ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Chronic granulomatous disease: clinical characteristics, follow-up, and therapy of 5 pediatric patients

Doença granulomatosa crônica: características clínicas, seguimento e terapêutica de cinco pacientes pediátricos

Karina Mescouto de Melo[1], Ludmila Gonçalves Ribeiro[1], Claudia França Cavalcante Valente[1], Carmem Maria Sales Bonfim[2], Antonio Condino-Neto[3], Shirley Claudino Pereira Couto[4], Maria Imaculada Muniz-Junqueira[4], Simone Castro Resende Franco[5], Thalita Dias[6], Fabíola Scancetti Tavares[1]

ABSTRACT

Introduction: Chronic granulomatous disease (CGD) is characterized by a defective microbicidal capacity of phagocytic cells (monocytes and neutrophils) with high mortality if not early diagnosed. Patients have recurrent or severe infections and are susceptible to granulomas in visceral organs, autoimmune diseases, and inflammatory bowel diseases. **Objective and Method:** To report the clinical features and treatment of 5 patients with CGD. **Results:** Five patients, 3 boys, with median ages at symptom onset and diagnosis of 8 months and 48 months, respectively, were followed for 10 years. Pneumonia (5/5) and mycobacterial disease (3/5) were the most common initial manifestations. Pulmonary changes were observed in all cases. Mutations in the *CYBB* and *NCF1* genes were identified in 3 cases. All patients received antibiotic prophylaxis. Three patients underwent a hematopoietic stem cell transplant (HSCT) at 7, 18, and 19 years, with current survival of 4 to 5 years. **Conclusion:** Careful monitoring for severe infection with prompt treatment was crucial for survival. Even though HSCT was performed in late adolescence, it promoted the cure of CGD in 3 patients.

Keywords: Chronic granulomatous disease, phagocyte, NADPH oxidases, bone marrow transplantation.

RESUMO

Introdução: A doença granulomatosa crônica (DGC) é caracterizada por um defeito na capacidade microbicida das células fagocíticas (monócitos e neutrófilos), com alta mortalidade se não diagnosticada precocemente. Os pacientes apresentam infecções recorrentes ou graves, suscetibilidade a granulomas em órgãos profundos, doenças autoimunes e doença inflamatória intestinal. **Objetivo e Método:** Relato de aspectos clínicos e do tratamento de cinco pacientes com doença granulomatosa crônica. **Resultados:** Cinco pacientes, três meninos, medianas de idade no início dos sintomas e diagnóstico de 8 meses e 48 meses, respectivamente, foram estudados por um período de 10 anos. Pneumonia (5/5) e doença micobacteriana (3/5) foram as manifestações iniciais mais comuns. Alterações pulmonares foram observadas em todos os casos. Mutações nos genes *CYBB* e *NCF1* foram identificadas em três casos. Antibioticoprofilaxia foi instituída em todos os pacientes e três foram submetidos ao transplante de células tronco-hematopoiéticas (TCH), aos 7, 18 e 19 anos e com sobrevida atual entre 4 a 5 anos. **Conclusão:** O monitoramento cuidadoso de infecções graves com tratamento imediato foi crucial para a sobrevivência. O TCH, mesmo ao final da adolescência, promoveu a cura da DGC em três pacientes.

Descritores: Doença granulomatosa crônica, fagócitos, NADPH oxidases, transplante de medula óssea

1. Hospital da Criança de Brasília José Alencar, Allergy and Immunology Service - Brasília, DF, Brazil.
2. Hospital de Clínicas da Universidade Federal do Paraná, Bone marrow transplant service - Curitiba, PR, Brazil.
3. Universidade de São Paulo, Department of Immunology, Institute of Biomedical Sciences - São Paulo, SP, Brazil.
4. Universidade de Brasília, Cellular Immunology Laboratory, Faculty of Medicine - Brasília, DF, Brazil.
5. Hospital da Criança de Brasília José Alencar, Onco-hematology Service - Brasília, DF, Brazil.
6. Hospital de Base do Distrito Federal Brasília, Allergy and Immunology Service - Brasília, DF, Brazil.

Submitted Aug 02 2023, accepted Aug 24 2023.

