

## Disseminated paracoccidioidomycosis prediagnosed as neoplasm: An important challenge in diagnosis using rt-PCR



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### ABSTRACT

This paper presents a case of disseminated paracoccidioidomycosis in a 62-year-old male patient, who lives in Belo Horizonte, MG, Brazil. The patient was hospitalized with icteric syndrome of cholestatic pattern and weight loss, with loss 30 kg in 5 months. The imaging of the abdomen showed lesion of infiltrative pattern, affecting gallbladder and intrahepatic bile ducts, suggesting neoplasia of malignant behavior, besides to presenting the yellow nail syndrome. Dermatological examination presented erythematous-infiltrated plaques in the occipital region. Also, the patient presented tegumentary lesions on the scalp and lumbar region from which the histopathological examination was carried out, which evidenced yeasts cells. The drug of choice for therapy was Liposomal Amphotericin-B. At the end of the antifungal treatment, liver enzyme dosages were normalized and there was improvement of the general condition of the patient, as well as the skin lesions. Here, we demonstrate the importance of molecular biology to confirm the diagnosis. Especially in cases of difficult diagnosis.

### 1. Introduction

Paracoccidioidomycosis (PCM) is a potentially lethal granulomatous mycosis, endemic in Latin America, caused by the fungi *Paracoccidioides brasiliensis* and *P. lutzii* [1–3]. Such as other systemic fungal infections, PCM can affect many organs as a result of dissemination of infection through the lymphohematogenic route with the installation of quiescent foci in different organs, allowing a future reactivation of the pathogen in any place where it may have settled [4,5]. Yellow nail syndrome (YNS) is a rare disease, characterized by the triad of lymphedema, pleural effusion and dystrophic nails with slow growth and yellowing [6,7]. We report a case of disseminated PCM misdiagnosed as gallbladder carcinoma and associated with the classic YNS. The study presents clinical, histological and molecular findings.

### 2. Case

A 62 years old immunocompetent and previously healthy male patient, presented with cholestatic pattern icteric, reporting 30 kg weight loss in the last 5 months. He also reported a chronic and progressive shortening of breath, upper right abdominal pain, and swollen lower limbs, besides development of skin rashes on scalp, face and

trunk during last two months. He also complained of changes in the 20 nails that started 3 years ago, with yellowing and slow growing nails and absence of cuticles (Fig. 1a).

At dermatological examination, the patient presented crusted erythematous and infiltrated plaques in the occipital (Fig. 1b) and right infra-auricular region (Fig. 1c), associated with erythematous-infiltrated, sometimes confluent papules and nodules located in the trunk, interscapulum region, right malar and nasal dorsum.

He was submitted to an ultrasound imaging and computed tomography (CT) scan that presented an infiltrative pattern lesion affecting gallbladder and intrahepatic bile ducts, resembling malignant neoplasm. The patient was hospitalized in the Santa Casa de Misericórdia de Belo Horizonte Hospital/ Minas Gerais, Brazil for further investigations. A magnetic resonance imaging (Fig. 2), also presented an infiltrative pattern lesion affecting gallbladder and intrahepatic bile ducts, with bilateral pleural effusion and hilar, per pancreatic and gastric curvature lymph node enlargement, with were highly suspicion for gallbladder malignant neoplasm.

At his tenth in hospital day was performed a biopsy of the scalp and chest lesions that showed pseudoepitheliomatous hyperplasia associated with dense and diffuse mixed inflammatory infiltrate, micro-abscesses and several multinucleated giant cells on the dermis. The

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**Fig. 1.** (A) Photo of nails of the hands and feet affected by yellow nail syndrome (YNS). Before treatment. (B)- Erythematous-Infiltrates and exulcero-crostosias in the occipital region. Before and during treatment. (C) -Lesion right infra-auricular, associated with erythematous-infiltrated papules and nodules. Before and after treatment.

Schiff Periodic Acid (PAS) staining revealed circular structures, sometimes with buds, suggesting yeasts of *Paracoccidioides* genus (Fig. 3). The direct examination of the pus of the lesion on the patient's nose showed yeast cells in multiple shoots, typical of *P. brasiliensis* (Fig. 4). The serology was performed by double radial immunodiffusion (ID) for detecting antibodies against gp43 antigens, specific for the genus *Paracoccidioides*, using the kit produced in Federal University of Paraná, Brazil. Molecular diagnosis in tissue and peripheral blood DNA through real-time PCR (rtPCR) was performed using TaqMan system, using primers and probe for detecting the gene for Pb27 protein, specific for *Paracoccidioides* genus, according standardized by Rocha-Silva et al., 2016 [8]. Although serology (Fig. 5) and rtPCR (Fig. 6) were positive, suggesting infection caused by *Paracoccidioides* sp, the culture for fungi in artificial medium did not show growth.

