Paracoccidioidomycosis

Carlos P Taborda, University of Sao Paulo, Sao Paulo, Brazil
Luiz R Travassos, Federal University of São Paulo, Sao Paulo, Brazil
Gil Benard, University of Sao Paulo, Sao Paulo, Brazil

Introduction

Paracoccidioidomycosis (PCM) is the prevalent systemic mycosis in Latin America, with 80% of cases being registered in Brazil, followed by Colombia, Venezuela and Argentina (Brummer *et al.*, 1993; Martinez, 2015). In Brazil, most cases are concentrated in the southeast region (States of São Paulo, Rio de Janeiro, Minas Gerais and Espírito Santo), west and middle-west (Mato Grosso do Sul and Goiás) and South (Paraná, Santa Catarina and Rio Grande do South). However, other regions have reported cases, especially the amazonian States of Pará, Tocantins, Maranhão and Rondônia (Martinez, 2015). Paracoccidioidomycosis has also been reported in Europe, the USA, Africa and Asia, but all cases are imported since the affected individuals were visitors or had worked in endemic areas in Latin America (Martinez, 2015). The fungal agent *Paracoccidioides* spp. preferably inhabits in a moist environment, with average annual temperature of 18–24°C and rainfall range of 900–1800 nm (Restrepo, 1985).

The disease was described by Adolfo Lutz, a Brazilian physician, in 1908 after examining oral lesions in two patients from São Paulo City (reviewed by Taborda *et al.*, 2016; Moreira, 2008) and originally designated as pseudococcidial hyphoblastomycosis to differentiate from coccidioidomycosis and hyphoblastomycosis (Lacaz *et al.*, 1991). Splendore in 1912, suggested the classification of the agent in the genus *Zymonema*, thus creating the name *Zymonema brasiliense*. The disease was designated as Brazilian blastomycosis, but, since it had been reported in different countries in South America, the disease was assigned as South American blastomycosis or Lutz and Splendore-Almeida disease (Lacaz, 1982). The term Paracoccidioidomycosis was established in 1971 in Medellín, Colombia, during a meeting of the American Continent Mycologists, widely accepted since then (Lacaz, 1982). After systematic studies, in 1930, Floriano Paulo de Almeida officially named the fungal agent as *Paracoccidioides brasiliensis* (Almeida, 1930) and classified by Ajello (Ajello, 1977) as in Kingdom Fungi, Phylum Eumycota, Subdivision Deuteromycotina, class Hyphomycetes, Order Moniales, Moniliaceae Family, Genus and Species *Paracoccidioides brasiliensis*. Based on phylogenetic studies using molecular tools *P. brasiliensis* was positioned along with other dimorphic fungi (*Coccidioides posadasii*, *Coccidioides immitis*, *Blastomyces dermatitidis* and *Histoplasma capsulatum*) in the Kingdom Fungi, Phylum Ascomycota, Plectomycetes class, Order Onygenales, Onygenaceae Family, Genus and Species *Ajellomyces brasiliensis*, then again called *Paracoccidioides brasiliensis* (San-Blas *et al.*, 2002; Arantes *et al.*, 2015).

Paracoccidioidomycosis is a chronic granulomatous systemic mycosis, caused by a fungus of genus *Paracoccidioides*. One of the main characteristics of this genus is the thermal dimorphism, mycelial form at 25°C characterized as saprophytic and yeast as the pathogenic form at 35–37°C (reviewed by (Taborda *et al.*, 2018)). The habitat of *Paracoccidioides* spp. has not yet been determined precisely. Soil samples from different locations in Venezuela, Argentina and Brazil (reviewed by (Franco *et al.*, 2000)) support the hypothesis that the soil is the natural habitat of the fungus. In addition, isolations of *P. brasiliensis* from wild animals such as armadillos (Bagagli *et al.*, 1998), bats (Grose and Tamsitt, 1965) and even dog food (Ferreira *et al.*, 1990) have been reported. Also, genomic analysis of samples from animals living in the soil of endemic areas, such as guinea pig (*Cavia aperea*), raccoon (*Procyon cancrivoros*) and armadillo (*Dasypus septemcinctus*), further indicates acquisition of the infection from the soil in these areas. (Richini-Pereira *et al.*, 2008).

It is important to stress that except for humans, the most frequent host among mammalians is the armadillo ((mainly *Dasypus novemcintus*) but the fungus can occur in other species). *Paracoccidioides* spp. has been isolated ca. 75%–100% from armadillo captured in hyper-endemic areas of PCM, showing a high frequency of infection (Corredor *et al.*, 1999; Hrycyk *et al.*, 2018). The armadillos seem to favor the infection by *Paracoccidioides* spp. yeasts, such as the body temperature (between 32.7 and 35°C), low cellular immunity and constant contact with the soil (Bagagli *et al.*, 2008). It is hypothesized that parasitism in these animals would make it possible to preserve the saprophyte form and contribute to sexual reproduction, by promoting the proximity of mating types in a protected environment, such as the armadillo tissue or organs (Bagagli *et al.*, 2006).

For more than 100 years, only one species was recognized in the Genus: *Paracoccidioides brasiliensis*. Phylogenetic studies have classified *P. brasiliensis* into four cryptic species (S1, PS2, PS3 and PS4) with different evolutionary characteristics and geographic distribution. S1 is considered a recombinant monophyletic group with a wide distribution in South America, being responsible for most cases of PCM. PS2 is also recombinant but paraphyletic, found in Brazil and Venezuela. PS3 is a clonal and monophyletic population, registered only in Colombia. The PS4 group is classified as monophyletic with clinical isolates from Venezuela (Matute *et al.*, 2006; Theodoro *et al.*, 2012). In parallel, a second species was proposed based on phylogenetic and comparative genomic data, recombination analysis, and morphological characteristics and designated as *P. lutzii* (formally known as Pb01-like) (Desjardins *et al.*, 2011; Teixeira *et al.*, 2014). It was estimated that *P. lutzii* monophyletic group was separated from groups S1, PS2 and PS3 approximately 30 million years ago (Teixeira *et al.*, 2014). This species is endemic in the North and Midwest regions of Brazil (mainly in States of Rondônia, Mato Grosso and Goiás) and shares some geographical areas with the S1 group (Bocca *et al.*, 2013; Teixeira *et al.*, 2014). Turissini *et al.* (2017) expanded the phylogenetic studies and showed that despite low levels of genetic difference among the four cryptic species of the *P. brasiliensis*, they are indeed well separated species indicating that cryptic species (S1, PS2, PS3 and

PS4) should be elevated to taxonomical species status (Turissini *et al.*, 2017). The authors propose therefore to adopt the names *P. lutzii*, *P. brasiliensis* (S1), *P. americana* (PS2), *P. restrepiensis* (PS3) and *P. venezuelensis* (PS4) (Turissini *et al.*, 2017).

Morphological Aspects

Paracoccidioides spp., are considered dimorphic fungi depending on temperature to change their shape. The fungal species develop as mold at room temperature (19–25°C) and are then known as the saprophytic phase. Otherwise, at temperatures ranging 35–37°C, fungi become unicellular yeasts (**Fig. 1**), consisting in the parasitic phase (reviewed in Taborda *et al.*, 2018).

Depending on the culture medium, nutrients and temperature, colonies assume different characteristics. At room temperature, white fungal colonies grow slowly well adherent to the medium (**Fig. 2(A)**). When examined under the microscope, and using specific culture media, fine, septated mycelial filaments with conidia, arthroconidia, aleurioconidia and arthroconidia are seen (Conant and Howell, 1942; Neves and Bogliolo, 1951; Pollak, 1971). Conidia are small, less than 5 μm, with different shapes and sites on the parental mycelium (Bustamante-Simon *et al.*, 1985). The conidia-to-yeast transition occurs in 96 h at 37°C and colonies now show transition to cream colonies known as cerebriform and yeast-like which appear after 10–20 days (Brummer *et al.*, 1993; **Fig. 2(B)**). The yeast cells are similar to those detected in patients. Spherical cells (3–30 μm but some cells

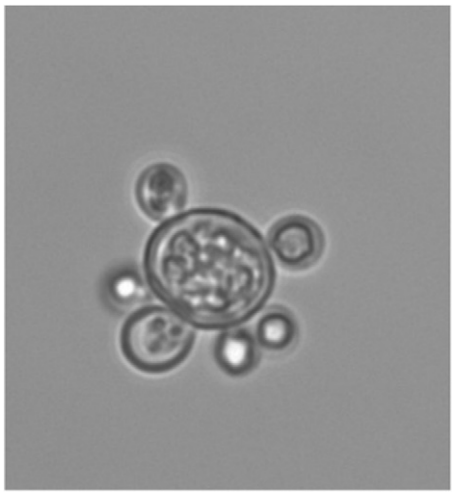


Fig. 1 Micrograph of typical "pilot wheel" yeast cells of *P. brasiliensis* seen on direct examination of culture (120x).

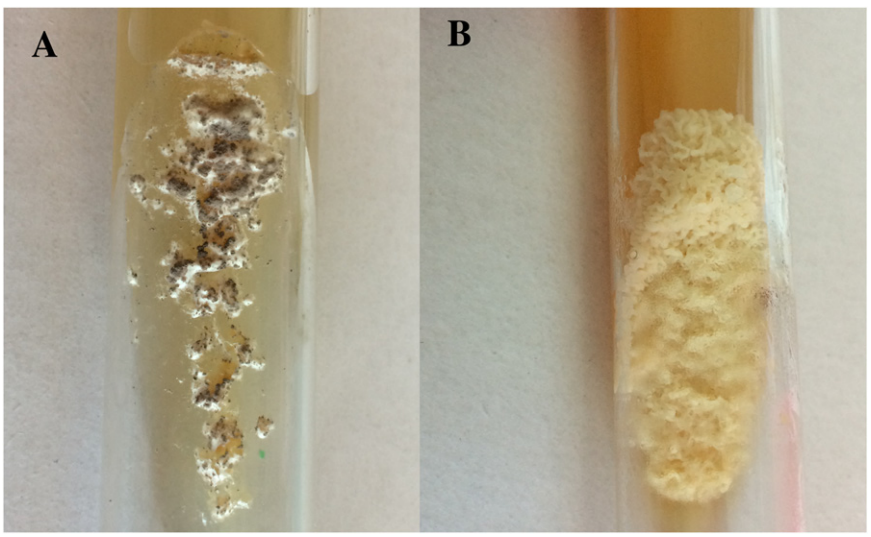


Fig. 2 Macroscopic colonies: (A) of mycelium (room temperature) and (B) yeasts (37°C) in BHI Agar.

may reach 60 μm) with thin and thick walls are seen; along the cell surface single or multiples narrow-necked buds (2–10 μm) (Brummer *et al.*, 1993).

Epidemiological Aspects

The infection is acquired when propagules from the mycelial phase of the fungus are inhaled and, through the respiratory tract, install primarily in the pulmonary alveoli. The transition to the yeast phase starts in the lung and cell transformation is essential for the infection to establish the disease that can spread via hematogenous and or lymphatic routes to any part of the organism (reviewed by Souza and Taborda, 2020). PCM is mostly manifested in male individuals, between 30 and 60 years of age, mostly rural workers in endemic areas. People from urban regions may also have contact with the fungus when visiting endemic areas. Other conditions such as smoking and excessive alcohol consumption are considered risk factors for the establishment of the disease (Shikanai-Yasuda *et al.*, 2017; Souza and Taborda, 2020). The hormone 17-β-estradiol is related to the fungal dimorphism and may protect women against the disease. Studies show that this hormone inhibits the transition from mycelium to yeast in a dose-dependent manner (Aristizábal *et al.*, 2002). It is believed that the Estradiol Binding Protein – PLE – present in the fungal cytoplasm binds to the 17-β-estradiol and blocks the conversion from the infective phase to the pathogenic phase (Shankar *et al.*, 2011). In addition to the hormones, PCM has already been reported in patients undergoing cytotoxic treatment or immunosuppressive therapy against cancer (Marques, Lastória and Marques, 2011), transplant patients (Shikanai-Yasuda *et al.*, 1995) and rheumatoid arthritis (Woyciechowsky *et al.*, 2011). It is assumed that cytotoxic drugs can reactivate a latent lesion, causing an acute lung infection, probably due to the impairment of the immune system due to chemotherapy and radiotherapy.

