

Fatal septic shock caused by *Paracoccidioides brasiliensis* phylogenetic species S1 in a young immunocompetent patient: a case report

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Abstract

The authors report the first case of fatal septic shock, a rare clinical presentation of paracoccidioidomycosis (PCM) caused by *Paracoccidioides brasiliensis* S1. We also provide an immunological evaluation of the patient. Severe clinical signs such as organ dysfunction and digital gangrene occurred in this case. The patient presented a remarkable cell activation profile and diminished percentage of peripheral blood T regulatory cells. A decrease in anti-inflammatory IL-1RA plasma level showed the potential for endothelium damage, probably contributing to a vasculitis process. Together with *P. lutzii*, *P. brasiliensis* appears to be involved in severe cases of PCM.

Keywords: *Paracoccidioides brasiliensis* S1. Septic shock. Host-parasite interaction.

INTRODUCTION

Paracoccidioidomycosis (PCM) is a severe systemic mycosis with a broad spectrum of clinical and immunological manifestations. These range from chronic PCM (adult type), accounting for 80–90% of cases that are mostly among male farm workers and usually affecting the lungs and oral mucosa, to acute/subacute PCM (juvenile type) in young susceptible patients presenting a more severe clinical condition because of intense reticuloendothelial system involvement¹. Although it has been hypothesized that *Paracoccidioides lutzii* is associated with severe clinical forms, *P. brasiliensis* has also been recently reported to cause serious and unusual clinical pictures^{2,3}.

CASE REPORT

A 19-year-old man from the Rio de Janeiro metropolitan area was admitted to Evandro Chagas National Institute of Infectious Diseases. His family reported a 6-month history of

adenomegaly initially misdiagnosed as mumps. The patient's clinical condition had worsened during this time, with progressive lymph node enlargement, 10-kg weight loss, high fever, abdominal pain, and vomiting during the 2 months before admission. There was no report of travel, rural activities, or comorbidities, except marijuana smoking and bathing in the waterfalls of Gericinó Natural Park (Brazilian Atlantic Forest, Rio de Janeiro). Five days before hospital admission, the patient had increased vomiting frequency, abdominal distention, jaundice, and decreased consciousness level. At admission, the patient presented anasarca, bulky cervical lymph nodes, oliguria, and acute respiratory failure (oxygen saturation 60% by pulse oximetry) requiring tracheal intubation (**Figure 1**). Other important clinical signs were tachycardia (132bpm); bedside echocardiography showing inferior vena cava collapse, and fluid-refractory hypotension (mean arterial pressure - MAP 70 mmHg after 3L of volume expansion and epinephrine infusion rate 14mL per hour). Laboratory results after support treatment showed hemoglobin 6.6g/dL (reference value [RV]: 13-18g/dL); leukocytosis 23,760/mm³ (RV: 4,200-10,000/mm³) with 33% band cells (RV: up to 7%); elevated amounts of nitrogenous compounds in the blood (urea 459mg/dL [RV: 15-38mg/dL], creatinine 2.74 mg/dL [RV: 0.7-1.3 mg/dL]); serum lactate 6.3 mmol/L (RV: 0.5–2.0 mmol/L); hypoalbuminemia 0.9 g/dL

