LETTER TO THE EDITOR
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Macrophage Activation Syndrome Secondary to Histoplasmosis in an Adult Female Carrier of X-linked Chronic Granulomatous Disease with Extreme Lyonization

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Dear Editor:

Chronic granulomatous disease (CGD) is an inborn error of immunity (IEI) characterized by recurrent, life-threatening bacterial and fungal infections and inflammatory complications.[1] Deficiency in phagocyte NADPH oxidase function due to pathogenic variants in *CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*, or *CYBC1* has been recognized as a genetic cause of CGD.[1] Abnormal NADPH oxidase activity leads to abnormal production of reactive oxygen species (ROS).[1] The most common form of CGD is X-linked (XL) due to a mutation in the *CYBB* gene.[1] XL-CGD female carriers have a dual phagocyte population due to lyonization, with 20% to 80% functioning neutrophils.[2] In XL-CGD carriers, infections occur when the percentage of functioning neutrophils ranges from 10 to 5%. However, the inflammatory and autoimmune manifestations do not correlate with a given percentage of functioning neutrophils,[3] and the mechanisms that produce the inflammation and autoimmunity in carriers are unknown.[4] Histoplasmosis is a sporadic endemic mycosis with environmental, occupational, and wilderness exposure-related risk factors.[5] We present a carrier of XL-CGD with a minimal percentage (10.8%) of a functioning neutrophil population and fatal histoplasmosis.

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A 28-year-old female from an eastern rural region of Mexico was identified as an XL-CGD carrier when her son was diagnosed with CGD. Her dihydrorhodamine (DHR) assay showed extremely skewed X chromosome inactivation, with only 10.8% of ROS-producing neutrophils (Figure 1 A-B). Sanger sequencing confirmed a heterozygous p. G412V deleterious variant in *CYBB*. She had a history of recurrent oral ulcers and denied other manifestations; she did not receive clinical follow-up as a carrier. Three years later, she was admitted to the hospital due to a two-month history of progressive asthenia, myalgia, diarrhea, productive cough, and fever that progressed to dyspnea. She was diagnosed with bilateral necrotizing pneumonia and was started on vancomycin, imipenem, and fluconazole. Due to her increased oxygen requirements and persistent symptoms, she was admitted to the intensive care unit with assisted ventilation. On physical examination at the referring hospital, she presented with mild tachypnea, bilateral crackles and hepatosplenomegaly. After seven days of hospitalization, she was placed on mechanical ventilation. Laboratory findings revealed anemia (hemoglobin 7.1 g/dL [14-17.5]), leukocytosis (17,800/mm^3 [4,500-11,000]), neutrophilia (15,800/mm^3 [2,000-7,000]), thrombocytosis (769/mm^3 [150,000-450,000]), elevated C-reactive protein (35 mg/dL [<0.3]), procalcitonin (18.3 ng/mL [<0.5]), hyperferritinemia (2,101 ng/mL [40-200]), hypertriglyceridemia (521 mg/dL [35-160]) and increased IgE levels (740 UI/L [<110]). Chest computed tomography showed diffuse bilateral lung consolidation with ground-glass opacities, mediastinal

lymphadenopathy, and pleural effusion. The lung lesions progressed during the disease, with the development of nodules and cavitary lesions of the lung parenchyma (Figure 1C).

Serial blood cultures and bronchoalveolar lavage cultures for bacteria (including mycobacteria) and fungi were negative. Bronchioalveolar lavage and transbronchial biopsy were also negative for *Pneumocystis jirovecii* pneumonia. Transbronchial biopsy showed necrosis and acute inflammation associated with microorganisms that were morphologically compatible with *Histoplasma* spp. (Figure 1D). We suspected that the patient had diminished her ROS-producing neutrophil percentage; however, the DHR assay showed the same percentage of ROS-producing neutrophils (10.8%). On the other hand, knowing that CGD predisposes to autoimmunity, the patient was tested for anti-Beta-2 glycoprotein 1 antibodies, anti-nuclear antibodies, anti-cardiolipin antibodies, anti-neutrophil cytoplasmic antibodies, and rheumatoid factor, all of which were within normal reference values. The HIV serology test was negative. Liposomal amphotericin B (3 mg/kg/day) and methylprednisolone were added to the meropenem and vancomycin treatment for severe acute diffuse pulmonary histoplasmosis. Given the severity of the clinical course, she received a single dose of intravenous gammaglobulin (2 g/kg). She had an unsatisfactory course, with persistent fever, cytopenias (hemoglobin 8.9 g/dl [14-17.5]), lymphocytes 600/mm^3 [1000-4800]), platelets 148 000/mm^3 [150,000-450,000]) and increased ventilatory support parameters, leading to a fatal outcome after 32 days of hospitalization and 30 days of antifungal treatment.

