ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Intrathoracic tuberculosis in the pseudotumoral and bone form as a manifestation of chronic granulomatous disease

Tuberculose intratorácica na forma pseudotumoral e óssea como manifestação de doença granulomatosa crônica

Priscilla Filippo A. M. Santos[1], Antonio Condino-Neto[2], Lillian Nunes Gomes[2], Claudete Araújo Cardoso[3]

ABSTRACT

Chronic granulomatous disease (CGD) is an inborn error of phagocyte immunity and occurs as a result of mutations that affect components of the NADPH oxidase enzyme. Patients are susceptible to serious and lethal fungal and bacterial infections. The aim of this paper is to report a case an infant with CGD who presented clinical manifestations of intrathoracic tuberculosis (TB) in the pseudotumoral and bone form, which started in the neonatal period. The diagnosis of CGD was performed using the DHR test and, after starting prophylaxis with sulfamethoxazole-trimethoprim and itraconazole, the patient remained clinically stable. The mother and sister also had altered DHR, genetic analysis revealed an X-linked mutation in exon 2 of the CYBB gene c.58G>A, leading to an alteration in G20R. It is essential that the diagnosis is made as early as possible, in order to establish guidelines for Family members and adequate treatment, thus reducing infectious complications and improving prognosis.

Keywords: Chronic granulomatous disease, primary immunodeficiency diseases, tuberculosis.

RESUMO

A doença granulomatosa crônica (DGC) é um erro inato da imunidade de fagócitos, e ocorre em decorrência de mutações que afetam componentes da enzima NADPH oxidase. Os pacientes são suceptíveis a infecções graves e letais por fungos e bactérias. O objetivo deste trabalho é relatar o caso de um lactente com DGC que apresentou manifestação clínica de tuberculose (TB) intratorácica na forma pseudotumoral e óssea iniciada no período neonatal. O diagnóstico de DGC foi realizado através do teste de DHR e, após o início da profilaxia com sulfametoxazol-trimetroprima e itraconazol, o paciente manteve-se estável clinicamente. A mãe e a irmã também apresentaram DHR alterados, a análise genética revelou uma mutação ligada ao X no exon 2 do gene CYBB c.58G>A, levando uma alteração em G20R. É fundamental que o diagnóstico seja realizado o mais precocemente possível, a fim de instituir as orientações aos familiares e tratamento adequado, reduzindo assim complicações infecciosas e melhorando prognóstico.

Descritores: Doença granulomatosa crônica, doenças da imunodeficiência primária, tuberculose.

Introduction

Chronic granulomatous disease (CGD) is a heterogeneous genetic disease, which was first described in the 1950s. It is a rare inborn error of immunity, characterized by severe and recurrent infections, due to the functional impairment of the NADPH oxidase complex in monocytes and neutrophilic granulocytes.[1,2] This enzyme complex generates superoxide and is essential for the intracellular killing of pathogens, fungi and bacteria by phagocytes.In addition to having direct cytotoxic

1. Hospital Municipal Jesus, Specialty Ambulatory - Rio de Janeiro, RJ, Brazil.
2. Instituto de Ciências Biomédicas da Universidade de São Paulo, Department of Immunology - São Paulo, SP, Brazil.
3. Faculdade de Medicina da Universidade Federal Fluminense, Maternal and Child Department - Niterói, RJ, Brazil.

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effects, the production of reactive oxygen species appears to be important for other innate immune functions.The incidence is variable: 1:1 million in Italy,[2] 1:300,000 in Japan,[4] 1:250,000 in the United States[1] and 1: 70,000 in the Israeli Arab population.[5]

Lungs, skin, lymph nodes and liver are the most affected organs.[1] Patients with CGD may have tuberculosis (TB), which is an opportunistic infection with high worldwide prevalence, especially in tropical countries.[6,7] The development of disease by the TB bacillus depends on the interaction between the host's immunological factors and the aggressiveness of the infectious agent. TB in the pseudotumor form is a rare entity, the diagnosis is difficult and can be confused with primary or secondary neoplasm. Bacteriological samples are often negative, which can cause a delay in diagnosis and therapy.[6] The diagnosis of CGD can be made by the dihydrorhodamine test (DHR), which measures intracellular digestion of phagocytes, and is confirmed by genotyping studies.[8]

Recent studies have reported a survival rate of approximately 90% at 10 years of age, attributed to early diagnosis and institution of antibiotic prophylaxis, use of interferon-gamma, antifungal prophylaxis and hematopoietic stem cell transplantation.[9] However, the average age of death of these patients remains around 30 to 40 years.[10]

The aim of this study is to report a case of intrathoracic TB in the pseudotumor and bone form that is uncommon in the neonatal period as the first manifestation of chronic granulomatous disease. The project was approved by the Research Ethics Committee (CAAE: 46255021.4.0000.5259), and an informed consent form was signed by the patient's guardian.