PCM is the most important systemic mycosis in Latin America and the countries with the highest number of cases are Brazil, Colombia, Venezuela and Argentina. Brazil has 80% of the total cases, and the southeast, south and midwest regions are the most prevalent (Martinez, 2017; Souza and Taborda, 2020). PCM is the eighth cause of mortality from chronic infectious diseases, reaching rates of 1.65 deaths per million inhabitants (Bocca *et al.*, 2013). In the period from 1996 to 2006 there were 517,058 deaths from infectious and parasitic diseases in Brazil. Systemic mycoses were responsible for 3583 deaths, with paracoccidioidomycosis being cited in approximately 51.2% deaths recorded in this period, occupying the tenth position, in this survey, among infectious and parasitic diseases with high mortality (Prado *et al.*, 2009). In 2010, it was estimated that 3360 new cases of PCM per year occur in Brazil alone (Martinez, 2010).

There are two progressive clinical forms of the disease: acute or subacute (juvenile type), which involves 3%–5% of cases, is characterized by having a severe faster course (weeks or months), with involvement of the reticuloendothelial system (spleen, liver, lymph nodes and spinal cord) rather than pulmonary involvement. Active replicating fungi and poor granuloma formation are found in the tissues. The chronic form (adult type) occurs in more than 90% of cases, as the disease takes months or years to establish, being, in most cases, asymptomatic. Pulmonary manifestations are the best indicators of this form of the disease, which can also spread to other organs and / or systems. Patients with the acute form of mycosis have high levels of antibodies depending on the mycosis severity. The reason for this clinical dichotomy is not clear and it is assumed that specific characteristics of the parasite-host relationship contribute to it (Benard, 2008; Benard *et al.*, 2012b; Vidal *et al.*, 2014).

Laboratorial Diagnosis

The laboratory diagnosis is relevant since some clinical aspects are shared with other fungal diseases such as: cryptococcosis, histoplasmosis, chromoblastomycosis, coccidioidomycosis and other diseases including tuberculosis, toxoplasmosis, leishmaniasis, cysticercosis, infectious mononucleosis, leprosy, sarcoidosis, syphilis, lymphoma, leukemia, neoplasms, pneumoconiosis, interstitial pneumonitis and Crohn's disease (Shikanai-Yasuda *et al.*, 2017).

The gold standard diagnosis of PCM is based on visualization of yeast cells in biological specimens and isolation of the fungus in culture (Moreira, 2008; Souza and Taborda, 2020). For isolation of the fungus it is recommended the use of Sabouraud dextrose agar or brain heart infusion agar (BHI) containing chloramphenicol and cycloheximide. The clinical specimens (sputum, bronchoalveolar lavage, scraped injury, ganglion aspirate, biopsy fragment) in culture medium are kept 15–30 days at 25–30°C. In the mycelial form, the fungus grows as white, cottony or glabrous colonies and there are no structures that can be associated to *Paracoccidioides* spp. Reversion to yeast form is achieved by changing the temperature to 36–37°C for at least 15 days resulting in cerebriform colonies. In this phase, it is possible to visualize yeast with multiple peripheral buds characteristic of the fungus (Shikanai-Yasuda *et al.*, 2017; Souza and Taborda, 2020). The clinical specimens treated with 10%–20% of potassium hydroxide (KOH) can be used for yeast cell visualization under an optical microscope (**Fig. 3**). Usually, oval, elliptical cells with 3 μm to 30 μm in diameter with refringent double contour wall and multiple peripheral buds in the typical "pilot wheel" (Shikanai-Yasuda *et al.*, 2017; Souza and Taborda, 2020) are of diagnostic value. Another important alternative as a diagnostic tool is the lesion biopsy. Special stains such as Gomori-Grocott or Schiff's periodic acid can assist in the diagnosis by detecting granulomas and the presence of typical yeast with multiple peripheral buds (**Fig. 4**; Taborda *et al.*, 2018).

Other important tools for diagnosis and follow-up of patients are the serological tests. Specific antibodies to *Paracoccidioides* spp. correlate to the severity of the clinical form, being high in the acute/subacute form and in the disseminated form (Vidal *et al.*, 2014). Although different techniques have been described in the last years, immunoprecipitation methods

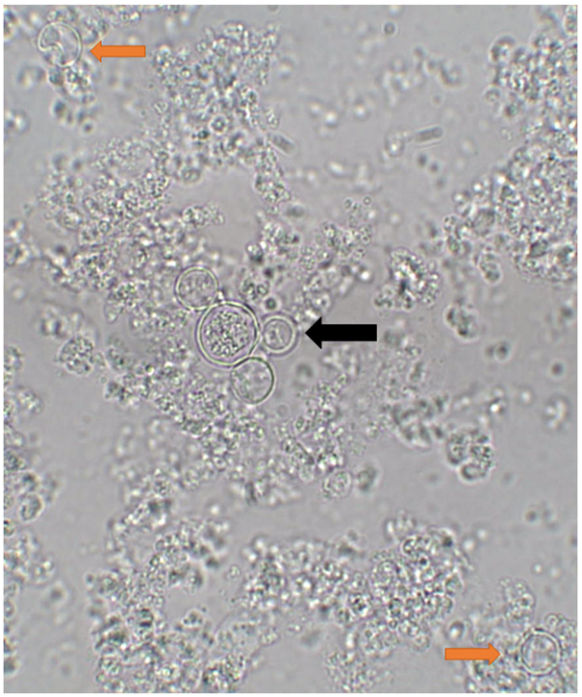


Fig. 3 Smear of a patient's superficial lesion on a KOH mount showing (black arrow) a multi-budding yeast cell, pathognomonic of Paracoccidioides spp. Two isolated yeast cells (red arrows) are also seen, which do not permit the specific diagnosis of PCM.

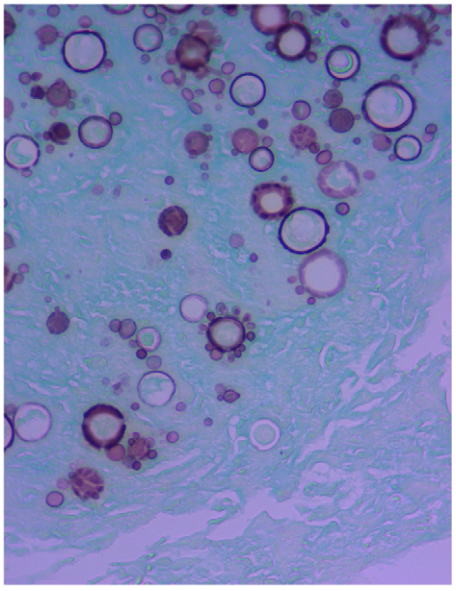


Fig. 4 Lung from infected mice with *P. brasiliensis* and stained by Gomori-Grocott. Multiple budding yeast cells characteristic of genus *Paracoccidioides*.

as in the counterimmunoelectrophoresis and double immunodiffusion still show excellent sensibility and specificity with 80%–95% diagnostic potential (Vidal *et al.*, 2014; Shikanai-Yasuda *et al.*, 2017). Other assays as enzyme linked immunosorbent assay (ELISA) or immunoblot/dot-blot techniques can also be used (de Camargo, 2008; Kamikawa *et al.*, 2017).

The immunoprecipitation methods are widely used in Latin America; however, the lack of standardization of methods and production of antigens sometimes present in the tests used may lead to controversial results (Vidal *et al.*, 2014). False negative results may be obtained in patients with localized lesions or with immunosuppression as in AIDS (Shikanai-Yasuda *et al.*, 2017). False positive results may occur in patients with other fungal infections such as histoplasmosis and aspergillosis or with leishmaniosis (de Camargo, 2008; Shikanai-Yasuda *et al.*, 2017). The main diagnostic antigen of *P. brasiliensis* is the 43 kDa glycoprotein (Puccia *et al.*, 1986) largely used in serological tests (Travassos *et al.*, 2004). The sensibility and specificity of gp43 for

P. brasiliensis and other cryptical isolates is excellent, but not for *P. lutzii* (Shikanai-Yasuda *et al.*, 2017). Although there is no consensus in the serology for *P. lutzii*, Gegembauer *et al.* (2014) developed a serological test with a specific antigen.

Additional diagnostic resources have been developed, such as polymerase chain reaction (PCR) tests, widely used in the diagnosis of infectious diseases. Specifically, in PCM, several authors have described variations in the methods with good results (Bialek *et al.*, 2000; Gomes *et al.*, 2000; Motoyama *et al.*, 2000; Rocha-Silva *et al.*, 2018) however, PCR in the laboratory routine still needs more experimentation. MALDI-TOX MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) has been used for differentiation of isolates of *P. brasiliensis* and *P. lutzii*, previously characterized by molecular techniques, and all isolates were correctly identified (de Almeida *et al.*, 2015).

Pathogenesis and Histopathology

The influence of genetic traits on the mycosis has yet to be elucidated. The observation that only a very small proportion (estimated as less than 2%) of the individuals in endemic areas exposed to the fungus develops the mycosis (Martinez, 2017), points to idea that the host's immunogenetic background plays a role in the susceptibility/resistance to PCM-disease. The occurrence, although rare, of PCM in individuals with inborn errors of the immune system such as defects of the IL-12/IL-23-IFN-γ axis also gives support to this possibility (de Moraes-Vasconcelos *et al.*, 2005; Schimke *et al.*, 2017). PCM has been associated with polymorphisms in some (e.g., IL12RB1, IL4, IL10) but not other (CTLA4, TNF and IFNG) genes related to the immune response (Bozzi *et al.*, 2006; Lozano *et al.*, 2011; Mendonça *et al.*, 2015; Carvalho *et al.*, 2016). The same holds to some class I and class II HLA alleles (Montoya de Restrepo *et al.*, 1983; Lacerda *et al.*, 1988; Goldani *et al.*, 1991). Probably the patient's clinical presentation also bears some relation with his genetic background. A small study suggested that in patients with the unifocal chronic form of the disease (a mild clinical presentation in which lesions are restricted or localized), the HLA allele that was most commonly seen was DRB1*11 (Sadahiro *et al.*, 2007). Clearly, large-scale genetic studies in human PCM are still required to elucidate these aspects.

The role of the host's genetic background in susceptibility to PCM has been more clearly demonstrated in mice models of the mycosis, where resistance and susceptibility varied according to the mouse strain (Singer-Vermfs *et al.*, 2008). Differences not only in adaptive immune response patterns but also in innate immunity (e.g., magnitude of neutrophils influx into the lungs) and type of extracellular matrix composition of the granulomatous lesions were described in these models (Xidieh *et al.*, 1999; Pina, 2006; Sperandio *et al.*, 2015).