Arq Asma Alerg Imunol. 2023;7(3):267-72.

http://dx.doi.org/10.5935/2526-5393.20230039-en

Introduction

Chronic granulomatous disease (CGD) is a severe primary immunodeficiency or inborn error of immunity characterized by defective microbicidal function in phagocytic cells (neutrophils and monocytes).[1,2] CGD has an estimated incidence of 1 in 200,000 live births.[2] Symptom onset occurs in the first years of life, with severe infections in the skin, respiratory tract, lymph nodes, liver, and bones, in addition autoimmune diseases and susceptibility to granuloma formation in many organs.[2-4]

CGD is caused by mutations in a gene that encodes proteins of the nicotinamide adenine dinucleotide oxidase enzyme complex in phagocytic cells. The *CYBB* gene, which encodes the gp91phox protein, causes X-linked CGD, and approximately 70% of CGD cases are X-linked.[1,2] There are also autosomal recessive forms resulting from mutations in the *NCF1*, *NCF2*, *CYBA*, *NCF4*, and *CYBC1* genes.[1,3]

Diagnosis is based on: (I) clinical criteria, including severe bacterial and/or fungal infection (abscesses, osteomyelitis, lymphadenitis), recurrent pneumonia, lymphadenopathy and/or hepatomegaly and/or splenomegaly, obstructive/diffuse granulomas (gastrointestinal or urogenital tract), and chronic inflammatory manifestations (colitis, liver abscess, and fistula formation)[4]; and (II) laboratory criteria, including phagocyte function according to the nitro blue tetrazolium test (optical microscopy) or a flow cytometry assay using dihydrorhodamine,[2,4] or identifying mutation in a gene associated with CGD.[1-3]. or a definitive diagnosis, at least 1 clinical and 1 laboratory criterion are necessary.[4]

Conventional treatment for CGD is prophylaxis with trimethoprim-sulfamethoxazole (5 mg/kg/day of trimethoprim) and itraconazole 100 mg daily for patients < 13 years old or < 50 kg; 200 mg daily for those ≥ 13 years or ≥ 50 kg.[2,3] However, the only curative treatment is hematopoietic cell transplantation (HCT).[2,3] In Brazil, data on HCT in CGD are scarce.[5,6] In this study, we describe 10-year clinical follow-up in 5 pediatric cases, highlighting successful HCT in 3 patients.

Description

Five patients with CGD were followed up in the immunology service of a tertiary pediatric hospital in the Federal District. Data were collected from patient records between 2011 and 2021. The confirmatory tests were performed in cooperation with the University of Brasília's cellular immunology laboratory for the nitro blue tetrazolium test and the University of São Paulo's human immunology laboratory for the dihydrorhodamine tests and genetic analysis. This study was approved by the Plataforma Brasil Research Ethics Committee.

Clinical features

Three of the 5 patients were boys (60%); the median age at symptom onset and diagnosis was 8 and 48 months, respectively. Severe pneumonia was the main initial clinical manifestation of the disease, being observed in all cases. One child (P-1) had a history of early deaths in the family due to pneumonia (2 maternal uncles, 5 maternal cousins, and 1 brother) (Table 1). One patient (P-3) was referred from the onco-hematology service due to autoimmune hemolytic anemia associated with severe pneumonia. Prior to diagnosis, all patients required hospitalization due to infection; all are currently alive.

Complementary laboratory tests

In the initial immune system assessment, hypergammaglobulinemia[7] was observed in 4 of the 5 patients. CD3+ T lymphocytes were elevated in 2 patients (P-1 and P-3), and the absolute number of natural killer cells[8] was low in all 5 (Table 1). Tests to assess the microbicidal capacity of neutrophils (nitro blue tetrazolium or dihydrorhodamine) and/or genetic sequencing were performed to confirm diagnosis in all patients (Table 1). Two patients presented the following mutations in the CYBB gene: c.951T>A, in exon 8 (P-1) and c271c>T in exon 3 (P-4), confirming the diagnosis of X-linked CGD. One patient (P-3) presented the autosomal recessive form, with a c.75_76delGT mutation in exon 2 of the *NCF1* gene.

Complementary radiological exams

In all patients, pulmonary sequelae were assessed with chest computed tomography at diagnosis and every 2 years thereafter. All patients had abnormal results on examination, and 3 have had them since diagnosis (atelectasis [P-1] and pneumonia [P-2 and P-3]). The most recent radiological findings included: bronchiolitis obliterans (P-1 and P-5), bronchiectasis (P-2 and P-3), ground glass opacity (P-1, P-3 and P-4), and pulmonary nodules (P-1 and P-4).