Case Report

A 12-month-old boy was referred for immunological investigation with a history of intrathoracic tuberculosis (TB), in the pseudotumor and bone form (Figure 1). She received the BCG vaccine in the neonatal period, without complications. At 19 days of age, he was hospitalized with daily fever, edema and erythema of the first left finger. During the investigation of the child, there was no microbiological confirmation of *Mycobacterium tuberculosis*, but the radiological aspect of the pulmonary image made the diagnosis of TB, and at the time isoniazid, rifampicin and pyrazinamide were started, with regression of the pseudotumor and complete remission of the bone condition after 12 months of treatment. The parents and sister were investigated for TB, with normal chest X-ray and reactive tuberculin skin test, and treatment for latent tuberculosis infection (LTBI) was prescribed at the time. Tumor markers were negative. Subsequently, the patient was hospitalized twice for pneumonia, without the need for oxygen therapy, with positive PCR for SARS-CoV2 on the second hospitalization. Growth and development were age appropriate. There was no family history of inborn errors of immunity, recurrent miscarriages, and parental consanguinity. He had a BCG scar greater than 3 mm on physical examination, with no other changes.

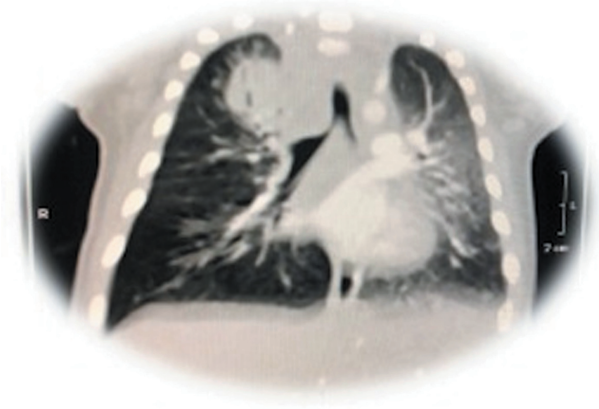

Figure 1
Chest CT angiography - intrathoracic tuberculosis pseudotumor form

Serology for HIV I and II, HTLV I and II were negative. Blood count showed anemia.Dglucose-6-phosphate dehydrogenase assay, Immunoglobulins, lymphocyte profile with normal results. Measles and mumps IgG reactive serologies. However,the dihydrorhodamine assay (DHR) showed an abnormal result of ROS production in granulocytes after stimulation and was suggestive of DGC (Figure 2).Prophylaxis with trimethoprim-sulfamethoxazole and itraconazole was started with good clinical outcome. HLA typing was requested and hematopoietic stem cell transplantation was indicated. The mother and sister had altered DHR, suggestive of carriers of X-linked CGD (Figure 2).

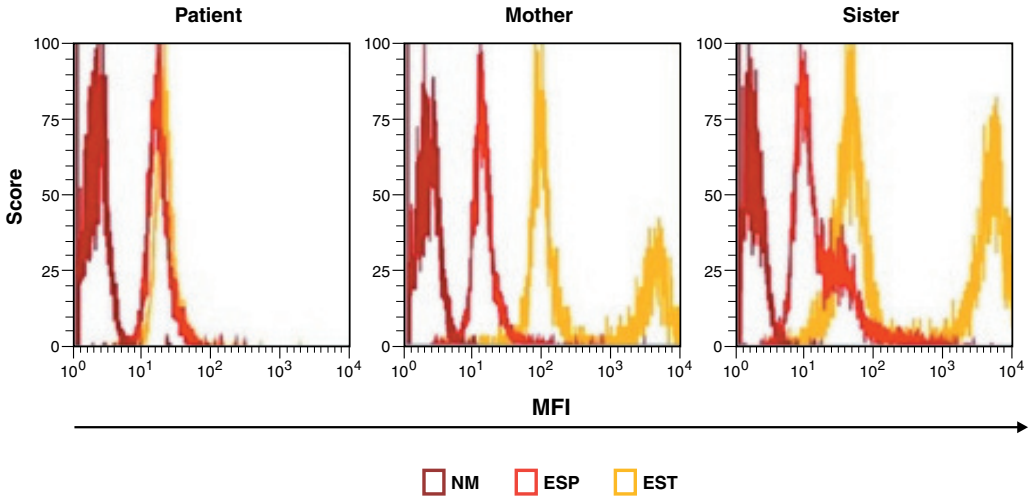

Figure 2
DHR test result. Spontaneous granulocyte (ESP) or PMA-stimulated (EST) and unlabeled, non-DHR (NM) granulocyte DHR from patient, mother, and sister samples, respectively. With altered patient result, and suggestive for X-linked CGD carrier