However, the difficulty in understanding many of the susceptibility/resistance aspects is largely due to the fact that the events that take place at the initial stages of the infective process are unknown, since patients are diagnosed months to years after the initial exposure to the fungus (Benard, 2008). Data from case-reports, small series of cases, and autopsies studies indicate that the primary PCM lesions occur in the lungs after inhalation of fungal propagules (Brass, 1969; Angulo-Ortega, 1972; Giraldo *et al.*, 1976; Franco *et al.*, 1987; Londero *et al.*, 1996). This has been has also been suggested in experimental models of PCM established through intra-nasal instillation of *P. brasiliensis* conidia (Mcewen *et al.*, 1987). As in other systemic mycoses, the infection initiates when conidia reach the terminal bronchi or alveolar spaces and transform into yeast cells. Most frequently, the local immune response controls this initial inoculum, either fully eradicating the fungus or leaving a few quiescent foci with dormant yeast cells located in the lungs and mediastinal lymph nodes. This has been illustrated by the observation in asymptomatic individuals the presence of residual or regressive pulmonary lesions (called paracoccidioidomas), witnessing the formation of a primary pulmonary complex much similar to that described in the pathogenesis of pulmonary tuberculosis (primary complex of Ghon) (Angulo-Ortega, 1972; Giraldo *et al.*, 1976; Severo *et al.*, 1979; Melo and Londero, 1983; Londero *et al.*, 1996; Santos *et al.*, 1997) Eventually, a few yeast cells may escape this initial local response and disseminate through the lymphohematogeneous route, giving rise to subclinical and quiescent foci in other organs and systems (Angulo-Ortega, 1972; Benard *et al.*, 2013). In most cases this prior initial pulmonary infection tends to pass unnoticed.

Rarely, for yet unknown reasons, the immune response fails to control the initial infection. In these cases, there is lymphohematogeous spread, possibly through phagocytic cells loaded with viable yeast cells, with the patient developing the A/SAF characterized by disseminated involvement of the reticuloendothelial system (Franco *et al.*, 1987). In these patients, the lungs are usually spared, at least clinical and radiologically since autopsy and in vivo studies of A/SAC-like cases showed that the lungs can harbor yeast cells or very small subclinical or radiologically unapparent granulomatous inflammatory foci along the bronchial tree (Angulo-Ortega, 1972; Giraldo *et al.*, 1976; Restrepo *et al.*, 1989; Londero *et al.*, 1996; Buccheri *et al.*, 2015). It has been proposed that, based on the fact that A/SAF patients have profoundly depressed immune response against the fungus and loose granuloma formation, there would be little inflammatory-mediated tissue damage while such granulomas would not mature and evolve to dense fibrosis (Benard, 2008). This would explain the lack of residual fibrosis or other architectural abnormalities in patients with the A/SAF, for whom pulmonary involvement is probably restricted to the initial phase of the disease and remains underdiagnosed because of the absence of clinical or radiological evidence (Campos *et al.*, 1992; Benard *et al.*, 2005). Alternatively, witnessing the wide gamut of clinical presentations of the mycosis, some reports described A/SAF patients with a progressive primary pulmonary PCM much like that seen in tuberculosis: they developed simultaneously clinically overt pulmonary involvement and systemic reticuloendothelial involvement (Ramos *et al.*, 1981; Bittencourt *et al.*, 1986; Martinez and Moya, 2009).

Nevertheless it is roughly estimated that 98% of the infected individuals the quiescent foci, pulmonary or extra-pulmonary, remain so throughout life, making the rate of subclinical infections to outnumber many fold the (low) frequency of PCM disease even in endemic regions (Martinez, 2017). The most common clinical presentation of the disease is the CF, considered to result from fungal reactivation in these foci (Franco *et al.*, 1987). Thus, not infrequently the disease manifests when the patient has left

the endemic area. Of note these cases may manifest decades after leaving the endemic area, occasionally in patients living outside Latin America, when they represent an otherwise diagnostic challenge (Chikamori *et al.*, 1984; Silletti *et al.*, 1996; Horré *et al.*, 2002; Slevogt *et al.*, 2004; Kayser *et al.*, 2019).

In the chronic form, lung involvement frequently occurs. The mycosis is restricted to the lungs in some of these patients, but in most, other organs also are involved due to lymphohematogeneous dissemination from reactivated pulmonary foci or directly from foci at virtually any organ (Angulo-Ortega, 1972; Franco *et al.*, 1987; Benard *et al.*, 2013). Actually, PCM, in the A/SAF and CF, is almost always a disseminated disease, even though clinical manifestations are apparently restricted to a single organ: the degree of dissemination certainly is influenced by the availability of diagnostic procedures (Yamaga *et al.*, 2003; Shikanai-Yasuda *et al.*, 2017). After appropriate therapy, residual fibrotic lesions, remain in significant proportion of patients and represent the actual main challenge in the treatment of PCM (Mendes, 1994; Tabón *et al.*, 1995, 2003; Weber *et al.*, 2006; Costa *et al.*, 2013; Lopera *et al.*, 2015).

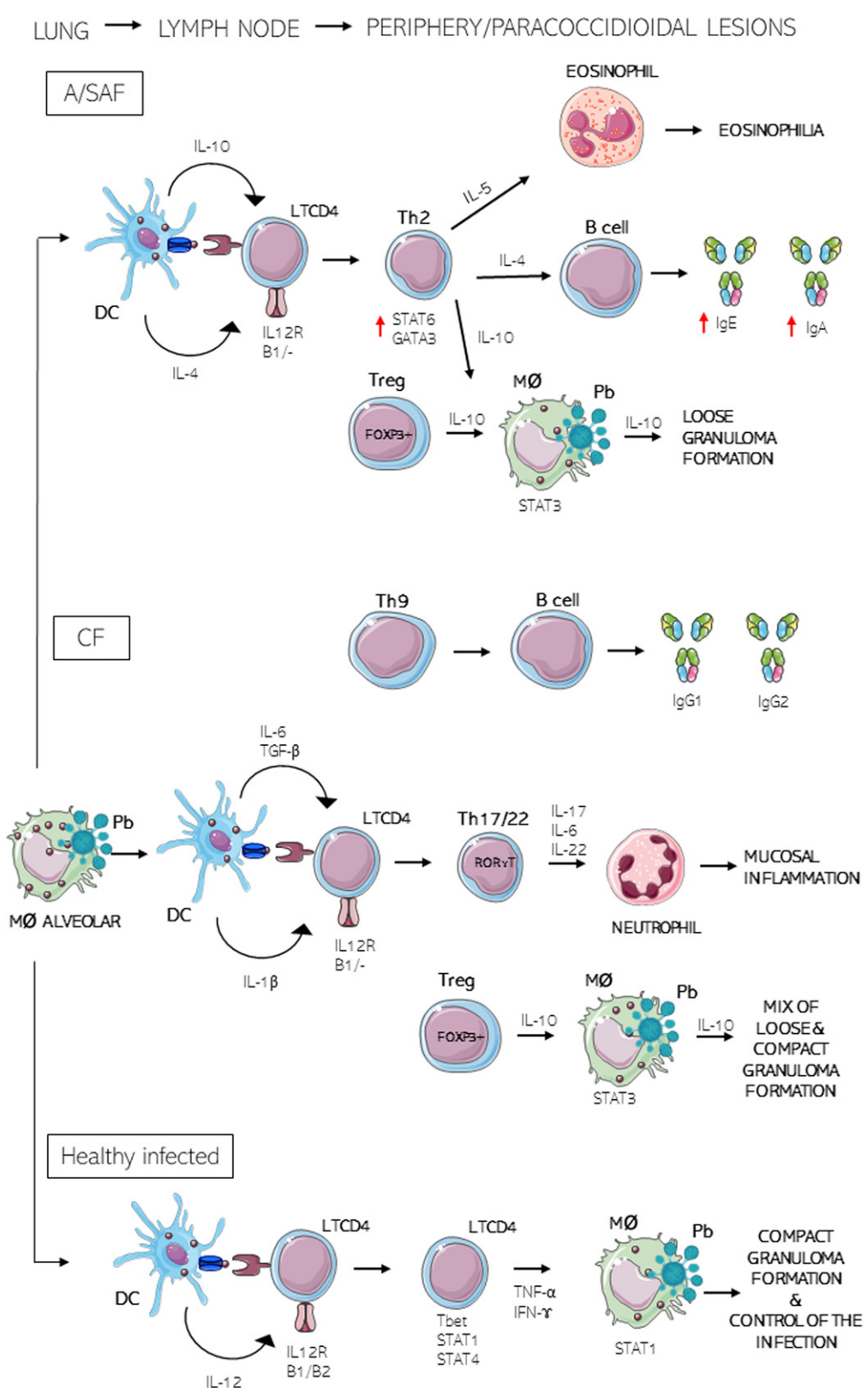
The time between infection and the onset of symptoms in the A/SAF of the disease is not precisely known, being estimated as weeks to many months; it lasted a few months according to the rare case reports with putative known date of infection (Franco *et al.*, 1987; Buccheri *et al.*, 2015). On the other hand, the time elapsed between infection and the onset of symptoms in the CF is estimated as years to decades (Franco *et al.*, 1987).

Histologically, the mycosis is characterized by the formation of compact granulomas containing few fungal elements in most patients; however, A/SAF or CF patients with severe disseminated disease have predominantly loose granuloma formation (Restrepo *et al.*, 1976; Brito and Franco, 1994). Lung involvement is secondary to a chronic lymphangitic process provoked by the fungus itself and the host's response represented by formation of granulomas and fibrosis, the latter of which predominates at the perihilar region (Tuder *et al.*, 1985). This aspect correlates with the butterfly-like (perihilar) micronodular and interstitial infiltration observed on plain films (Funari *et al.*, 1999; Souza *et al.*, 2006). Obstruction and reversal of lymphatic flow would lead to the spread of the inflammatory process throughout the lungs (Tuder *et al.*, 1985). The granulomatous inflammation is associated with a mixed pyogenic component, especially in the case of ulcerated skin lesions or ruptured lymph nodes (Franco *et al.*, 1989). Caseation and central necrosis may be present. In compact granulomas, abundant epithelial cells, Langerhans or foreign-body giant cells, plasmocytes, and lymphocytes are seen; often, phagocytosis of the yeast cells can be observed. $CD4^+$ lymphocytes dominate over $CD8^+$ lymphocytes and appear as peripheral mantles around aggregates of macrophages and histiocytes (Moscardi-Bacchi *et al.*, 1989). In the A/SAF and CF patients with disseminated disease, the inflammatory reaction is loose, with abundance of both mononuclear and yeast cells but sparse formation of compact granulomas (Tuder *et al.*, 1985; Franco *et al.*, 1989; Brito and Franco, 1994). Loose granulomas appear unable to circumscribe fungal antigens, and at their periphery, *Paracoccidioides* spp. antigens may permeate throughout the intercellular space (Sandoval *et al.*, 1996). Skin and mucous membrane lesions usually exhibit pseudoepitheliomatous hyperplasia and intraepithelial microabscesses (Franco *et al.*, 1989; Moscardi-Bacchi *et al.*, 1989). The role of neutrophils in the inflammatory response against paracoccidioides spp. has recently been reinvestigated and it was shown that neutrophil extracellular traps are present in tegumentary lesions of patients (Della Coletta *et al.*, 2015).

An important aspect drawn from the previously described histologic studies is the frequent description of areas of active disease characterized by pyogenic reaction and loose granulomata, rich in budding fungal cells, intermingled with areas with compact granulomas, rare fungal cells, and variable degrees of fibrosis (Tuder *et al.*, 1985). This mixed aspect can be observed in pulmonary, oro-pharyngeal and skin lesions, and even in lymph nodes, suggesting that the disease evolves through localized new bouts of fungal multiplication and tissue invasion, whereas the adjacent older lesions are in their way to fibrotic resolution. CT scans of the lung confirmed this aspect by depicting areas with alveolar condensation along with fibrotic and emphysematous zones and the simultaneous presence of features of more recent and chronic lesions (Funari *et al.*, 1999; Souza *et al.*, 2006).

Tissue reactions are nonspecific; thus, diagnosis depends on finding *P. brasiliensis*. If the parasite is abundant, it may be identified by hematoxylin and eosin stains. Special fungal stains (e.g., Grocott silver methenamine), however, always should be employed, especially when granulomata are examined. The typical multiple budding yeast cells must be found to establish a diagnosis. The presence of fungal cells of different sizes (2–40 μm) suggests the presence of *P. brasiliensis*. In some cases, short chains and cells with single buds also are observed, and in these patients, differentiation of *P. brasiliensis* from *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and even *Histoplasma capsulatum* and *Pneumocystis jirovecii* must be made (Brummer *et al.*, 1993; Silletti *et al.*, 1996). When the disease is chronic, most of the fungal cells are found inside the macrophages, but free yeast cells predominate in disseminated cases. Internalized yeast cells exhibit altered morphology (Restrepo, 2000).