Following confirmation the diagnosis of PCM infection, therapy with 3 mg/kg/day for 20 days of Liposomal Amphotericin B was initiated, followed by Itraconazole 200 mg twice daily plus sulfamethoxazole trimethoprim 800/160 mg twice daily. After antifungal treatment, liver enzymes and bilirubins dosages had an elevation, followed by slow complete normalization into the next thirty days, along

with clinical improvement and clearing of almost all cutaneous lesions.

Around sixty days after therapy start, new abdominal image showed involution of all abdominal mass previously seen. The disappearing of those masses after antifungal treatment, which previously suggested a primary biliary neoplastic process, confirmed multisystem involvement by the fungus. Subsequently, further investigation definitively out ruled the diagnosis of neoplasia of the digestive tract. The pleura lesion was also reversed with the treatment. The patient responded well to the treatment, the infection was discharged and the organs compromised resumed normal function without sequelae.

Nowadays, after 120 days of therapy beginning, the patient is still being regularly evaluated and remains in asymptomatic and in use of antifungal therapy, which is recommended for at least 18 months in severe cases, due to the possibility of quiescence foci of the fungi.

### 3. Discussion

PCM is seen in the majority cases in the chronic form. In this clinical presentation, the involvement of the skin, mucosal and lungs are very frequent [2]. However, due to hematogenic dissemination in