In addition, CYBB gene sequencing was performed to confirm the diagnosis of CGD. Sequence data were analyzed using the NCBI databases (www.ncbi.nlm.nih.gov), Ensembl SNP (https://www.ensembl.org/index.html), and the Mutation Taster (http://www.mutationtaster.org). This analysis confirmed a missense mutation (c.58G>A) in exon 2 that leads to a substitution of a single amino acid, G20R, in the gp91phox protein. The same mutation was observed in heterozygosity in the patient's mother and sister (Figure 3).

Discussion

CGD is a rare inborn error of immunity, with a variable incidence according to ethnicity, more prevalent in males (2:1), due to the predominant model of genetic transmission (X-linked disease).[2] The infant in the case report is male, and probably has an X-linked disease, since his mother and sister had altered DHR. However, defects in autosomal genes can also cause CGD in men and women.[11] X-linked CGD is more common in areas with miscegenation, while the autosomal recessive form is more common in areas with a history of inbreeding.[12] There is no report of consanguinity between the parents. Children with X-linked CGD have a more severe clinical presentation: earlier onset of the disease, frequent obstructions caused by granulomas, more frequent infections, and a higher mortality rate.[11] Patients with CGD have reduced expression and function of Toll-like receptors, complement receptors, and chemokine receptors that correlate with disease severity. The most common cause of CGD is a defect in the CYBB gene (gp91 phox), located on the short arm of the X chromosome (Xp21.1-p11.4).[2]

An Italian multicenter study showed that the most common infectious manifestations were pulmonary infection (pneumonia/abscess in 50%), dermatitis/subcutaneous abscess (46%), lymphadenitis (45%), osteomyelitis and liver abscess (16%) [3]. Arnold et al. observed that the lung, skin, lymph nodes and liver are the most affected organs.[1] Osteomyelitis is an important infection in GCD and can arise from the hematogenous spread of pathogens (*S. aureus*, *Salmonella spp.*, *S. marcescens*) or contiguous bone invasion.[1] Susceptibility to infections in these patients arises from the neonatal period to adulthood, and the outcome depends on prompt recognition and therapy for the underlying infection.[12]

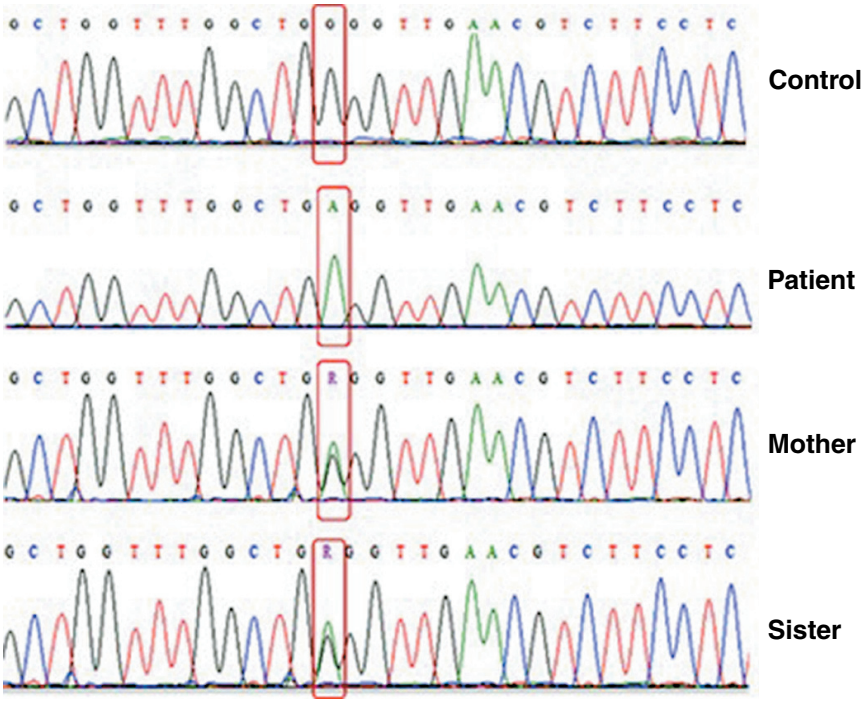

Figure 3
Sequencing results for CYBB gene. Chromatogram for the healthy, patient Control, her mother and her sister, from top to bottom, respectively. Shows in red highlight the single nucleotide substitution, c.58G>A, which results in a G20R change