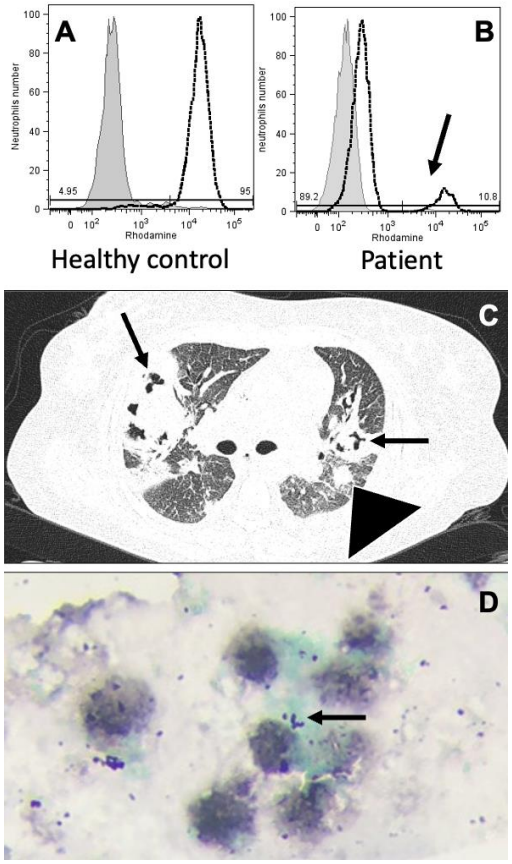

Figure 1. Paraclinical studies. Neutrophil histograms of the dihydrorhodamine (DHR) assay using phorbol myristate (PMA) as a stimulus of (A) the healthy control and (B) the patient. The gray area indicates the basal point, and the dotted line indicates the stimulated point; the arrow shows the percentage of functioning neutrophils. (C) Pulmonary tomography shows cavitated areas (arrows), pulmonary nodules (arrowhead), and a micronodular pattern. (D) Grocott-stained bronchoalveolar lavage showing intracellular and extracellular spherical to ovoid bodies surrounded by a thin halo (arrow), compatible with *Histoplasma* spp.

DISCUSSION

The present case is a woman with histoplasmosis who, three years earlier, was identified as a carrier of XL-CGD. At that time, she had 10.8% functioning neutrophils (Figure 1 A-B). In this hospitalization, the DHR test showed the same percentage of functioning neutrophils. We reviewed the literature showing that classical CGD does not confer susceptibility to dimorphic fungi such as Histoplasma.[6] However, disseminated histoplasmosis has been described in patients with p40^{phox} deficiency, and *an in vitro* assay showed abnormal ROS neutrophil production after stimulation with *H. capsulatum.*[7] The reason why p40^{phox} deficiency confers susceptibility to Histoplasma and that gp91^{phox}, p22^{phox}, p47^{phox}, and p67^{phox} deficiencies do not is unknown. Thus, in the reported patient, we considered that the low ROS production could not explain the increased susceptibility to histoplasmosis.

The reported patient fulfilled different macrophage activation syndrome (MAS) criteria, such as hepatosplenomegaly, lymphadenopathy, fever, pancytopenia, hypertriglyceridemia, and hyperferritinemia. However, when analyzing the possible causes that could explain the severity and fatal outcome, the diagnosis of MAS was made retrospectively. MAS is one of the inflammatory manifestations described in CGD, and the described triggers are infectious agents or inflammatory bowel disease.[8] Only one elderly female carrier of X-linked CGD with severe lyonisation deviation has been described to have MAS.[9,10] Among patients with CGD and MAS, *Burkholderia* spp. infections are the most frequent etiology. However, other described agents are associated, such as *S. epidermidis*, *E. cloacae*, *Salmonella* spp., *Pseudomonas* spp., *Klebsiella* spp., *S. maltophilia*, *F. noatuensis*, *Nocardia* spp., Bacille Calmette-Guerin infection, *Aspergillus* spp., *Candida* spp., visceral leishmaniasis and human herpes virus-6.[8] Histoplasmosis has been associated with MAS in patients with immunosuppressive agents, HIV, diabetes mellitus, renal transplantation or systemic lupus erythematosus.[11] To our knowledge, histoplasmosis has not been previously associated with MAS in CGD patients. The recognition of CGD as an etiology of MAS in female carriers of X-linked CGD with severe lyonization deviation has important diagnostic and therapeutic consequences. Steroids and high-dose immunoglobulin treatment might lead to the resolution of the hyperinflammation.[9]

Regarding treatment, XL-CGD carriers may benefit from antibiotic and antifungal prophylaxis, alongside regular medical review.[12] Regarding the treatment of infections and inflammatory and autoimmune manifestations, similar rules should apply for CGD patients and affected XL-CGD carriers. Hydroxychloroquine has been used in some XL-CGD carriers suffering from photosensitivity and lupus-like skin eruptions. Additionally, hematopoietic stem cell transplantation (HSCT) is a therapeutic option that could be explored for symptomatic XL-CGD carriers.[13]

XL-CGD carriers could present with severe clinical manifestations, especially those with extreme lyonization. Although patients with CGD do not have an increased genetic susceptibility to histoplasmosis, it can be fatal in association with MAS, as has been observed in the reported case.

STATEMENT OF ETHICS

Informed consent has been obtained from the patient involved in the study. The study protocol has received approval from the Research Ethical Committees of the participating centers. Ethics Committee Approval Code is 030/2020.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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