As other chronic granulomatous diseases, PCM is a spectral disease, with the A/SAF characterized by poor cellular immune responses, high fungal burden, high anti-*Paracoccidioides* antibody titers, including those of the IgG4 and IgE subclasses, eosinophilia, all of which typical of a predominance of Th-2 over Th-1 immune responses (Benard, 2008). Conversely, some patients with the chronic form have a more localized disease, low fungal burden, compact granulomas and low antibody titers, suggesting that they are still able to mount Th-1 responses. In between would stay the chronic multifocal patients with deficient Th-1 type responses without marked shift to Th-2 responses (Benard, 2008). However, the simplistic view of a Th-1/Th-2 paradigm of immune response has been challenged by the description of other subset of T cells such as T regulatory cells, Th-17 cells, Th-9 cells and Th-22 cells. The clinical phenotypes presented by PCM patients have been associated with these subsets. A/SAF patients displayed predominantly a mixed Th2/Th9 response while in CF patients there was a balance between Th-1, Th-17 and Th-22 responses depending on the extent of the disease in these patients (de Castro *et al.*, 2013). Recent data demonstrated that the NLRP3 inflammasome was essential for the activation and expansion of Th17 and Th1 cells upon challenge in vitro with *P. brasiliensis* yeast cells, while inhibition of this receptor by DC lead to increased Th2 and Treg frequency (de Castro *et al.*, 2018). **Fig. 5** describes a proposed scheme of the cellular immune response profiles of patients with PCM, based mostly on data from


LUNG → LYMPH NODE → PERIPHERY/PARACOCCIDIOIDAL LESIONS
A/SAF
EOSINOPHIL
EOSINOPHILIA
IL-10
LTCD4
Th2
IL-5
B cell
DC
IL-4
IL12R B1/-
STAT6 GATA3
IL-10
IgE
IgA
Treg
FOXP3+
MØ
Pb
IL-10
LOOSE GRANULOMA FORMATION
STAT3
CF
Th9
B cell
IgG1
IgG2
IL-6 TGF-β
Pb
MØ ALVEOLAR
DC
LTCD4
Th17/22
RORγT
IL-17 IL-6 IL-22
MUCOSAL INFLAMMATION
NEUTROPHIL
IL12R B1/-
IL-1β
Treg
FOXP3+
IL-10
MØ
Pb
IL-10
MIX OF LOOSE & COMPACT GRANULOMA FORMATION
STAT3
Healthy infected
DC
LTCD4
IL12R B1/B2
IL-12
LTCD4
Tbet STAT1 STAT4
TNF-α IFN-γ
MØ
Pb
STAT1
COMPACT GRANULOMA FORMATION & CONTROL OF THE INFECTION

studies of patients' immune responses, the focus of this review. Apparently, the severity of the disease is driven mainly, on one side, by the host's genetic background and immune response status and, on the other side, by the size of the inoculum, rather than peculiarities of fungus isolate. The immunogenicity and pathogenicity of *P. brasiliensis* samples freshly isolated from patients with PCM were tested in mice to compare with the severity of disease of the isolate's donor but a well defined relationship between pathogenicity of the fungal isolate in mice and the clinical findings of the correspondent patient was not evident (Sadahiro *et al.*, 2007). On the other hand, the mechanisms responsible in vivo for killing of the fungus are not yet known. *In vitro* studies showed that hydrogen peroxide (H_2O_2) released by normal human macrophages activated by Th-1 cytokines, kills yeast cells of *Paracoccidioides* sp. (Carmo *et al.*, 2006). Resting human macrophages are otherwise unable to kill the fungus or to handle the fungal cell wall of phagocytosed yeast cells (Lenzi HL., unpublished data). Others have shown that NK cells are able to recognize and kill in vitro *P. brasiliensis* as well as *P. brasiliensis*-infected monocytes (Longhi *et al.*, 2012). They also found that NK cells from PCM patients exhibit a lower cytotoxic response compared with healthy individuals.

Clinical Manifestations

As yet, there is no differentiation between the clinical manifestations caused by the two *Paracoccidioides* species, *brasiliensis* and *lutzii*, in a same fashion to what is observed with the disease caused by *C. immitis* and *C. posadasii*. PCM is a polymorphic disorder that may affect any system and organ. The current classification of the mycosis relies not only in the organs/systems involved but also in the host's immune status and the natural history of the disease (Franco *et al.*, 1987). The classification comprises a subclinical form (PCM-infection), the PCM-disease, which is subdivided into A/SAF, CF and mixed form, and a residual form. The A/SAF form predominates in children and young adults, though the denomination of juvenile form, while the CF affects adults and aged people, and is thus also named adult form. According to the severity of the disease, the A/SAF may be severe and moderate. The CF may apparently be restricted to the lungs or disseminated. As stated earlier, the degree of dissemination will depend on the availability of diagnostic procedures. Disseminated disease is characterized by involvement of the oral and upper respiratory mucosa, reticuloendothelial system, adrenal glands, skin and central nervous system; and, less frequently, gastrointestinal or genitourinary tract and bones (Giraldo *et al.*, 1976; Restrepo *et al.*, 1976; Mendes, 1994; Bellissimo-Rodrigues *et al.*, 2013). In more than 80% of the disseminated cases there is concomitant lung involvement, probably the initial localization from which distant foci were established through lymphohematogenous route. The CF can be mild, moderate, or severe (Franco *et al.*, 1987). It has been proposed that the disease presented by immunocompromised patients such as those with HIV-infection would correspond to a new, mixed form (Benard and Duarte, 2000). This mixed form was further substantiated by case-control studies (Morejón *et al.*, 2009; Almeida *et al.*, 2017). Finally, a fraction of the patients remains with fibrotic, residual lesions which may compromise their full health recovery, and thus are classified as having the residual form (Franco *et al.*, 1987; Shikanai-Yasuda *et al.*, 2017).

Fig. 5 Proposed schematic view of the immune network that takes place in the main outcomes of the paracoccidioidomycosis host-parasite interaction. Note that the three main outcomes, healthy infected, A/SAF and CF, are also an oversimplification of the polymorphic clinical spectrum of the disease. Infection initiates when inhaled *Paracoccidioides* conidia reach terminal bronchi or alveolar spaces, where they interact with and are phagocytosed by alveolar macrophages. These cells, harboring live and/or dead fungal elements, migrate to draining lymph nodes where transformation into dendritic cells and antigen presentation take place. DC-T cell interaction will then trigger three types, eventually overlapping, immune networks. In HI (lower part), DCs express NLRP3 and secrete predominantly IL-12 which drives activate activated T cells to differentiate into Th-1 cells, expressing the transcriptional factor Tbet and Th-1 cytokine signaling molecules STAT-1 and STAT-4, while expressing the β1 and β2 subunits of the IL-12R. These in turn release IFN-γ and TNF-α and activate macrophages to phagocytose and kill the yeast cells. Macrophages in this case express the pro-inflammatory cytokine signaling molecule STAT-1. Overall, this network leads to formation of compact granulomas and control/resolution of the infection. In the A/SAF (upper part), DCs do not express NLRP3 and release predominantly IL-10 and IL-4, which activate Th-2 cells; other T cell subtypes (Treg, Th-9) are also expanded and activated. Gata3 and STAT-6 expressing Th-2 cells induce eosinophilia (via IL-5 release), B-cells production of anti-Paracoccidioides IgE and Ig4 (via IL-4 release), but disarm macrophages via IL-10 release, which upregulate the inhibitory cytokine signaling molecule STAT-3, and as such *Paracoccidioides* yeast cells are not contained and loose granuloma formation ensues. The expanded *Paracoccidioides*-activated Tregs seen in these patients also contribute to the poor cellular immune response and poor granuloma formation. Expanded activated Th-9 cells drive massive *anti-Paracoccidioides* IgG1 and IgG2 isotype production. In the CF (middle part), which comprises patients with mild, localized disease to "localized" but severe lungs involvement to disseminated disease, present immune networks that may or may not be closer to either that of the HF or that of the A/SAF. In CF patients, the dormant yeast cells within clinically silent foci established during the initial infection, for yet unknown reasons, reactivate. DCs at these sites, upon challenge with the fungi, express NLRP3 but secrete predominantly the pro-inflammatory cytokines IL-6, IL1-β and TGF-β, which activates T-cells toward a Th-17 phenotype, the hallmark of the CF, but also Th-22. In parallel, the lack of Th-1 activation, resulting in less well activated (STAT-3 +) macrophages favor further destabilization of the granuloma. Th activated Th cells also lack expression of the β2 subunit of the IL-12R but express the intracellular transcriptional factor RORγT. Both Th subtypes are responsible for the secretion of several cytokines, among them IL-6, IL-22, IL-17, that induce mucosal inflammation with prominent participation of neutrophils. Tregs and Th-9 are also important participants in the CF immune network. The net result is a variable mix of well-formed granuloma that can limit the infection and loose granuloma that correspond to foci of active disease. Note that the arrows arising from the CF to both the A/SAF and HI outcomes indicate that both Th2 and Th-1 responses may be part of the CF network, depending on the extent and severity of the patient's disease. The factors that dictate the patient's progression to one or another outcome are as yet unknown. Adapted from Benard, G., 2008. An overview of the immunopathology of human paracoccidioidomycosis. Mycopathologia 165, 209–221. doi:10.1007/s11046-007-9065-0. de Castro, L.F., *et al.*, 2013. Characterization of the immune response in human paracoccidioidomycosis. Journal of Infection 67, 470–485. doi:10.1016/j.jinf.2013.07.019, with modifications.

Patients with A/SAF develop signs and symptoms of a wasting process. Fever, malaise, listlessness, weight loss, and emaciation are recorded frequently (Benard *et al.*, 1994; Londero *et al.*, 1996; Nogueira *et al.*, 2006; Benard and Mendes-Giannini, 2019; Romaneli *et al.*, 2019). The A/SAF is a disease of the reticuloendothelial system resulting in damage of the corresponding organs. Superficial lymph node enlargement is the predominant sign in over 80% of these cases (Londero *et al.*, 1996; Benard and Mendes-Giannini, 2019; Romaneli *et al.*, 2019). Cervical and submandibular lymph node chains are involved most commonly, followed by those of the supraclavicular and axillary regions; however, any chain can be affected. Lymph nodes may vary in size from slightly enlarged to large, painful, coalescent masses; some may progress to fistulization draining purulent material rich in yeast cells.