Table 1
Clinical and laboratory characteristics of patients with chronic granulomatous disease treated at a pediatric hospital in the Federal District of Brazil

Patient	P-1	P-2	P-3	P-4	P-5
Sex	M	F	F	M	M
Current age (years)	22	21	11	23	21
Consanguinity	No	No	Yes	No	No
Family history	Yes	No	No	No	No
Onset of symptoms	18 months	8 months	23 dias	4 dias	12 months
Age at diagnosis	48 months	96 months	36 months	9 months	228 months
Initial clinical picture	PNM	PNM, TB, sepsis, fungal meningitis	UTI, PNM+DP AHA	Reaction to BCG vaccine, UTI, PNM	PNM, intestinal TB
Clinical picture at follow-up	TB, abscess, esophageal granuloma, fungal PNM	Osteomyelitis	PNM +DP Skin abscess Fungal PNM	Skin abscess PNM Pulmonary granuloma	Oral thrush BOOP
Microorganism	Gram positive *Neisseria spp.*	*C. albicans* Zigomiceto *Aspergillus sp.*	*S. epidermidis, B. cepacea*	*Enterobacter S. aureus E.coli C. albicans*	NI
Leukocyte /µL	18,200	5500	10,400	6300	9500
Lymphocyte /µL	6734	1300 ↓	6400	2268	2565
Neutrophil/µL	10,010	3600	2500	3213	5510
IgG (mg/dL)	1020	2290 ↑	1510 ↑	1043 ↑	1640 ↑
IgM (mg/dL)	161 ↑	286 ↑	286 ↑	82	146
IgA (mg/dL)	332 ↑	357 ↑	277 ↑	226	641 ↑
IgE (mg/dL)	3.7	2240 ↑	26.3	410	274
CD3+/µL	2217 ↑	1009 ↓	2849	1083 ↓	ND
CD4+/µL	966	512 ↓	1406	560 ↓	494
CD8+/µL	1209 ↑	560	1226	464	227 ↓
CD19+/µL	179	16 ↓	188 ↓	260 ↓	121 ↓
CD56+/µL	131 ↓	48 ↓	14 ↓	125 ↓	28 ↓
Functional test	NBT + DHR	NBT + DHR	NBT + DHR	NBT	DHR
Mutated gene	*CYBB*	–	*NCF1*	*CYBB*	–

AHA = autoimmune hemolytic anemia, BOOP = bronchiolitis obliterans organizing pneumonia, DHR = dihydrorhodamine oxidation test, NBT = nitro blue tetrazolium, NI = not isolated, PD = pleural effusion, PNM = pneumonia, TB = tuberculosis, UTI = urinary tract infection; ↑ and ↓ = values above or below the reference standard for the Brazilian population.[7,8]

Follow-up and therapy

In all patients, prophylaxis with trimethoprim-sulfamethoxazole and antifungals was applied according to recommendations.[2] Itraconazole was the antifungal of choice. Ketoconazole and fluconazole were initially used in two patients (P-1 and P-2) and were later replaced with itraconazole. Liposomal amphotericin B and voriconazole were prescribed as specific treatments for disseminated fungemia and fungal pneumonia in 2 patients (P-1 and P-2) (Table 1). Interferon-gamma was used in 2 patients (P-1 and P-4). All patients had infectious conditions during follow-up that required short-term therapeutic intervention.

Three of the patients had a confirmed genetic defect and a more severe clinical condition (P-1, P-3 and P-4; aged between 7 and 19 years) and underwent HCT at the Federal University of Paraná University Hospital (Table 2). Their current post-HCT survival is 4 years and 4 months (P-1), 4 years and 11 months (P-3), and 5 years (P-4). In all cases, the graft was accepted without immunosuppressive treatment. One patient had a late rejection episode and underwent a successful second transplant. All patients had normal dihydrorhodamine test results after HCT.

