

## Case Report

# Case report of myeloperoxidase deficiency associated with disseminated paracoccidioidomycosis and peritoneal tuberculosis

**Maurício Domingues-Ferreira<sup>[1]</sup>, Ariel Levy<sup>[1]</sup>, Noac Chuffi Barros<sup>[1]</sup>, Dalton Luis Bertolini<sup>[1]</sup> and Dewton de Moraes Vasconcelos<sup>[1]</sup>**

[1]. Ambulatório das Manifestações Cutâneas das Imunodeficiência Primárias, Departamento de Dermatologia, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil.

### Abstract

Myeloperoxidase (MOP) is present in monocyte and neutrophil lysosomes, catalyzing hydrogen peroxide and chloride ion conversion to hypochlorous acid. MOP seems to destroy pathogens during phagocytosis by neutrophils and is considered an important defense against innumerable bacteria. We present a patient who had MOP deficiency, who presented with a subacute form of paracoccidioidomycosis and later with peritoneal tuberculosis. MOP deficiency leads to the diminished destruction of phagocytized pathogens. This case gives important evidence of an association between MOP deficiency and increased susceptibility to infection by *Paracoccidioides brasiliensis* and *Mycobacterium tuberculosis*.

**Keywords:** Myeloperoxidase deficiency. *Paracoccidioides brasiliensis*. *Mycobacterium tuberculosis*.

### INTRODUCTION

The enzyme myeloperoxidase (MOP) is present in the lysosomes of monocytes and neutrophils. Its main function is believed to be the destruction of pathogens through the process of phagocytosis. MOP catalyzes the conversion of hydrogen peroxide and chloride ions into hypochlorous acid<sup>1</sup>. It has been suggested that neutrophils use this process as a crucial mechanism in defending the body against innumerable bacteria<sup>2</sup>. Hypochlorous acid is a very potent agent in bacterial lysis that is released by lysosomal granules, which also contain other products of the respiratory burst, such as superoxide, hydrogen peroxide and similar reactive oxygen species that are fundamental to microbial destruction during phagocytosis. Hypochlorous acid is 50 times more potent at achieving microbial destruction than hydrogen peroxide. The MPO-H<sub>2</sub>O<sub>2</sub>-Cl system seems to play a fundamental role in microbial death<sup>2</sup>. These substances are essential for the process of phagocytosis. In addition to killing bacteria, the products of the MPO-H<sub>2</sub>O<sub>2</sub>-Cl system also play a fundamental role in the destruction of fungi, parasites, protozoa, viruses, and tumor cells<sup>3</sup>. Paracoccidioidomycosis, caused by the fungus *Paracoccidioides brasiliensis* is the most important and prevalent systemic mycosis in Latin America; Brazil accounts

for 80% of cases reported worldwide. It has been proposed that neutrophils play an essential defensive role against this fungus based on the number of neutrophils detected in *P. brasiliensis* granulomas. Another important observation was the verification of a functional deficiency of neutrophils against *P. brasiliensis* in individuals susceptible to this infection<sup>4</sup>. The pathogenicity of another infectious agent, *Mycobacterium tuberculosis*, has been associated with the ability to escape the bactericidal activity of macrophages. In this case, *M. tuberculosis* can reside within phagosomes without being destroyed. *M. tuberculosis* has been shown to inhibit phagosome maturation in phagolysosomes, diminishing lysosomal acidification and the fusion of lysosomes with phagosomes<sup>5</sup>.

This report describes a patient who developed tuberculosis while undergoing treatment for paracoccidioidomycosis. Once her symptoms had subsided, she was referred for tests to assess for a possible immunodeficiency.

### CASE REPORT

The patient, a white 20-year-old woman, was born in the State of Minas Gerais, Brazil. There was no indication of inbreeding in her family. She showed normal psychomotor development and had no major illnesses during her childhood up to the age of 13 years. She lived in an urban area, but sometimes went to rural areas, and had never been in contact with tuberculosis patients. At this time, she developed cervical, axillary and inguinal polyadenopathy, and weight loss, but no fever, for about six months. She was referred for a lymph-

**Corresponding author:** Prof. Maurício Domingues Ferreira.

**e-mail:** madofe@uol.com.br

**Received** 24 November 2016

**Accepted** 7 April 2017

**TABLE 1**  
Phenotyping of the patient's lymphocytes and the reference ranges.

	Number of cells	Reference
Total leukocytes	7,810	5,000-10,000 cells/mm <sup>3</sup>
Lymphocytes	2,038	1,000-3,000 cells/mm <sup>3</sup>
CD3+ T Lymphocytes	1,592	605-2,460 cells/mm <sup>3</sup>
CD4+ T Lymphocytes	882	493-1,666 cells/mm <sup>3</sup>
CD8+ T Lymphocytes	687	224-1,112 cells/mm <sup>3</sup>
CD19+ B Lymphocytes	169	72-520 cells/mm <sup>3</sup>
CD3- CD16+ CD56+ (Natural killer cells)	259	73-654 cells/mm <sup>3</sup>