Hepatomegaly and splenomegaly, usually asymptomatic, are the second most common finding in the A/SAF. In a report of 63 cases of PCM in children under age 16 liver involvement was detected in 40%, of whom 68% presented hepatomegaly and 29% jaundice (Pereira *et al.*, 2004). A more recent survey of 142 cases under 15 years-old found slightly lower frequencies of hepatomegaly ($\sim$30%) and splenomegaly (20%) (Romaneli *et al.*, 2019). Liver enzymes, especially alkaline phosphatase and gamma-glutamyl transferase are frequently but not markedly increased (Nogueira *et al.*, 2006). Gross hepatic lesions may not be apparent, but histopathologic examination regularly reveals fungal invasion of this organ in the more severe cases (Benard *et al.*, 1994; Boccalandro and Albuquerque, 1960; Londero *et al.*, 1996), as also revealed by a study of a series of fatal cases (Teixeira *et al.*, 1978). The presence of yeast cells in the liver was associated with a granulomatous tissue response. Splenic lesions are nodular or military. Portal hypertensions a rare occurrence. Importantly, a study showed that liver involvement was associated with younger age, more severe anemia, hypoalbulinemia and malnutrition and eventually higher risk of death (de Melo Braga *et al.*, 2013). Findings and complaints relating to the abdomen and digestive tract, such as presence of abdominal masses, lymph node enlargement, diarrhea, vomiting, abdominal distention or pain, and ascites, also have also been recorded in a sizable proportion of A/SA form patients (Benard *et al.*, 1994; Londero *et al.*, 1996; Pereira *et al.*, 2004; de Melo Braga *et al.*, 2013; Romaneli *et al.*, 2019). Hypertrophied lymph nodes, usually generalized but particularly periaortic, around the hepatic hilum and retroperitoneally, can be detected by US, CT scans or MRI (Martinez *et al.*, 1988). Signs and symptoms of an acute abdomen, caused by masses formed by hypertrophied lymph nodes that may become palpable or perforation, also have been reported. These coalescent masses may result in pathology caused by extrinsic compression of adjacent structures, such as jaundice by compression of the biliary duct, pancreatitis, intestinal occlusion, and blockade of lymphatic drainage followed by ascites (Benard *et al.*, 1994; Londero *et al.*, 1996; Pereira *et al.*, 2004; de Melo Braga *et al.*, 2013; Benard and Mendes-Giannini, 2019; Romaneli *et al.*, 2019). In fact, histopathologic examination of autopsies (likely the more severe cases) showed a specific granulomatous enteritis in 80% (Fonseca and Mignone, 1976). Additionally, in a MRI study of patients mostly with the A/SAF, 48% had abdominal lymph node enlargement, even if no abdominal signs and symptoms had been recorded (Martinez *et al.*, 1979b). This lymph node involvement can cause mesenteric lymphatic stasis and enteric mucosal edema that may progress to fungal enteritis accompanied by abnormal intestinal function such as reduced absorption of fat (Martinez *et al.*, 1979b; Shikanai-Yasuda *et al.*, 1992a,b; Benard *et al.*, 1996; Nogueira *et al.*, 2006; de Melo Braga *et al.*, 2013). Patients may thus develop a disabsorptive syndrome that aggravates the nutritional and immune status. Abdominal complications are still being sporadically reported despite improved diagnosis and treatments (Romaneli *et al.*, 2019). Digestive involvement may eventually be observed in CF patients with severe disseminated disease. The pathogenesis and manifestations are similar to those of the A/SAF, with primary involvement of the lymphatics causing lymph nodes enlargements, hepatosplenomegaly and ulcerative enteritis (Martinez *et al.*, 1979a,b).

Studies also have shown bone marrow infiltration mainly, but not exclusively, in the A/SAF of the disease (Resende *et al.*, 2006). Bone lesions and articular involvement may be important components of the severe forms of the disease, especially in younger children. In these patients, the long bones were frequently affected, with the lytic lesions located at the diaphyseal or metaphyseal-epiphyseal regions (Doria and Taylor, 1997; Pereira *et al.*, 2010; Correa-de-Castro *et al.*, 2012; Monsignore *et al.*, 2012), probably because of their higher vascularization, emphasizing the hematogenous dissemination that typically occurs in this form of the disease. Ribs, skull, phalanges, and vertebral lytic lesions also have been documented. Differently from the painful, motion-restriction joint lesions, the bone lesions can be silent of oligosymptomatic (Doria and Taylor, 1997; Monsignore *et al.*, 2012). A pathologic fracture may occasionally occur (Cimerman *et al.*, 1997).

Skin lesions were noted in 20%–30% of the A/SAF cases, with a tendency toward higher frequency with increasing patient age (**Fig. 6**). Distribution of cutaneous lesions was variable, but face and trunk were involved more frequently Acute-subacute paracoccidioidomycosis: A pediatric cohort of 141 patients, exploring clinical characteristics, laboratorial analysis and developing a non-survival predictor (Londero *et al.*, 1996; Nogueira *et al.*, 2006; Romaneli *et al.*, 2019). Lung abnormalities were recorded in a small proportion of cases. However, even in the absence of clinical and radiologic involvement, colonization of the lung by *Paracoccidioides* spp. can be demonstrated by direct examination and by culture (Restrepo *et al.*, 1989). When chest radiographs (**Fig. 7**) were abnormal, enlarged hilar lymph nodes was the most common abnormality; military or interstitial infiltrates were occasionally seen (Campos *et al.*, 1992; Londero *et al.*, 1996; Benard *et al.*, 2005; Martinez and Moya, 2009). In contrast with the CF, where adrenal involvement is a serious concern, it is uncommon in the A/SAF. A survey of adrenal function before and after treatment in 23 children with the A/SA disease showed normal adrenal function (Pereira *et al.*, 2006).

Anemia, an increased erythrocyte sedimentation rate, severe hypoalbuminemia, and hypergammaglobulinemia with high IgG serum concentrations are found regularly (Londero *et al.*, 1996; Nogueira *et al.*, 2006; Romaneli *et al.*, 2019). Nonetheless, anti–*Paracoccidioides* antibodies may prove undetectable in some patients with localized disease and low fungal burden or due to the production of low avidity antibodies (Neves *et al.*, 2003). Eosinophilia, as well as elevated IgE antibody titers, has been detected in most patients (Yarzábal *et al.*, 1980; Shikanai-Yasuda *et al.*, 1992a,b; Biselli *et al.*, 2001; Mamoni *et al.*, 2002; Nogueira *et al.*, 2006). Recently, it was proposed that the serum albumin level could predict the outcome of children with PCM: an albumin cutoff $\leq$ 2.18 g/dL on admission had a

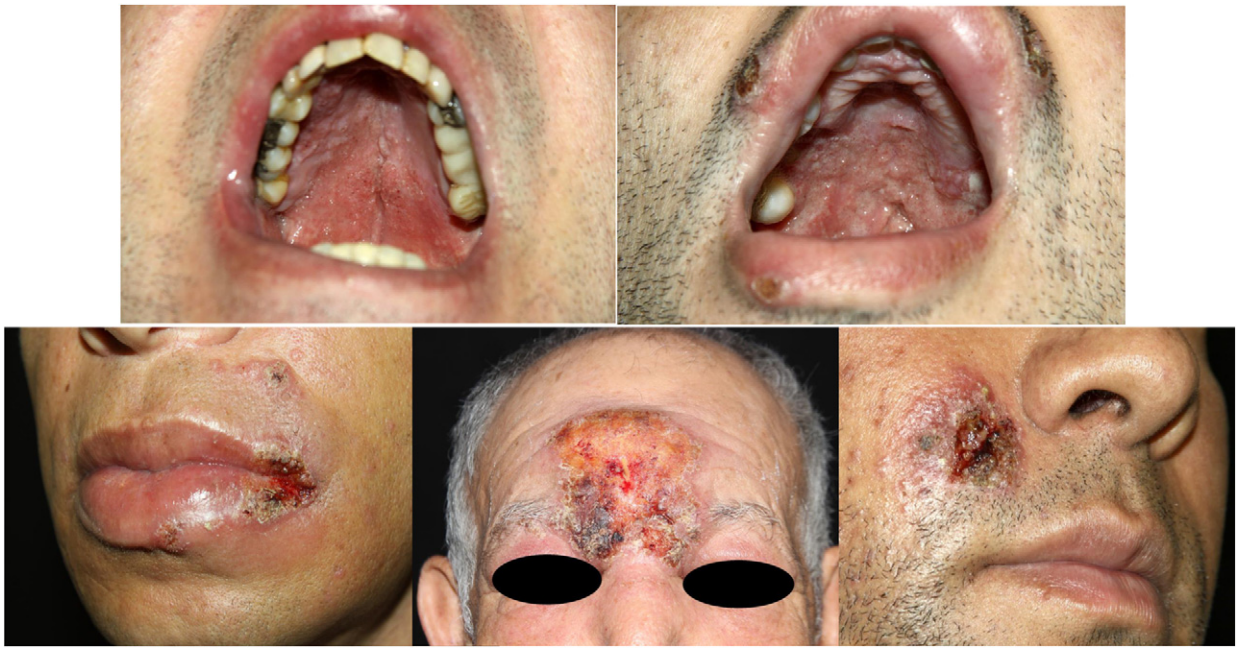


Fig. 6 Patients with the chronic form of the disease presenting characteristic muco-cutaneous lesions: hard palate ulcerated lesions with the typical moriform stomatitis aspect, ulcerated and infiltrative cutaneous facial lesions, and ulcerated lesions of the lips.

85.7% sensitivity and 85.4% specificity for non-survival outcome while values above 2.18 g/dL translated into a 99.1% chance of survival (Romaneli *et al.*, 2019). Studies of other cohorts should be performed to validate this prognostic factor.

The lungs are the target organ in the CF. The respiratory symptoms are nonspecific and, in most cases, evolve slowly, albeit progressively, thus delaying the diagnosis. Many patients attribute their symptoms to the smoking habit, related by ~90% of the patients with the CF (Costa *et al.*, 2013; Peçanha *et al.*, 2017). Smoking appears to be a risk factor for the development of the CF PCM (dos Santos *et al.*, 2003). Main symptoms are short breathiness, mild dyspnea, dry cough, occasionally with sputum production and, in lesser proportions, hemoptysis. These symptoms may be accompanied by systemic symptoms such as fatigue, weight loss and asthenia. Lung auscultation reveals abnormalities in less than half of the patients; furthermore, at diagnosis over 20% of patients with several alterations on lungs radiological exams exhibit very mild symptoms and no alterations on pulmonary auscultation, a clinic radiological dissociation early observed in this mycosis (Franco *et al.*, 1989; Mendes, 1994; Gomes *et al.*, 2008). Fibrosis is frequently observed already at diagnosis (Campos *et al.*, 2008; Valle *et al.*, 1992; Mendes, 1994; Tobón *et al.*, 2003; Costa *et al.*, 2013) In fact, patients seek medical assistance for oropharyngeal lesions with the pulmonary involvement being diagnosed subsequently through imaging exams. CT scans main findings, according to the Fleischner Society´s Glossary of Terms (Austin *et al.*, 1996), are ground-glass attenuation, small centrilobular nodules, cavitated nodules, large nodules, parenchymal bands, areas of cicatricial emphysema, interlobular septal thickening, and architectural distortion (Funari *et al.*, 1999; Souza *et al.*, 2006; Costa *et al.*, 2013). Cavitations and air-space consolidation are also found (Funari *et al.*, 1999; Souza *et al.*, 2006; Costa *et al.*, 2013). These abnormalities are bilateral and tend to be symmetrical. Hilar lymph node enlargements can be found. The reversed halo sign, defined as central ground-glass opacity surrounded by a crescent or ring of consolidation, is observed in up to 10% of patients with PCM (Gasparetto *et al.*, 2005). Pulmonary function tests show an obstructive pattern affecting mainly the small airways: probably both the fungus and smoking contribute the ventilation/perfusion alterations and alveolo-interstitial destruction (Lemle *et al.*, 1983; Campos *et al.*, 2008; Costa *et al.*, 2013). Smoking alone could probably explain these alterations in some patients (Gomes *et al.*, 2008). However, in 30% of the patients receiving an adequate course of therapy, pulmonary fibrosis will appear de novo or will consolidate if preexisting, such that by the end of the treatment period this sequela will be documented in over 50% of the cases by chest X-ray, or even higher percentages when CT scans are employed (Tobón *et al.*, 2003; Costa *et al.*, 2013).