Table 2
Patients with chronic granulomatous disease (CGD) who underwent hematopoietic cell transplantation

Patient	P-1	P-3	P-4
Sex	M	F	M
HCT age (years)	19	7	18
Donor type and compatibility	Haploidentical, compatible 5/10	Unrelated, compatible 10/10	Unrelated, compatible 10/10
Cell source	PBSC	BM	BM
Conditioning	BU+FLU+ATG+TBI 200 rads	BU+FLU+ATG	BU+FLU+ATG
GVHD prophylaxis	CY-pós+SIR+MMF	CSP +MTX	CSP+MTX
Post-HCT complications	Acute GVHD in skin and liver (grade II)	Acute skin GVHD, CMV reactivation, SAH	Graft failure/ retransplantation
Post-HCT time	4y 4m	4y 11m	5y
Current situation	Alive; 100% donor cells, clinically stable, no immunosuppressants, asthma	Alive; 100% donor cells, no immunosuppressants, bronchiolitis obliterans, asymptomatic AHA pre-BMT	Alive, 100% donor cells, clinically asymptomatic, no immunosuppressants

AHA = autoimmune hemolytic anemia, BMT = bone marrow transplant, BU = busulfan, CMV = cytomegalovirus, CSP = cyclosporine, CTSP PBSC = peripheral blood stem cell, CY = cyclophosphamide, FLU = fludarabine, GVHD = graft-versus-host disease, HCT= hematopoietic cell transplantation, MMF = mycophenolate mofetil, BM = bone marrow, MTX = methotrexate, SAH = systemic arterial hypertension, SIR = sirolimus, TBI = total body irradiation.

Discussion

Although the prevalence of CGD has been estimated at 1 in 200,000 in the United States and Europe[2,3], it is still unknown in Brazil. In a series of 71 CGD cases in Latin America (39% Brazilian), Oliveira-Junior et al.[9] reported a lower rate based on Brazil's population size and international statistics. The present study included 5 patients with CGD, most of them male, with symptom onset in the first year of life but diagnosis around 4 years of age. This is relevant, since CGD leads to serious (and often fatal) infections and/or sequelae in various organs, especially the lungs, early in life. The diagnostic delay may be due to the fact that patients have difficulty accessing professionals who are knowledgeable about the clinical manifestations of inborn errors of immunity, as observed by Dantas et al. when they interviewed physicians about the relevant warning signs.[12]

All patients had respiratory tract infections, some of them with serious clinical manifestations, such as the pleural effusion and fungal pneumonia. The spread of fungal infection to other organs, mainly by *Aspergillus spp.*, has a high mortality rate and is common in patients with CGD.[2,10] In this study, however, *C. albicans* was the most common species, which may be related to patient characteristics or technical difficulties in isolating other fungi. There were no cases of liver abscess, which are commonly described in patients with CGD.[2,3] However, *E. coli* and *Enterobacter spp.* were observed, which are uncommon in CGD.

Mycobacterial infection, either by *M. tuberculosis*, which causes tuberculosis, or by *M. bovis*, which can be introduced through the BCG vaccine, is part of the clinical spectrum of CGD in countries where tuberculosis is endemic, as was observed in this study.[9,11] A severe adverse reaction to the BCG vaccine is also an early clinical manifestation of other inborn errors of immunity, such as severe combined immunodeficiency.[9,13] In countries where the vaccine is mandatory, such as Brazil, it has a major impact on patient survival. Thus, there have been proposals to postpone applying the vaccine in children until immune disorders have been ruled out.[9,11,13] The lung abnormalities observed in chest computed tomography generally result from pneumonia cases occurring prior to diagnosis, in addition to a chronic inflammatory process, and can influence patient survival and quality of life.[2,3] Gastrointestinal manifestations, such as esophageal granuloma, are common, although, unlike other studies, we found no cases of colitis.[2,3,10]

All patients received prophylaxis with trimethoprim-sulfamethoxazole associated with antifungals, and there were no relevant adverse effects. Prophylaxis with trimethoprim-sulfamethoxazole has been an established practice for more than 40 years and is effective in reducing infections.[2,3] Since the 1990s, antifungals, especially itraconazole, have been part of the therapeutic arsenal for CGD, considerably reducing deaths due to fungal pneumonia.[2,14]

Three patients underwent HCT. Brazilian studies on HCT for CGD are still scarce.[5,6] HCT is indicated for all patients with poorly controlled CGD and for those with significant morbidity, such as recurrent life-threatening infections or progressive lung disease.[14] Among patients with CGD, survival after HCT has increased due to several factors, such as reduced-toxicity conditioning, better cell sources and donor selection, advances in antimicrobial therapy, and younger age at HCT.[6,14,15] However, the procedure is still considered high-risk and is associated with significant morbidity and mortality, especially for patients under 14 years of age.[14,15] Two of the transplant patients were teenagers with several comorbidities and a high risk of both short- and long-term complications. They received reduced-toxicity conditioning and the graft was successful. Two had mild graft-versus-host disease, which was controlled with corticosteroids, and they are not immunosuppressed. The other patient developed secondary graft failure, which was successfully resolved with a second transplant. This patient is also alive and has been clinically stable for more than 4 years since transplantation.