In Brazil, a series of 18 patients identified as the most common manifestations of CGD: lymphadenopathy, hepatosplenomegaly, pneumonia and abscesses. A second study, with seven Brazilian patients, showed that pneumonia was the most frequent clinical manifestation, followed by skin infections, sinusitis, otitis and liver abscess.[13]

In Europe and North America, the most common pathogens are *Aspergillus spp.*, *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia spp.* and *Salmonella*.[1,3] In these places, mycobacterial infection is not common. CGD is the primary immunodeficiency in which invasive fungal infection is most common, affecting the lungs and chest wall.[1] In developing countries, Bacillus Calmette-Guerin (BCG) and *Mycobacterium tuberculosis* are the most important pathogens.[11] There are reports of mycobacterial infection after BCG and *Mycobacterium tuberculosis* in China, Iran and Latin America. However, patients develop a severe form localized and not disseminated by BCG and pulmonary and not miliary TB.[11]

In countries such as Brazil, where tuberculosis is endemic, BCG vaccination is carried out in the first months of life, and is usually applied in the neonatal period, and patients with immunodeficiency may have adverse reactions to BCG vaccination. A multicenter study (Latin America, Africa, Europe and Asia) carried out by Conti F. et al. showed that the adverse reaction to BCG was the first sign of the disease in 39 (55%) of the 53 children with this reaction. A local or regional reaction (BCG-ites) was reported in 33 (63%) of the 53 patients with an adverse reaction, and a disseminated reaction (BCG-osis) was observed in the other 20 (37%) patients.[14] Furthermore, patients with any form of CGD may fail to develop a protective immune response against *Mycobacterium* species, and may develop active TB at any stage of life. Clinically, BCG infection was in some cases the first manifestation of CGD in Brazil.[14]

In Argentina, Hong Kong and Iran, up to 11%, 54.5% and 31.7% of patients with CGD, respectively, had TB. Children with severe forms of tuberculosis should be investigated for inborn errors of immunity.[14]

In a study carried out by Oliveira Júnior et al. with data from LASID (Latin American Society for Immunodeficiencies), 71 patients with CGD were evaluated with the following distribution by country: 39% from Brazil, 36% from Argentina, 16% from Mexico, 6% from Chile and 3% from Colombia. Of the patients evaluated, 30% had a reaction to BCG with an increased prevalence in the group with mutation in the CYBB gene (85.7%), compared to the group with mutation in the NCF1 gene (14.3%). Of these patients who had a reaction to BCG, 30% had a disseminated reaction with a severe clinical picture.[13]

Patients with CGD may present alterations in the inflammatory response and autoimmunity.[1] Inflammatory manifestations are common in patients with CGD and are most often seen in the gastrointestinal tract, urogenital tract, lungs, and eyes.[2]

One of the characteristics of GCD is the formation of granulomas, which can cause clinical symptoms of obstruction, such as vomiting, dysphagia, slow gastric emptying, weight loss, bronchial obstruction, bladder obstruction, etc.[11] In the case reported, the first clinical manifestation was intrathoracic TB in the pseudotumor form, which regressed after the initiation of specific treatment.

Brazil is among the 30 countries with the highest TB burden in the world, with an incidence rate of 80,000 cases/year. Children account for 10% of total TB cases. Children under five years of age are at greater risk of becoming ill after primary infection when compared to adults and adolescents. The pulmonary form is the most frequent, highlighting the greatest potential for progression to severe forms of TB, such as meningoencephalitis and miliary. The BCG vaccine protects against these severe forms and in Brazil, its application is recommended at birth. TB patients should be screened for HIV.[15] The most frequent radiological manifestations in childhood are: lymph node enlargement (hilar or mediastinal), miliary disease (diffuse reticulonodular type), parenchymal disease (pulmonary condensation), atelectasis and pleural effusion (rarely in children under five). Alterations on computed tomography are lobar consolidations and hypodense areas, found in more than 80% of cases, and, in less than 25%, cavitations.[16] Computed tomography angiography of the patient's chest showed a mediastinal mass with a hypodense center and a small excavation inside, associated with hilar, paratracheal, and infracarinal adenopathy suggestive of TB.