Lesions in the oropharyngeal and laryngeal mucosa occur frequently in the CF (Mendes, 1994; Sant'Anna *et al.*, 1999; **Fig. 5**). Hoarseness, localized pain, dysphagia, and dyspnea (due to tracheal stenosis) are main complaints. Deaths due to suffocation have been recorded, and immediate laryngoscopic evaluation is highly recommended in patients reporting laryngeal stridor and dyspnea. Urgent tracheostomy may be indicated in these cases (Campos *et al.*, 1986). Lesions in the oral cavity are particularly painfull, and can affect the teeth and gum, which results in difficulties in chewing the aliments and significant weight loss. There is not a uniform or typical aspect of the lesions: they may be infiltrative, ulcerated, nodular, or vegetative, and may mimick squamous cell carcinoma. However, they usually have a granulomatous superficial aspect (Mendes, 1994; Migliari *et al.*, 1998; Sant'Anna *et al.*, 1999): the base of the ulcerated lesions usually is covered by small abscesses (the mulberry-like lesions) that probably represent fungal dissemination through the lymphatic system because they usually are accompanied by regional lymph node involvement (Uribe *et al.*, 1987; Castro *et al.*, 1999). In the A/SAF, mucosal lesions are rather exceptional, but skin

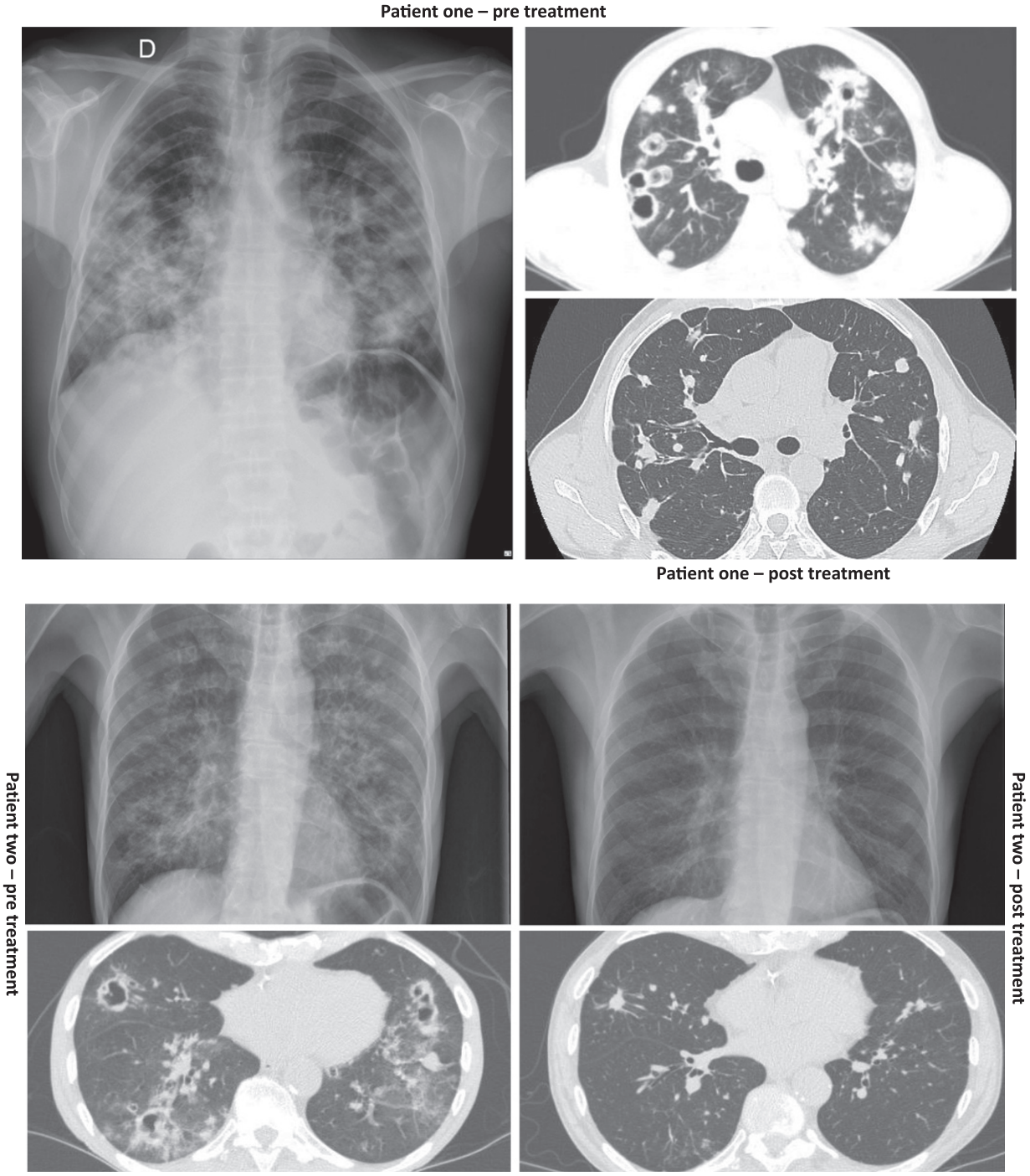


Fig. 7 Typical pulmonary involvement in the chronic form of the disease. Patient one, chest X-ray (left) showing typical bilateral infiltrates on admission, and thoracic CT scans showing the parenchymatous lesions before (upper) and their improvement during antifungal therapy (lower). Patient two, on the left, chest X-ray showing typical bilateral infiltrates and thoracic CT scan showing the parenchymatous lesions on admission; on the right, chest X-ray and CT scan taken at the end of the antifungal therapy: note the presence of residual abnormalities such as small opacities and nodules, fibrosis, bronchial thickening and small area still with ground glass aspect.

involvement occurs more commonly and tends to be multiple, in contrast with the CF (Mendes, 1994; Marques *et al.*, 2007). In the latter, lesions are represented mostly by contiguous involvement of the periorificial mucosal lesions or draining lymph nodes. In both the A/SA and chronic severe disseminated form, they represent hematogenous spread of the fungus. In this case, the lesions may appear as ulcerated or ulcero-vegetative lesions, papules, or crust-covered ulcers and warts, usually at the same stage of

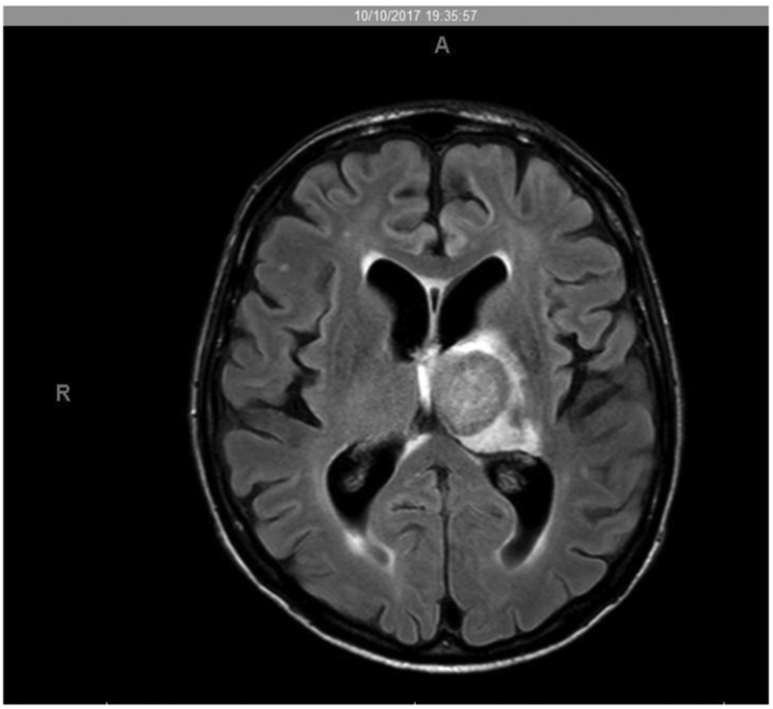


Fig. 8 Brain CT scan showing a pseudotumoral lesion in a patient with the chronic form of the disease.

development (Mendes, 1994; Marques *et al.*, 2007) Skin involvement may rarely present as sarcoidic like lesions (Coelho *et al.*, 2016). Mucosal and skin lesions usually are the first ones to resolve with treatment.

The adrenals often are involved in patients with the chronic form of the mycoses, with a small proportion of the patients evolving to overt adrenal hypofunction or insufficiency (Addison disease). A study showed that adrenal reserve was reduced in 44% of the patients, mostly with the CF (del Negro *et al.*, 1980). Main manifestations of chronic adrenal insufficiency are malaise, fatigue, anorexia, weight loss, arterial hypotension, orthostatic hypotension, hyperpigmentation of the skin, nausea, and reduced libido. The glands contain multiple granulomatous foci, and diffuse necrosis may be seen in the most severe cases. Hyperplasia of the adrenal glands also occurs commonly (Tendrich *et al.*, 1991). The adrenal insufficiency has been documented in patients even after prolonged post-therapy follow up (Tabón *et al.*, 1995), although in a few patients the adrenal function has been reported to fully recover (do Valle *et al.*, 1993).

Reports on the frequency of CNS involvement are quite variable, from 3.4% to 25.45% (Almeida, 2005); it is however more frequent than it has been admitted in the past. NeuroPCM (**Fig. 8**) should always be considered in the differential diagnosis of meningoencephalitis and in expansive processes of the CNS, especially in endemic areas (de Almedia *et al.*, 2004). Most patients with neuroPCM have the CF and involvement of other organs (particularly lungs) at the time SNC symptoms manifest. Involvement of the CNS is considered to be secondary to a primary focus: not infrequently the pulmonary involvement is diagnosed after that of the CNS. Rarely it is the sole localization of the disease (Almeida, 2005). A review showed that while in 21% of the cases the onset of neurological symptoms occurred before the onset of systemic symptoms, in 33% they happened simultaneously and in 46% they appeared after the onset of the systemic symptoms (de Almedia *et al.*, 2004). Pseudotumoral lesions in the brain hemispheres is the most typical presentation, although it can occur in any location of the CNS (Gasparetto *et al.*, 2003; Rosa Junior *et al.*, 2019). Clinical manifestations are non-specific and related to the location of the CNS lesion: according to a review, seizure in 33%, hemiparesis in 25%, cerebellar signs in 25%, headache in 21%, and hydrocephalus in 21%; less frequent symptoms include paresthesia in 13%, confusion in 13%, and bulbar signs in 8% (Almeida, 2005). CT scan images are usually hypodense, with annular or nodular enhancing, surrounded by mild edema after contrast injection. In 65% of the patients, there were multiple mass lesions, and 35% had a single mass lesion (Almeida, 2005). Meningitis associated with pseudo tumoral lesions can also occur. It usually presents as a skull base meningitis, with mild to moderate lymphomononuclear pleocytosis and elevated protein levels, but the finding of yeast cells in the CSF is an exception. Neuro-PCM is a serious condition: in a case series of 24 neuroPCM patients (de Almedia *et al.*, 2004), four patients died while 20 responded to the antifungal treatment, but most remained with residual lesions after treatment, characterized in the CT scan as hyperdense lesions, with irregular contrast enhancement, eventually residual calcified lesions. Neurological sequels are not uncommon in these patients.

Reports of septic shock caused by septicemia by *Paracoccidioides* spp. show that fungemia can occur (Azulay *et al.*, 1988; Londero *et al.*, 1996). However, probably death is not caused by the fungemia per se, but by the severely dysregulated immune response triggered by it which culminates in an Inflammatory cytokine storm and extensive tissue necrosis (Benard *et al.*, 2010, 2012b).