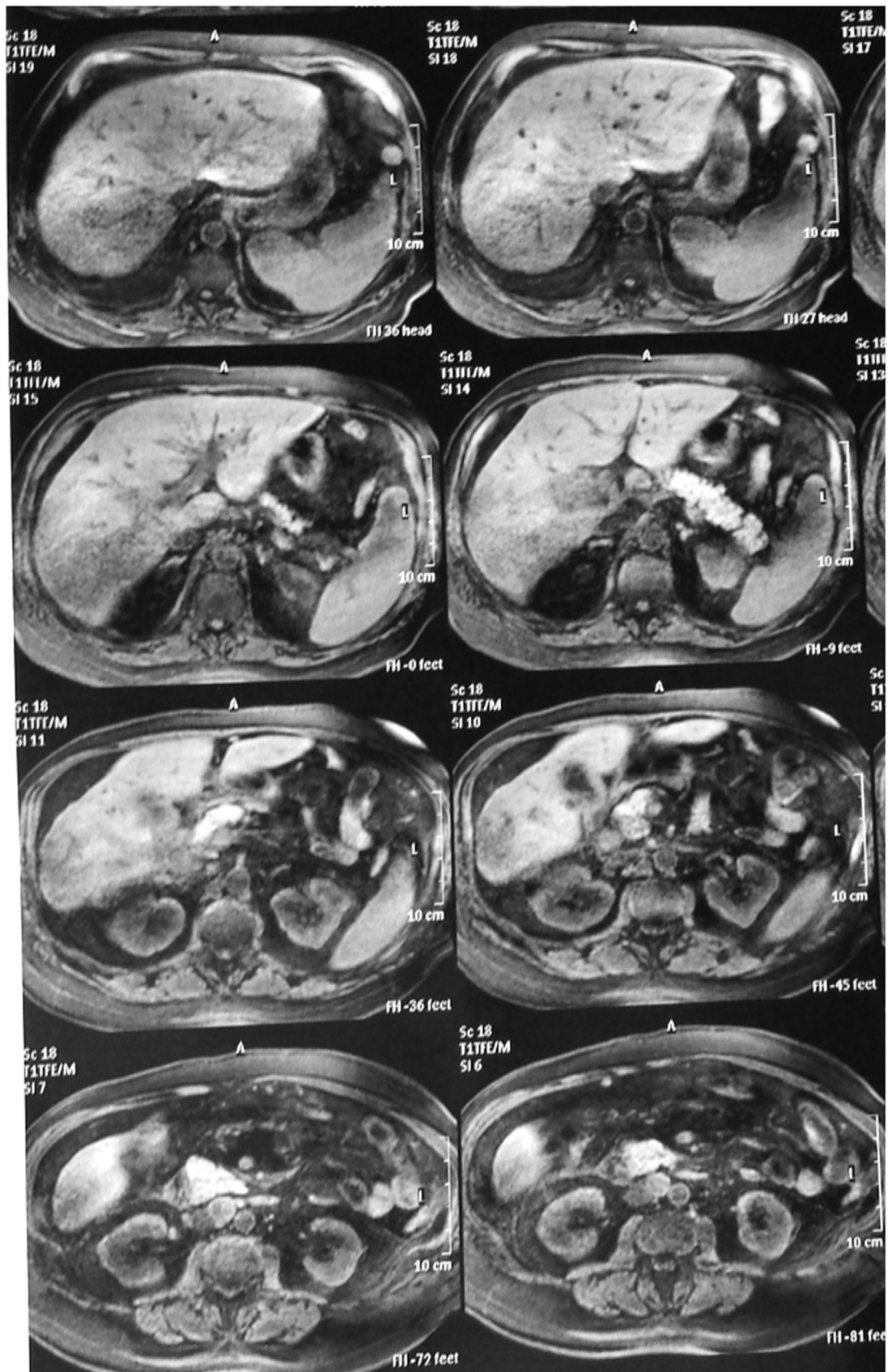
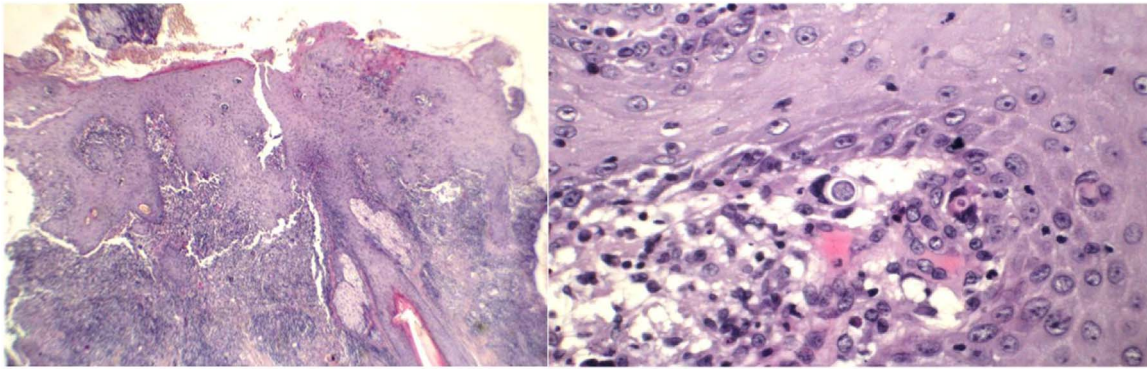
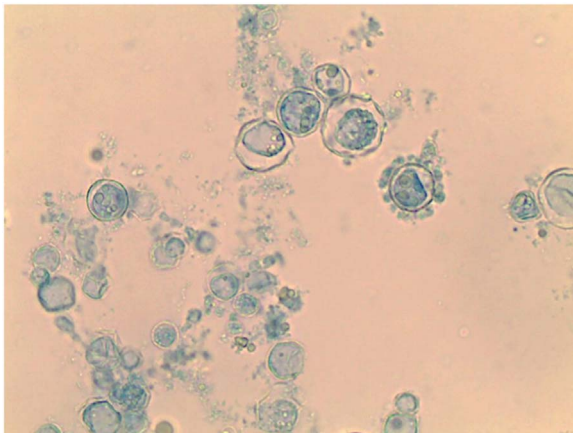


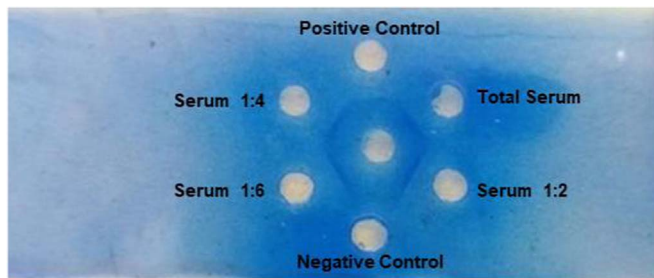
Fig. 2. Magnetic resonance imaging presenting an infiltrative pattern lesion affecting gallbladder and intrahepatic bile ducts, with bilateral pleural effusion and hilar, per pancreatic and gastric curvature lymph node enlargement.



**Fig. 3.** Histological findings in the dermal fragment: Fragment showing pseudoepitheliomatous hyperplasia. There is, in the dermis, dense and diffuse inflammatory infiltrate of mono- and polymorphonuclear cells with formation of microabscesses and some multinucleated giant cells.