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(RV: 3.4-5.0g/dL); C-reactive protein 98.7mg/dL (RV: 0.3mg/dL), and remarkable hypercalcemia (Ca^{2+} 48mg/dL [RV: 8.5–10.1mg/dL]). Antibody detection tests such as ELISA for detection of anti-HIV, syphilis, and hepatitis (B and C) were negative. Chest radiographs showed lower lobe pulmonary consolidation with left pleural effusion. Abdominal ultrasonography revealed massive hepatosplenomegaly. Upper digestive endoscopy showed extensive flat ulcers secondary to mediastinal lymph node enlargement fistulized to the esophagus. Acute juvenile PCM was diagnosed by direct examination of cervical lymph node aspirate that revealed large, yeast-like cells with a thick birefringent cell wall and presenting “cryptosporulation” typical of *Paracoccidioides* spp. (Figure 2A). Blood cultures were negative for bacteria, but Gram staining revealed *Paracoccidioides* yeast-like cells (Figure 2B). Lymph node aspirate and blood cultures were grown on Sabouraud dextrose agar and mycobiotic agar at 25°C, and the isolated pure fungus was subcultured on Fava-Netto Agar at 37°C for dimorphism confirmation. All cultures yielded *Paracoccidioides* spp. The result of serological testing for PCM using the Ouchterlony double immunodiffusion test was positive (1:256). Liposomal amphotericin B was prescribed (cumulative dose 9 g) together with sulfamethoxazole/trimethoprim (1,600 mg/320 mg t.i.d.) and corticosteroids (hydrocortisone 50 mg t.i.d.). Despite subsequent negative blood cultures and partial improvement in specific PCM clinical signs such as adenomegaly and hepatosplenomegaly, the patient’s general clinical condition deteriorated and he developed multiple digital gangrene (Figure 3A and 3B) and worsening renal function. The patient died 47 days after admission (Figure 3C).

Ethical considerations

The Research Ethics Committee of INI/Fiocruz approved this study (CAAE 42590515.0.0000.5262). The patient’s mother signed an institutional written consent form authorizing description of the case and the release of patient photographs.

Molecular identification of fungal strains

Genomic DNA was extracted from the yeast phase of isolates from lymph nodes and blood cultures. Molecular identification was obtained by the amplification of partial sequences of *arf* and *gp43* genes^{3,4}. Using the Basic Local Alignment Search Tool, sequences were compared with those previously deposited in GenBank® by Matute et al⁵. Sequences showed 100% homology with *P. brasiliensis* S1. The sequences generated in this study are available from GenBank® (accession numbers MF066648, MF066649, MF066650, and MF066651).

Immunological evaluation

Lithium heparin plasma samples were stored at –80°C until use. IL-1RA and IL-6 cytokines were measured using ELISA (R&D Systems, Minneapolis, MN, USA), according to the manufacturer’s instructions; results were expressed as picograms per milliliter. The detection limits for IL-6 were 3.1-300.0pg/mL and 31.2-2,000 pg/mL for IL-1RA. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation through a Histopaque gradient (Sigma–Aldrich, St. Louis, MO, USA).



FIGURE 1: Patient at admission presenting anasarca, bulky cervical lymph nodes, and acute respiratory failure requiring tracheal intubation for oxygen supply.

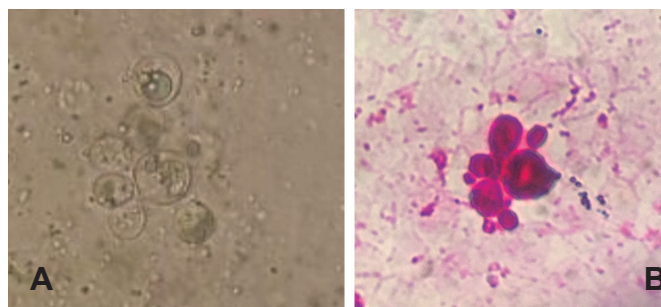


FIGURE 2: (A): Direct microscopic examination of cervical lymph node aspirate with potassium hydroxide preparation reveals large, multiple, budding yeast-like cells typical of *Paracoccidioides* spp. (B): Direct microscopic examination of blood culture with Gram staining reveals *Paracoccidioides* spp. yeast-like cells.



FIGURE 3: (A, B): Multiple digital gangrene as a severe complication in this patient. (C): Terminal patient with severe cachexia as a consequence of disease severity.