The diagnosis of TB in 80% of cases in children younger than 5 years is made without bacteriological evidence, due to the paucibacillary characteristic of the disease in the pediatric age group, which makes it difficult to perform sputum bacterioscopy in younger children.[16] At the time of the diagnosis of TB in the child, one should always research the history of the disease in the contacts, in order to identify the source case of TB. In the case reported, the parents and the sister did not have the disease. The patient had no bacteriological confirmation, and presented complete regression of the clinical picture with the institution of treatment with a RIP regimen.

Marine B. et al. reported that the diagnosis of CGD was made at an average age of 4.4 years (median 2.5 years, range 0 to 38 years) and the mean age of symptom onset was 1 year (median 7.5 years, range from 0 to 10 years).[3] The patient started symptoms early, in the neonatal period, and the diagnosis of CGD was performed at 13 months, through the DHR, which was performed and repeated after 15 days. The DHR is a very sensitive and specific assay that reliably detects all NADPH oxidase deficiencies in neutrophils.[18] Early diagnosis is essential for the proper institution of treatment, with the use of prophylactic antibiotics and antifungal agents, avoiding infectious complications, hospitalizations and interfering with the patient's quality of life. In addition, it is important to find the genetic mutation, for genetic counseling and eventually choose a suitable bone marrow donor or perform gene therapy.[10,17] In Brazil, we still do not have gene therapy available.

In some cases, parents of a CGD patient may consider having an additional child to serve as a hematopoietic stem cell donor for the affected sibling.[12] In this case report, the parents, in principle, do not think about having more children. Thinking about genetic counseling, DHR and maternal and sister genetic analysis were requested. The results of the DHRs had altered results and genetics confirmed that both have the same variant found in the patient (c.58G>A), in the heterozygous form. This is a missense mutation in exon 2 of the CYBB gene, resulting in the p.G20R amino acid change in the N-terminal domain. This is an already described mutation.[18] The dataof this study highlight the relevance of the diagnosisfor definitive family counseling, since a new pregnancy of the

mother has a 50% chance of having a boy with CGD, the same for future pregnancies of the sister.

Long-term prophylactic use with trimethoprim-sulfamethoxazole or doxycacilcin and itraconazole has been shown to reduce infections in patients with CGD, and is recommended during infection-free periods.[19,20] From the beginning of prophylaxis with these medications, the patient was clinically stable, without further hospitalizations due to infectious complications. It is also recommended to prevent infections through immunizations.[11]

BCG is a mandatory vaccine in Latin America. It is usually given during the neonatal period. This practice is potentially harmful for patients with DCG, SCID, or another immunodeficiency that affects phagocytes or T cell function. Newborn screening tests for DCG and other phagocyte defects should be performed prior to BCG vaccination.[13]

In Mexico and Argentina, patients with CGD have access to routine interferon-gamma therapy, given subcutaneously, three times a week. In Brazil, this drug is not registered by the national regulatory agency, limiting access for use.[13]

In the study by Oliveira-Junior et al. Bone marrow transplants have been performed in patients in Brazil, Mexico and Argentina, depending on the availability of a compatible donor. Gene therapy for primary immunodeficiencies is not yet available in Latin America.[13]

Agudelo-Flórez et al. described a series of 14 patients with CGD from Latin America (10 Brazilians, 2 Chileans and 2 Mexicans), in which all of them started to become infected before 2 years. None had a serious reaction to BCG. All were on prophylactic use of sulfamethoxazole/trimethoprim, 11 were on itraconazole and two were regularly given recombinant gamma interferon.[21]

Other differential diagnoses should be excluded, such as G6PD deficiency, because this deficiency in the severe form can lead to insufficient formation of NADPH in leukocytes, hindering the activity of NADPH oxidase.[11]

Martire B. et al. reported a survival rate of 97%, 83%, and 46% at 10, 20, and 25 years, respectively, from the diagnosis of the disease.[3]

We report a rare case of an infant with a pseudotumor and bone form of TB, and it is important to rule out other causes with similar clinical and imaging findings and to investigate inborn errors of immunity in these cases, which is relevant for early treatment and genetic counseling.

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Corresponding author:
Priscilla Filippo A. M. Santos
E-mail: priscillaalvim05@gmail.com