Conclusion

Since CGD is a serious disease with onset of symptom in childhood, diagnostic suspicion and early therapeutic intervention can increase survival. Careful monitoring for serious infections and prompt treatment are crucial for long-term survival. Periodic evaluation of pulmonary changes is suggested for all patients. In Brazil, HCT can be considered a viable treatment for patients with CGD, even in late adolescence.

References

1. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. Clin Immunol. 2020;40,24-64.

2. Arnold DE, Heimall JR. A Review of Chronic Granulomatous Disease. Adv Ther. 2017;34(12):2543-57.
3. Holland SM. Chronic Granulomatous Disease. Hematol Oncol Clin North Am. 2013 Feb;27(1):89-99, viii.
4. ESID Registry - Working definitions for clinical diagnosis of PID [internet]. Available from: https://esid.org/Working-Parties/Registry-Working-Party/Diagnosis-criteria.
5. Fernandes JF, Kerbauy FR, Ribeiro AAF, Kutner JM, Camargo LFA, A Stape, et al. Transplante alogênico de células-tronco hematopoéticas em crianças com imunodeficiências primárias: a experiência do Hospital Israelita Albert Einstein. Einstein. 2011 Apr 1;9(2 Pt 1):140-4.
6. Fernandes JF, Nichele S, Arcuri LJ, Ribeiro L, Zamperlini-Netto G, Loth G, et al. Outcomes after Haploidentical Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Patients with Primary Immunodeficiency Diseases. Biol Blood Marrow Transplant. 2020 Oct;26(10):1923-9.
7. Fujimura MD. Níveis séricos das subclasses de IgG em crianças normais e nefróticas [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 1991.
8. Moraes-Pinto MI, Ono E, Santos-Valente EC, Almeida LC, Andrade PR, Dinelli MI, et al. Lymphocyte subsets in human immunodeficiency virus-unexposed Brazilian individuals from birth to adulthood. Mem Inst Oswaldo Cruz. 2014;109(8):989-98.
9. Oliveira-Junior EB, Zurro NB, Prando C, Cabral-Marques O, Pereira PV, Schimke L, et al. Clinical and Genotypic Spectrum of Chronic Granulomatous Disease in 71 Latin American Patients: First Report from the LASID Registry. Pediatr Blood Cancer. 2015 Dec;62(12):2101-7.
10. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic Granulomatous Disease: the European experience. PLoS One. 2009;4(4):e5234.
11. Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira Jr E, et al. Mycobacterial disease in patients with Chronic Granulomatous Disease: a retrospective analysis of 71 cases. J Allergy Clin Immunol. 2016 Jul;138(1):241-248.e3.
12. Dantas EO, Aranda CS, Nobre FA, Fahl K, Mazzucchelli JTL, Felix E, Friedlander-Del Nero DL, et al. Conhecimento médico sobre as imunodeficiências primárias na cidade de São Paulo, Brasil. Einstein. 2013;11(4):479-85.
13. Mazzucchelli JTL, Bonfim C, Castro G, Condino-Neto A, Costa NMX, Cunha L. Severe Combined Immunodeficiency in Brazil: management, prognosis, and BCG-Associated complications. J Investig Allergol Clin Immunol. 2014;24(3):184-91.
14. Segal BH, Veys P, Malech H, Cowan MJ. Chronic granulomatous disease: lessons from a rare disorder. Biol Blood Marrow Transplant. 2011 Jan;17(1 Suppl):S123-31.
15. Connelly JA, Marsh R, Parikh S, Talano JA. Allogeneic hematopoietic cell transplantation for Chronic Granulomatous Disease: controversies and state of the art. J Pediatric Infect Dis Soc. 2018 May 9;7 (Suppl 1):S31-S39.

No conflicts of interest declared concerning the publication of this article.

Corresponding author:
Karina Mescouto de Melo
E-mai: karinamescouto@gmail.com