**Fig. 4.** Direct examination of pus from the patient's nose injury, showing yeast cells with multiple shoots.



**Fig. 5.** Double Radial immunodiffusion using Gp43 antigen. Serum is the patient sample and its dilutions. The antigen and the positive control were assigned by the Technological Center of Paraná (PR, Brazil).

the infection phase, the disease can be expressed in many organs, and it can be often difficult to diagnose [4]. This study reports a case of PCM in chronic multi-systemic form, simulating clinically and radiologically a gallbladder carcinoma. The examination of the skin and the molecular analysis using peripheral blood, made possible the diagnosis of this mycosis, avoiding an intra-abdominal biopsy, which can be a very invasive procedure given the patient's clinical and nutritional state. Subsequently, there was a prompt response to antifungal therapy, with normalization of liver tests, improvement of clinical parameters, and the involution of abdominal masses, confirming that all clinical manifestations of the patient could be attributed to systemic infection by the fungus.

We highlight the finding of cutaneous lesions with sarcoid pattern (Fig. 1), rarely related in this mycosis [9]. The patient presented Yellow Nail Syndrome (YNS) which is characterized by the triad of

lymphedema, yellow dystrophic nails and pleural effusion [10], an unusual clinical condition in the PCM carrier. It nail's manifestations such as opacity, thickening, lack of cuticle and slow growth may be related to long-term changes in lymphatic drainage of the toes [7]. Pathogenesis of YNS involves changes in lymphatic circulation, associated with neoplasia, infections, collagenases and endocrinopathies, among others [6]. In this study, it is theorized that the formation of abdominal masses and lymphadenopathy secondary to fungal infection may have generated changes in the lymphatic circulation and triggered the syndrome. However, it is not possible to establish a definitive causal association between the entities, considering the rarity of YNS, the low understanding of its pathophysiology and that there is no similar case report in the literature.

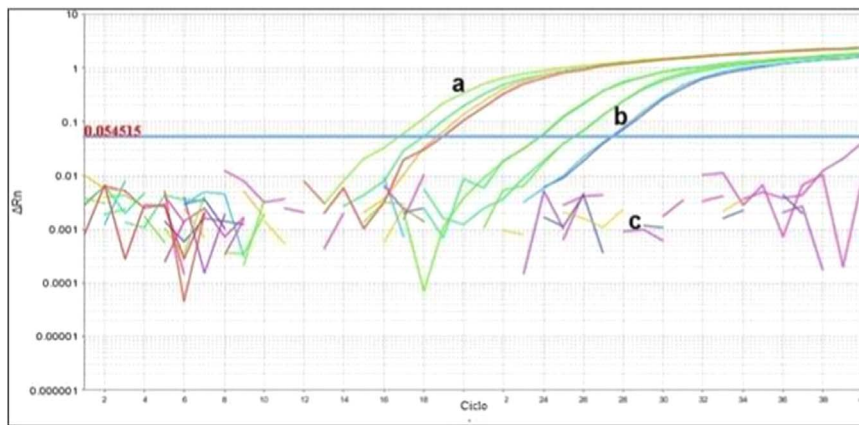
It is important to note the contribution of the molecular biology, by means of specific rt-PCR to confirm the infection. In this case, it was decisive, leading to rapid and safe diagnosis, essential for the prompt treatment of the patient. This case study confirms the standardization of the rt-PCR technique for molecular diagnosis of PCM, which was patented last year and has already been published [8]. Without a doubt, this technique will contribute a lot to help, especially in cases of challenging diagnosis. The detection of DNA in the blood of the patient, suggesting continuous circulation of the agent, strongly suggests the diagnosis of the multi-system form of PCM.

Diagnoses of invasive fungal infections (DIFIs) are usually based on isolation of the fungus in culture and histopathological techniques [11]. Nevertheless, these methods contain many limitations which frequently delay the definitive diagnosis. The molecular diagnostics methods have emerged as an appropriate alternative for DIFIs diagnosis. When there is no clinical, culture and histopathological definite diagnosis of the agent involved in the DIFIs, fungal real-time PCR assays have been used, allowing amplification of fungal DNA. This procedure is fast, sensitive, and specific and promises to be useful for improving early diagnosis of DIFIs [12]. The development of real-time PCR technique has improved the diagnosis of PCM and appears to be essential for a rapid and accurate diagnosis [7,12].

This work highlights the need to implement a molecular diagnostic method when culture is negative. However, a positive PCM result must necessarily be interpreted in the clinical setting. Standardized rules of interpretation of PCM results are required and should include sample type and quality, and the entire clinical and radiological context of the assessed patient.

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**Fig. 6.** Amplification Curve by the rtPCR technique, using primers species-specific for the genus *Paracoccidioides*. A: Positive control; B: Patient sample; C: Negative control.

### Conflict of interest

The authors have no conflicts of interest to declare and confirm that each one has made substantial contributions to the information or materials submitted for publication.

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