**CD:** cluster of differentiation.

node biopsy which showed granulomas with central necrosis and the presence of giant multinucleated cells and fungal structures that were identified as *Paracoccidioides* spp. She was diagnosed with a subacute form of paracoccidioidomycosis. She was prescribed fluconazole (600mg/day) resulting in a quick remission of the mycosis. However, five days after the start of treatment, one of the lymph nodes in the right cervical chain fistulized and a purulent, bloody discharge was expelled for 15 days. She continued using this medication. At the age of 14, she developed ascites, weight loss, and vomiting. Peritoneal tuberculosis was diagnosed following a biopsy, and rifampicin, isoniazid and pyrazinamide were prescribed. She never presented with pulmonary tuberculosis and was treated for a year with a complete resolution of the symptoms. She was subsequently referred to our department for assessment regarding a possible immunodeficiency. Her human immunodeficiency virus (HIV) serology test yielded negative results, and she showed normal levels of glucose 6-phosphate dehydrogenase. Flow cytometric phenotyping showed normal expression of cluster of differentiation (CD) CD3+, CD4+, CD8+, CD19+, CD3- CD16+ CD56+ (Table 1), and normal expression of interleukin 12 (IL-12) receptors and interferon (IFN)-gamma in monocytes and lymphocytes. Her dihydrorhodamine assay was normal, as were the lymphocyte proliferation assays for mitogens, phytohemagglutinin, pokeweed, anti-CD3 antibody, gp43 antigen, *Candida*, purified protein derivative (PPD), toxoplasmosis, tetanus toxoid, and cytomegalovirus. Her immunoglobulin, ferritin, and glucose levels were normal.

Flow cytometric immunophenotyping showed an expression level of 100% for CD45+ cells, with a partially positive expression of CD33+, strong expression of CD15+, and weak expression of CD13+ and MPO antigens. In the immunocytochemical assay, 97% of neutrophils with low MPO activity was detected. The opposite should be the norm; in healthy individuals, most of the neutrophils have high MPO activity. Immunophenotyping

showed the presence of MPO, though at reduced levels, and immunocytochemistry demonstrated that the function of MPO was greatly reduced.

## DISCUSSION

Our patient experienced two consecutive systemic granulomatous infections, a subacute form of paracoccidioidomycosis and peritoneal tuberculosis. The systemic nature of both infections and atypical localization of her tuberculosis suggested an immunological defect. During the investigation, abnormal expression of the receptors of the interleukin-12/interferon gamma (IL-12/IFN- $\gamma$ ) axis was not observed. This abnormality, if it had occurred, would easily explain this susceptibility because immune defects in the IL-12/IFN-gamma axis lead to a high susceptibility to mycobacterial disease, systemic fungal infections and dissemination, and salmonellosis<sup>6</sup>. Immunological parameters, such as mitogen and antigen lymphoproliferation assays, were normal, including positive proliferative response to glycoprotein 43 (gp43) (an important *P. brasiliensis* antigen) and PPD. These positive responses suggest that the patient's T cells respond to and proliferate appropriately upon exposure to the antigens of *M. tuberculosis* and *P. brasiliensis*. We therefore did not consider a T lymphocyte (cellular immunity) defect, as confirmed by the lymphoproliferation assay. The only serious abnormality was that of MOP activity, which was extremely low. MOP is an essential enzyme in the production of one of the most potent compounds, hypochlorous acid<sup>1,2</sup>, which is particularly important in the process of removing pathogens phagocytized by neutrophils and monocytes. A defect in the production of this enzyme could cause a malfunction in the phagocytosis of monocytes and neutrophils, thereby favoring infection. Kutter et al.<sup>3</sup> demonstrated that MOP-deficient patients show a higher prevalence of serious infections and inflammatory processes.

A study by Dias et al.<sup>4</sup> demonstrated a defective neutrophil function against *P. brasiliensis* in susceptible individuals. This study showed that the neutrophils of patients treated for

paracoccidiodomycosis degenerated during phagocytosis, which suggests that this immunological defect could be the basis for susceptibility to infection by *P. brasiliensis* in certain individuals<sup>4</sup>. In another study, Gohman-Yahr et al.<sup>7</sup> reported that peripheral blood polymorphonuclear neutrophils (PMN) from patients with paracoccidiodomycosis were much less capable of destroying *P. brasiliensis* than PMN from normal individuals or those with other diseases. This neutrophil dysfunction is caused by a defect in the digestion of *P. brasiliensis* after phagocytosis by neutrophils<sup>7</sup>. Using an animal model, Meloni-Bruneri et al.<sup>8</sup> demonstrated parallels between the defective production of reactive oxygen species in PMN and lower fungicidal activity for paracoccidiodomycosis. *Mycobacterium tuberculosis* is an intracellular pathogen that is also subject to the action of superoxides produced by macrophages when phagocytized. When the production of superoxides is insufficient, *Mycobacterium* resistance to phagocytosis can occur<sup>9</sup>.