Phenotypic analysis of T cells was conducted after incubation of 1×10^5 PAMC with the following specific monoclonal antibodies: anti-CD38 Pe-Cy7, anti-HLA-DR V500, anti-CD69 Pe-Cy7, anti-CD3 APC-H7, anti-CD4 PercP, and anti-CD8 APC (BD Biosciences, San Jose, CA, USA). Forty thousand events were acquired using CytoFLEX flow cytometry (Beckman Coulter Inc., Brea, CA, USA) into a lymphocyte electronic region. Subsequently, the percentage of activated T cells was determined using Kaluza software (Beckman Coulter Inc.). Regulatory T cells were characterized using a commercial kit containing anti-CD4 Pe-Cy5, anti-CD25 PE, and anti-FOXP3 FITC (BioLegend, San Diego, CA, USA). Limits for the quadrant markers were always set based on negative populations and isotype controls. Results were expressed as percentage of positive cells. The patient presented CD38⁺HLA-DR⁺=37.5%, CD69⁺=85.8%, and CD4⁺CD25⁺Foxp3⁺=3.9%. Moreover, a three-fold increment of inflammatory IL-6 plasma level (D1=71.0ng/mL; D6=237.0ng/mL) and a decrease in anti-inflammatory IL-1RA plasma level (D1=1,456.1ng/mL; D6=918.9ng/mL) were observed after 6 days of disease progression.

DISCUSSION

PCM is a life-threatening, neglected systemic mycosis that is usually misdiagnosed, as in this case, thus protracting the correct treatment. Late diagnosis, especially of the acute juvenile clinical form, induces serious complications including death⁶. The mononuclear phagocytic system is the main site of infection. The lymph nodes are involved in nearly 100% of acute PCM cases, followed by the liver, spleen, and skin^{1,6}.

Although systemic bloodstream dissemination of *Paracoccidioides* spp. is a typical event in PCM pathogenesis, isolation of the fungus from blood samples is unusual, being mostly reported in cases of immunosuppression^{7,8}. The direct observation of *Paracoccidioides* structures in blood smears is even more rare because it indicates a high fungal burden. Both laboratory findings were observed in the present case. Together, these observations could explain a septic embolization inducing the digital gangrene observed in this patient because *Paracoccidioides* spp. yeast-like cells are larger than the lumen of small blood vessels in the extremities. However, the vascular and inflammatory events in septic shock are more likely to explain digital gangrene as well as organ dysfunction, such as renal failure. Septic shock can cause peripheral hypoperfusion and hypoxia, inducing gangrene of the extremities. Another even stronger hypothesis for the digital gangrene in this case is that an intense inflammatory response damaged the endothelium, causing a severe vasculitis process⁹.

The severity of this case can likely be ascribed to an exacerbated host response against the pathogen, as demonstrated by the immunological analysis, which presented a remarkable cell activation profile without a satisfactory T cell regulation response^{10,11}. Notably, the patient showed a diminished percentage of peripheral blood T regulatory cells in comparison with healthy individuals (CD4⁺CD25⁺Foxp3⁺=11.1%). High titers of circulating antibodies specific to the fungus (typical in acute forms of PCM) along with a high fungal burden in the

bloodstream are very likely to have induced immune complex formation and complement activation. In addition, the detected decrease in anti-inflammatory IL-1RA plasma level indicates a potential for endothelial damage, which contributed to the vasculitis process. The lack of biopsy or necropsy investigation for possible vasculitis causes, including the presence of fungi, fungal antigens, or host elements, is a limitation of the present study, which was justified by the ethical considerations.

P. lutzii has been previously described in the context of worse clinical conditions, such as fatal fungemia². To our knowledge, this is the first report of fatal septic shock, a rare clinical manifestation, caused by *P. brasiliensis* S1. Although there is a paucity of clinical information about this phylogenetic species, together with *P. lutzii*, *P. brasiliensis* also seems to be involved in other severe cases of PCM^{3,6}. These findings indicate the need for more studies on *Paracoccidioides*-host interaction, with a higher number of clinical isolates obtained from patients with different clinical manifestations, treatment responses, and outcomes, to better understand the physiopathology of this neglected disease and prevent unfavorable outcomes.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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