Association between human immunodeficiency virus (HIV) infection and PCM has been reported (Benard and Duarte, 2000; Morejón *et al.*, 2009; Almeida *et al.*, 2017). PCM was also diagnosed occasionally in patients with other immunossupressive conditions such as malignancies, transplant, and immunosuppressive therapies (Shikanai-Yasuda *et al.*, 2008; Ruiz e Resende *et al.*, 2015; de Almeida *et al.*, 2019). The number of cases in HIV-infected patients was much lower than it was initially expected from what was observed with the association of other systemic mycoses with HIV (e.g., histoplasmosis). However the actual number of cases may be underestimated since PCM still is not a compulsory notification disease in most endemic countries. Clinical manifestations of these patients often do not fit into the A/SAF or CF patterns. They frequently present a disseminated disease, with lymph nodes involvement, hepatosplenomegaly and cutaneous lesions, suggestive of the A/SAF, concomitant with pulmonary and/or oropharyngeal mucosal involvement, more frequently seen in the CF (Benard and Duarte, 2000; Morejón *et al.*, 2009; Almeida *et al.*, 2017). The likely explanation is that the disease is due to reactivation of a latent infection as in the CF, but with higher lymphohematogenous dissemination due to the immunosuppression, as seen in the A/SAF. In fact, most patients were living in urban areas when they developed the (Benard and Duarte, 2000; de Almeida *et al.*, 2019). Thus the proposition of the classification as mixed form. Most patients with HIV-PCM had CD4 cell counts below 200 cells/mL (Benard and Duarte, 2000; Morejón *et al.*, 2009; Almeida *et al.*, 2017; de Almeida *et al.*, 2019). Mortality was higher than in non-HIV infected, but it was usually attributed to other HIV-infection related comorbidities than to PCM itself (Morejón *et al.*, 2009; Almeida *et al.*, 2017; de Almeida *et al.*, 2019).

Treatment

Several classes of drugs can be used to treat PCM. Historically sulfonamides were the first class of compounds to be effective in the treatment of PCM (Ribeiro, 1940), followed by the introduction of amphotericin B in 1958 (Lacaz and Sampaio, 1958). Imidazole derivatives were introduced in the end of 1970s' with ketoconazole. This drug is now rarely considered in PCM treatment, having been replaced in the end of the 1980s' by the triazole derivative itraconazole (Negroni *et al.*, 1987; Restrepo *et al.*, 1987). Fluconazole is another option for PCM treatment, but is has less often been used for its lower in vitro activity compared with itraconazole, although it can be indicated for neuro-PCM cases for its low binding to plasma proteins, allowing diffusion into the CSF (Diaz *et al.*, 1992; Shikanai-Yasuda, 2015). Voriconazole, posaconazole, and isavuconazole showed inhibitory activity in vitro against *Paracoccidioides* spp. isolates and, therefore, are potentially useful in the treatment of PCM. These as yet high cost drugs are not easily accessible in the endemic areas. Thus, except for two pilot studies showing voriconazole and isavuconazole effectiveness against PCM, mostly in CF patients without severe disseminated disease, accumulated experience with these drugs is still lacking (Telles *et al.*, 2007; Thompson *et al.*, 2016). Voriconazole was used at 8 mg/Kg/day dose, usually 200 mg PO two times daily, while isavuconazole was used at 200 mg PO 3 times daily for 2 days followed by 200 mg once-daily (Telles *et al.*, 2007; Thompson *et al.*, 2016). Drug interactions and adverse events of prolonged therapy (as required for PCM treatment) may be issues with these drugs.

Until recently there was no consensus on treatment decisions and protocols: these varied considerably among reference centers, for several reasons. Total duration of therapy is quite variable according to the severity of the disease and the patients' clinical, radiological and serologic responses to treatment. Neither the classification of the severity of the disease nor the local availability of antifungal drugs are equal among the reference centers. There are scarce comparative studies on PCM treatment, most of them bearing important limitations (Queiroz-Telles *et al.*, 1992; Shikanai-Yasuda *et al.*, 2002; Borges *et al.*, 2014; Cavalcante *et al.*, 2014). A *quasi-experimental* study of 177 patients showed the superiority of itraconazole over the association of sulfamethoxazole and trimethoprim (SMX-TMP), the most commonly used drugs for PCM treatment in Brazil (the country that contributes with over 80% of the cases worldwide) (Cavalcante *et al.*, 2014). These studies nevertheless support the indication of itraconazole as the first-choice treatment for PCM. However, there are some situations in which SMX-TMP is preferred, including patients with neuro-PCM (itraconazole does not cross the blood brain barrier), and patients with gastrointestinal tract involvement, as itraconazole already has erratic absorption in the gastrointestinal tract (Shikanai-Yasuda, 2015). SMX-TMP may be a viable option for the treatment of severe disseminated life threatening PCM in cases amphotericin deoxycolate is contraindicated or lipid formulations are not accessible. An updated guideline on the management of PCM has recently been published by Brazilian experts (Shikanai-Yasuda *et al.*, 2017).

One of the major issues in the treatment of PCM is compliance for the patients often require at least one year of therapy. This issue is sibling of the other major issue in PCM, the development of sequels after treatment in a sizable proportion of patients, discussed in the next section. A retrospective study of SMX-TMP therapy in a poor resource endemic area showed that only 68.3% of 244 treatment records that fulfilled the study's inclusion criteria reported adequate compliance. However, this proportion is likely an overestimation because from the other 283 patients' records that did not fulfill the inclusion criteria, in 67.8% this was due to the patient's missing more that 50% of the appointments (Nery *et al.*, 2017). In fact, a recent study from another reference center in the same poor resource area reported a compliance rate of only 44.6% (Andrade *et al.*, 2019). The importance of continuous treatment must always be emphasized because relapses and increasing risk to developing sequels occur if the drug is not taken regularly. As the CF is strongly associated with smoking habit and alcohol abuse (Martinez and Moya, 1992; dos Santos *et al.*, 2003), particular care should be provided to these issues, in order to assure better compliance, better response to treatment, and lower risk of developing sequels (especially pulmonary damage).

Itraconazole is administered in 100 mg capsules that should be given short after a meal. The Brazilian guidelines recommend 200 mg/day for low to moderate severity forms (Shikanai-Yasuda *et al.*, 2017). Several groups reported good results with this therapy but relapses may still occur (Marques, 2002; Shikanai-Yasuda, 2015). However, we have now successful experience in treating patients with the more severe forms (either A/SAF and CF) of the disease with higher doses of itraconazole (400–600 mg/daily) provided

there is such an involvement of the digestive tract that could further impair the variable absorption rate of the drug. (amphotericin B, the conventional choice for these severe cases, has been reserved to patients with life threatening conditions). Absorption is also impaired by antacids or inhibitors of gastric acid secretion. Side effects have been few and include transient elevation of hepatic enzymes (Naranjo *et al.*, 1990; Shikanai-Yasuda, 2015). Itraconazole in children is recommended at a dose of 5–10 mg/kg daily, with a maximum dosage of 200 mg twice daily (Shikanai-Yasuda *et al.*, 2017).

SMX-TMP (400 mg/80 mg per tablet) is widely used in Brazil, mainly because of its easier availability within Brazil's public health system than itraconazole (Shikanai-Yasuda *et al.*, 2017). It has also been used in association with amphotericin B (Marques *et al.*, 1985). It has good absorption with predictable serum levels, as well as good tolerability, with myelotoxicity (leukopenia) the main side effect, which can be monitored and controlled by folinic acid administration without modification of the therapeutic regimen (Telles *et al.*, 2007). Interstitial nephritis manifested as hyperkalemia may occasionally occur. The Brazilian guideline recommends 8–10 mg/kg of trimethoprim daily (Shikanai-Yasuda *et al.*, 2017). However, in adults it is given generally at a dose of two tablets PO at 12-h (low to moderate cases) or 8-h (moderate to severe cases) intervals with good results. This combination has the advantage of permitting alternative parenteral administration whenever necessary; in this case it can be administered from 12 to up 6-h intervals. Duration of the treatment with this drug varies in each case, but it usually lasts for no less than nine months. Maintenance treatment can be achieved by using one tablet at 12-h intervals. Development of SMX-TMP resistance is occasionally clinically suspected but has rarely been documented in vitro. There is a single report of resistance of an isolate from an A/SAF patient (Hahn *et al.*, 2003).

Amphotericin B should be reserved for severe disseminated or life threatening cases (Shikanai-Yasuda *et al.*, 2017). It also can be used by patients who relapse during the course of or after treatment with orally administered drug because gastrointestinal involvement may impair drug absorption in these cases. The effectiveness of therapy with azoles has curtailed the need for more aggressive regimens. There is still scarce data regarding effectiveness of the new amphotericin B lipid formulations. However, a series of 28 severe patients treated with amphotericin B lipid complex showed good results in all cases (Peçanha *et al.*, 2016).

Fluconazole is an alternative treatment, although not as effective at least in vitro. High doses, up to 600 mg/day, and longer treatment periods that itraconazole should be considered. Recrudescence and relapse of disease apparently occur more frequently than when itraconazole is used (Diaz *et al.*, 1992). Fluconazole may be useful in severely ill patients who must be treated intravenously (Shikanai-Yasuda, 2015). No experience with the A/SAF has yet been reported.

Aids patients can be treated initially intravenously with amphotericin B or SMX-TMP IV (800/160 mg thrice daily), than moved to itraconazole 400–600 mg/day or SMX-TMP P.O. 800/160 mg thrice daily. Alternatively, patients may also be treated orally with higher doses of itraconazole (400–600 mg/day) or SMX-TMP (800–160 mg PO or IV at 12 8-h intervals). Good therapeutic response is generally observed, achieving control of the PCM. Treatment can be moved to the lower regular doses of both drugs until the TCD4 + counts reach 200 cells/μL (or eventually at least 100 cells/μL), provided the HIV viral load remains undetectable (Shikanai-Yasuda *et al.*, 2017). Pharmacological interaction with antiretroviral drugs is an important issue to be addressed in the treatment of Aids patients. For more details regarding treatment of specific conditions such as HIV-infection, pregnancy, children severe forms, the reader is referred to the Brazilian guidelines (Shikanai-Yasuda *et al.*, 2017).

Many issues remain to be addressed regarding optimal treatment of PCM. As stated earlier, treatment of PCM still faces many challenges such as the low compliance rates and strategies that could curtail the development of sequels. The role of adjunct therapy with immunomodulators, such as peptides derived from the *P. brasiliensis* immunodominant glycoprotein that act by augmenting the Th-1-mediated immune responses, was tested in vivo in experimental models only (Taborda *et al.*, 2015). A few studies demonstrated some in vitro reactivity of patients' T-cells to these peptides (Leo *et al.*, 2007). Further studies are required to demonstrate their utility in promoting protective cell mediated immune responses in the human host. In the opposite direction, some investigators have administrated short courses of corticosteroids as adjunct therapy to severe cases, based on the rationale that some patients suffer from an uncontrolled or excessive inflammatory response (Gryschek *et al.*, 2010; Benard *et al.*, 2012a). In support of this, some fatalities were attributed to the exacerbated inflammatory response (Benard *et al.*, 2010, 2012b). Patients who benefited from adjunct prednisone administration (0.5–1.0 mg/kg) for 1–2 weeks were those with intense inflammation in CNS, obstructive lesions of the larynx or trachea, lung lesions resulting in respiratory insufficiency and unremitting fever and weight loss (Gryschek *et al.*, 2010; Benard *et al.*, 2012a). The clinical and laboratory criteria that allow reduction in the antifungal dose or that could be used to end treatment with the assurance that the patient is cured are not established. Progressive decrease in the titers of the serologic tests currently available is one of the laboratory parameters used most frequently. Newer methods (e.g., antigenemia detection, proteomic biomarkers) still need further standardization and clinical studies and have not yet become widely available (Gómez *et al.*, 1998; Salina *et al.*, 1998; Marques da Silva *et al.*, 2003; Marques Da Silva *et al.*, 2004; Sylvestre *et al.*, 2018). Meanwhile, most specialists agree that treatment decisions need to be tailored according to the patient. **Table 1** includes the first line drugs recommended in the therapy of PCM.