It was shown that superoxide radicals are protective against systemic infections by mycobacteria. A study using an animal model showed that knockout mice that did not produce superoxide radicals showed a significant increase in susceptibility to infection by *M. tuberculosis* in the lungs compared to mice showing normal superoxide production<sup>9</sup>. In humans, some reports have shown that patients with chronic granulomatous disease, characterized by a defect in the production of superoxide radicals in phagocytic cells, demonstrated high susceptibility to infection by different mycobacterial species<sup>10</sup>. It has been reported that *M. tuberculosis* and *Salmonella typhimurium* possess certain genes that confer greater resistance to the effects of free radicals derived from oxygen and nitrogen produced by macrophages. This genetic effect is believed to confer resistance on mycobacteria against phagocytic cells<sup>10</sup>.

We uncovered other studies that suggested that neutrophils are important cells in protection against *M. tuberculosis*. Borelli et al.<sup>11</sup> demonstrated that purified MPO in the presence of hydrogen peroxide exerts a consistent destructive action against *M. tuberculosis* H37Rv. This activity is dose- and time-dependent, and requires the presence of chloride ions<sup>11</sup>. Appelberg<sup>12</sup> showed that mice infected intraperitoneally with *Mycobacterium bovis* [Bacillus Calmette-Guérin (BCG)] responded with a higher concentration of neutrophils than uninfected mice. These studies suggest that neutrophils could be important cells in resistance to infection by *Mycobacterium* and the main defense mechanism is hydrogen peroxide produced by neutrophils<sup>12</sup>. These findings support the idea that our patient had a greater susceptibility to *M. tuberculosis* and *P. brasiliensis* due to a defect in MPO production that resulted in a critical decrease in or loss of hypochlorous acid production.

Herein, we reported on a patient with a deficiency in the production of the enzyme MOP, who suffered from systemic infections in the form of intraperitoneal tuberculosis and paracoccidiodomycosis. Following our analysis of her

immunodeficiency profile, it seems most likely that the MOP deficiency we observed was the principal cause of her heightened susceptibility to these infections.

#### Acknowledgments

We offer our deepest thanks to the Department of Dermatology, Faculty of Medicine, University of São Paulo Hospital, and the Research Laboratory in Dermatology and Immunodeficiencies (LIM 56), that provided technical support for the development and implementation of this study.

#### Conflict of interest

The authors have not conflicts of interest to declare.

## REFERENCES

1. Dale DC, Boxer L, Liles WC. The phagocytes: neutrophils and monocytes. *Blood*. 2008;112(4):935-45.
2. Klebanoff SJ, Kettle AJ, Rosen H, Winterbourn CC, Nauseef WM. Myeloperoxidase: a front-line defender against phagocytosed microorganisms. *J Leukoc Biol*. 2013;93(2):185-98.
3. Kutter D, Devaquet P, Vanderstocken G, Paulus JM, Marchal V, Gothot A. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit? *Acta Haematol*. 2000;104(1):10-5.
4. Dias MF, Mesquita J, Filgueira AL, De Souza W. Human neutrophils susceptibility to *Paracoccidioides brasiliensis*: an ultrastructural and cytochemical assay. *Med Mycol*. 2008 46(3):241-9.
5. Oiso R, Fujiwara N, Yamagami H, Maeda S, Matsumoto S, Nakamura S, et al. Mycobacterial trehalose 6,6'-dimycolate preferentially induces type 1 helper T cell responses through signal transducer and activator of transcription 4 protein. *Microb Pathog*. 2005;39(1-2):35-43.
6. Ramirez-Alejo N, Santos-Argumedo L. Innate defects of the IL-12/IFN- $\gamma$  axis in susceptibility to infections by mycobacteria and salmonella. *J Interferon Cytokine Res*. 2014;34(5):307-17.
7. Gohman-Yahr M, Pereira J, Istúriz G, Vilorio N, Carrasquero M, Saavedra N, et al. Relationship between digestive and killing abilities of neutrophils against *Paracoccidioides brasiliensis*. *Mycoses*. 1992;35(11-12):269-74.
8. Meloni-Bruneri LH, Campa A, Abdalla DS, Calich VL, Lenzi HL, Burger E. Neutrophil oxidative metabolism and killing of *P. brasiliensis* after air pouch infection of susceptible and resistant mice. *J Leukoc Biol*. 1996;59(4): 526-33
9. Adams LB, Dinauer MC, Morgenstern DE, Krahenbuhl JL. Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to *Mycobacterium tuberculosis* using transgenic mice. *Tuber Lung Dis*. 1997;78(5-6):237-46.
10. Shiloh MU, Nathan CF. Reactive nitrogen intermediates and the pathogenesis of *Salmonella* and mycobacteria. *Curr Opin Microbiol*. 2000;3(1):35-42.
11. Borelli V, Banfi E, Perrotta MG, Zabucchi G. Myeloperoxidase exerts microbicidal activity against *Mycobacterium tuberculosis*. *Infect Immun*. 1999;67(8):4149-52.
12. Appelberg R. Mycobacterial infection primes T cells and macrophages for enhanced recruitment of neutrophils. *J Leukoc Biol*. 1992;51(5):472-7.