eosinophilia, no adverse reactions, and adequate adherence to glucocorticoid treatment.

DISCUSSION

The present case report describes a patient with nonspecific systemic manifestations and multiorgan involvement, initially characterized by the medical team as HIV infection after analyzing the symptoms and the positive results of the fourth generation ELISA test. Subsequently, upon completion of the diagnostic algorithm to confirm this suspicion, negative results were obtained in the confirmatory tests (viral load), so small vessel vasculitis was suspected due to the presence of polyneuropathy, asthma, eosinophilia and the positive ANCA results, which in the clinical context described allowed reaching the diagnosis of eosinophilic granulomatosis with polyangiitis (EGPA).

EGPA, also known as Churg-Strauss syndrome, is a type of small vessel vasculitis of low prevalence, with an incidence of approximately 2.6 cases per million people, so the information available in clinical trials is scarce. The presence of rheumatoid factor has been described in 60% of EGPA cases, and ANCA positivity has also been documented in only 40% of these patients (8). Recent studies have proposed the active role of ANCAs in the pathogenesis of the disease, revealing differential clinical profiles and increased expression of HLA-DRB4 in these patients (8,9).

On the other hand, the incidence of false-positive results of the fourth generation ELISA test is low (0.5%) (6). Autoimmune diseases are an important factor in the occurrence of these results, with SLE being one of the diseases most associated with false positives to date since the description of the first cases by Perentice in 1980 (6). However, it was not until 1992 that Esteva *et al.*, in a report of two cases of false positive results for HIV, proposed molecular mimicry mechanisms (described in the 1980s) as a possible explanation for this clinical phenomenon, which are accepted today (4).

It has been proposed that molecular mimicry between the different autoantibodies and HIV envelope glycoproteins results in cross-reactivity. This mimicry would be the main pathophysiological mechanism that explains this phenomenon. The correlation between the presence of autoantibodies targeting different antigens, such as major histocompatibility complex (MHC-I) molecules, and the positivity of serological tests (non-specific) for these antibodies has been known since the 1980s when, according to Jian *et al.* (4), a study by Okudaira *et al.* described the presence of anti-HLA-DR antibodies in the serum of patients with SLE, demonstrating the plausibility of a cross-reaction. Furthermore, the group of Golding *et al.* demonstrated the similarity between two highly conserved sequences in the B1 domain of HLA-II and the HIV gp41 molecule, which may be the reason for the cross-reactivity that explains the false positives of the HIV ELISA test (4). Subsequent studies confirmed these findings (4,10).

Similarly, the relationship between the incidence of false positive ELISA results and various autoimmune diseases other than SLE has been noted. Silverstein *et al.* (11) reported the case of a man with rapidly progressive glomerulonephritis,

developed in the context of a small vessel vasculitis and positive ANCAS. During the diagnostic approach, HIV serology was performed on multiple occasions, all of them with positive results. Finally, the diagnosis of HIV infection was ruled out given the impossibility for the detection of confirmatory genetic material by polymerase chain reaction, suggesting that the previously performed immunoassays actually represented false positive results.

The work by Gervorkian *et al.* (12) describes the potential difficulties in accurately diagnosing HIV infection in Hispanic patients with seropositive rheumatoid arthritis and neurocysticercosis. The authors were able to identify at least 7 false positive results in the study population and proposed a phenomenon of cross-reactivity between the C1q complement fraction and the glycopeptides gp120, p51, p24 and, to a lesser extent, gp41, found in the virus envelope as the cause of these results.

The detection of the p24 antigen is the differentiating factor of the fourth generation ELISA, and this factor is responsible for its high diagnostic efficiency. However, cross-reactivity with small ribonucleoprotein and other retrovirus antigens has been reported, especially in patients with hemolytic anemia, leading to false positive results in these patients (6).

It should be noted that no case reports on the presence of false positive HIV ELISA in the setting of patients with ANCAS-associated small vessel vasculitis have been found. This leads to consider that the case presented here is exceptional and, possibly, the first case reported in Colombia.

CONCLUSIONS

Myeloperoxidase-ANCA-positive vasculitis could cross-react with HIV antigens in the ELISA test, as occurred in the case reported here. Although this is a unique case, it demonstrates that HIV diagnosis should be approached with caution given the implications of a positive result. It is still unclear how a sensitive test such as the fourth generation ELISA can generate false positive results, especially in relation to small vessel vasculitides. In any case, as this is a unique case in Colombia, it is considered a contribution to the existing literature that allows for more research into the pathophysiology of this disease. Further studies on the cross-reactivity of HIV diagnostic tests in patients with small-vessel vasculitis are needed.

ETHICAL CONSIDERATIONS

This case report was prepared upon obtaining verbal and written informed consent of the patient.

CONFLICT OF INTEREST

None stated by the authors.

FUNDING

None stated by the authors.

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