Prognosis

Prognosis depends on the status of the patient at the time of diagnosis. Children and adults in whom fungemia has taken place and who have multiple organ involvement may respond less well to therapy and require critical care. Fatality rate due to PCM is progressively being reduced as a result of earlier diagnoses and better treatments. In a report describing eight deaths in a cohort of 141 pediatric cases, fatality rate was 9.5% in the 1981–2001 period and 2.6% in the 2002–2019 period (Romaneli *et al.*, 2019). As stated

Table 1 First line drugs recommended for therapy of PCM

Drugs	*Administration*	*Treatment schedule*
Itraconazole	Adult: 200 mg/day	For ≥ 9 months of treatment. In adults and adolescents the 200 mg dose can be reduced to 100 mg/day with clinical/mycological cure and low antibodies titers in sorological tests
	Children 5–10 mg/kg/day	
SMX-TMP	Adult: SMX 800 mg/TMP 160 mg (PO 8/8 or 12/12hs)	For ≥ 9 months of treatment. In adults and adolescents the dose can be reduced to 400/160 mg/Kg PO 12/12hs with clinical/mycological cure and low antibody titers in sorological tests
	children 5MX 40–50 mg/Kg / TMP 8–10 mg/kg (PO 12/12hs)	
Amphotericin B	deoxycholate 0.5 to 0.7 mg/kg/day (IV)	Attack treatment for few (usually 2–4) weeks then move to itraconazole or SMX-TMP
	lipid formulation 3–5 mg/kg/day (IV)	

Note: Adapted with modifications from Shikanai-Yasuda, M.A., *et al.*, 2017. Brazilian guidelines for the clinical management of paracoccidioidomycosis. Revista da Sociedade Brasileira de Medicina Tropical, 50 (5), 715–740. doi:10.1590/0037-8682-0230-2017.

earlier, albumin level on admission could be a predictor factor for death. Other authors indicated that abdominal complications, especially liver involvement, ascites and malnutrition, were associated with the lethal prognosis (Nogueira *et al.*, 2006; de Melo Braga *et al.*, 2013). In the A/SAF, mortality rates are lower. Complete remission is to be expected for most but not all patients with the A/SAF and CF since sequels may develop and certain organ involvements may be definitive (e.g., pulmonary fibrosis, adrenal insufficiency, neurological sequels). Thus, some clinicians consider the term *cure* inappropriate because of the inability to confirm complete eradication of the organism; the term *apparent cure* should instead be used (Shikanai-Yasuda *et al.*, 2017).

PCM persists as a disease with relatively low mortality but considerable morbidity. Complications vary and, like prognosis, their occurrence depends on the extent of fungal invasion. In the A/SAF, early complications that may lead to surgical intervention are intestinal obstruction and jaundice, both of which result from enlarged mesenteric lymph nodes, or emergency tracheostomy due to laryngeal lesions leading to tracheal stenosis. Sequels are more common in adults than children. In general, fibrosis is the cause of serious problems in patients who respond to therapy, particularly when extensive pulmonary infiltrates are present. Despite the newer, very effective therapies, these sequelae preclude, in a proportion of the cases, the complete restoration of the patients' previous health status (Tobón *et al.*, 2003; Lopera *et al.*, 2015). Scarring and fibrosis of the affected nodes and residual pulmonary fibrosis have been noted in earlier studies (Londero *et al.*, 1996). A study showed that almost all recently treated CF patients remain with at least one abnormality on CT scan (Costa *et al.*, 2013), the most frequent of which were architectural distortion, reticulate and septal thickening, centrilobular and paraseptal emphysema and parenchymal bands. These findings likely reflect the pattern of dissemination of the fungi through the lymphatics and subsequent fibrosis previously described in histopathology studies (Tuder *et al.*, 1985). Functionally, patients typically presented with a mild obstructive disorder and a mild reduction in diffusion capacity with preserved exercise capacity. However, one third of patients presented significant oxygen desaturation upon exercise that was associated with respiratory distress (Costa *et al.*, 2013). In addition, centrilobular and paraseptal emphysema were also observed in most patients, reflecting the high tobacco exposure in this population, and emphasizing the importance of tobacco exposure cessation for better treatment results. A recent study of post-treatment patients with a longer follow-up showed persistence of the fibrosis and emphysema abnormalities; all patients were smokers (Pina, 2006). Attempts to reduce post-treatment pulmonary fibrosis have been tested in experimental models only but may be promising (Lopera *et al.*, 2015). Rarely, a patient may remain with significant enough fibrous scarring to result in development of cor pulmonale (Campos *et al.*, 1986). Other important sequels that may be present in the CF are hoarseness, tracheal stenosis leading to definitive tracheostomy, microstomia, which requires surgical reconstruction, adrenal insufficiency, which requires long life hormonal replacement, and neurological sequels (do Valle *et al.*, 1995; Tabón *et al.*, 1995; Londero *et al.*, 1996; Weber *et al.*, 2006; Francesconi *et al.*, 2011; de Pina *et al.*, 2018).

Human PCM has no recognized prophylactic treatment. Vaccine strategies are being developed but are still restricted to experimental models of the disease (Taborda *et al.*, 2015).

Vaccine Development

The antifungal treatment of PCM requires prolonged drug use plagued by relapses and fibrotic sequelae that incapacitate individuals. This lead scientists to look for new tools (reviewed by Taborda *et al.*, 2018; Shikanai-Yasuda *et al.*, 2017). In this sense, the use of prophylactic or therapeutic vaccines would be an option to protect the host or to reduce the treatment time (Travassos and Taborda, 2012).

One of the most promising vaccine studies concerns the P10 peptide, derived from the *P. brasiliensis* 43 kDa glycoprotein (gp43) (Taborda *et al.*, 1998).

The epitope was mapped to a 15-mer peptide (QTLIAIHTLAIRYAN), immunodominant and protective in murine models against a virulent isolate of *P. brasiliensis*. This effect was attributed to a strong cellular immune response mediated by the secretion of IFN-gamma and IL-2 (Travassos and Taborda, 2012). Very importantly, P10 is also immunodominant and promiscuous in PCM patients, being recognized by 9 predominant HLA molecules, thus becoming a promising candidate for an effective vaccine against PCM in humans (Iwai *et al.*, 2003).

An interesting approach was carried out aiming at associating the P10 peptide with antifungal chemotherapy. Treatment of mice (anergic or not) with antifungal drugs such as itraconazole and sulfamethoxazole/trimethoprim plus P10 showed a protective reduction of the fungal load in the lungs, preserving the alveolar structure and preventing the spread of the fungus to the spleen and liver (Travassos and Taborda, 2012). Based on the peptide P10, different vaccine models with anergic or immunocompetent mice were developed involving: dendritic cells (Magalhães *et al.*, 2012; Silva *et al.*, 2017, 2019); hepatitis B virus-derived particle (VLP) as an antigen carrier (Holanda *et al.*, 2017); Different adjuvants such as aluminum hydroxide, flagellin, cationic lipid dioctadecyl-dimethylammonium bromide (DODAB) and complete Freund's adjuvant (CFA) (Mayorga *et al.*, 2012; Braga *et al.*, 2009); P10 incorporated into PLGA (Amaral *et al.*, 2010), etc.

DNA Vaccine

A vaccine against *P. brasiliensis* using plasmid DNA was first tested in 2000 (Pinto *et al.*, 2000) with a mammalian expression vector carrying the full gene of the gp43 under the control of CMV promoter with Freund's adjuvant resulting in the induction of both B and T cell-mediated immune responses, modulated by IFN-γ. This immunization method was protective in mice prior to challenge with virulent *P. brasiliensis*. Similar DNA vaccines were carried out using P10 as the minigene in the plasmid construction (Pinto *et al.*, 2000).

When given prior to or after infection with a *P. brasiliensis* virulent isolate, plasmid-vaccination with P10 successfully reduced the fungal burden in lungs of infected mice. Intramuscular injection of a combination of plasmids expressing P10 and IL-12 given weekly for one month, followed by single injections, restored normal lung architecture and eradicated the fungus in mice infected one month before treatment (Rittner *et al.*, 2012).

Mice immunized with another plasmid carrying the P10 minigene (pcDNA3-*P10*) were examined, before and after infection with *P. brasiliensis*, for the expression of memory CD4 + $CD44^{hi}$ T cells together with Foxp3 + Treg cells in the spleens and lungs, post infection. Both T lymphocyte types increased corresponding to a lung histopathology with minimal inflammation. The repeated immunization with the.

DNA-P10 plasmid which generated long-term memory and regulatory T cells, replaced the initially protective pro-inflammatory T effector cells, and were thus effective while preserving the integrity of the infected tissue (de Amorim *et al.*, 2013).

Other researchers have also studied different vaccine models such as: DNA vaccine based on HSP65 from *Mycobacterium leprae*, rPb40 and rPb27 recombinant proteins in association with fluconazole reviewed by (Rossi *et al.*, 2019), radio-attenuated yeast cells (Demicheli *et al.*, 2006).

Antibodies

Clinical studies showed that patients with paracoccidioidomycosis develop antibodies, although only a few of them are protective against the mycosis reviewed by (Boniche *et al.*, 2020). Therapy based on passive transference of monoclonal antibodies showed efficient anti-fungal activity in an experimental model using mice. de Mattos Grosso *et al.* (2003) demonstrated that monoclonal antibodies against a 70 kDa glycoprotein of *P. brasiliensis* protected mice infected with the fungi (de Mattos Grosso *et al.*, 2003). Buissa-Filho *et al.* (2008) showed protective and non-protective monoclonal antibodies against gp43, the main diagnostic antigen of *P. brasiliensis* (Buissa-Filho *et al.*, 2008). Other protective antibodies were described: anti-melanin polyclonal antibodies obtained by immunization of mice with melanin ghosts of *P. brasiliensis* reduced fungal burden (da Silva *et al.*, 2006); monoclonal antibodies generated against heat shock protein 60 from *H. capsulatum* also reduced fungal burden from mice infected with *P. lutzii* (Thomaz *et al.*, 2014), polyclonal antibodies to acidic glycosphingolipids (GSL) purified from *P. brasiliensis* reduced infection (Bueno *et al.*, 2016). The results showed that passive transference of antibodies can be a therapeutic strategy associated or not with antifungal chemotherapy.

Acknowledgments

We would like to thank Ronaldo C. B. Gryschek for critical review of the chapter and Thalyta Nery C. Pinto for preparing the **Fig. 5**. This work was partly supported by Fundação de Amparo à Pesquisa do Estado de São Paulo 2016/08730-6, CAPES, and CNPq 420480/2018-8. CPT, LRT and GB are senior researchers of the Conselho Nacional de Desenvolvimento Científico e Tecnológico.

References

Ajello, L., 1977. Medically infectious fungi. Control Microbiology and Immunology 3, 7–19.

Almeida, F.A., 1930. Estudos comparativos do granuloma coccidioidico nos Estados Unidos e no Brasil. Novo gênero para o parasito brasileiro. Annaes da Faculdade de Medicina de São Paulo 5, 125–142.

Almeida, F.A., *et al*., 2017. Paracoccidioidomycosis in brazilian patients with and without human immunodeficiency virus infection. American Journal of Tropical Medicine and Hygiene 96 (2), 368–372. doi:10.4269/ajtmh.16-0254.

Almeida, S.M., 2005. Central nervous system paracoccidioidomycosis: An overview. Braz J Infect Dis 9 (2), 126–133.

Amaral, A.C., *et al*., 2010. Poly(lactic acid-glycolic acid) nanoparticles markedly improve immunological protection provided by peptide P10 against murine paracoccidioidomycosis. British Journal of Pharmacology 159 (5), 1126–1132. doi:10.1111/j.1476-5381.2009.00617.x.

Andrade, U.V., *et al*., 2019. Treatment compliance of patients with paracoccidioidomycosis in Central-West Brazil. Jornal brasileiro de pneumologia: Publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia 45 (2), e20180167. doi:10.1590/1806-3713/e20180167.

Angulo-Ortega, A., 1972. Calcification in paracoccidioidomycosis. Are they morphological manifestation of subclinical infections? Proceedings of the First Panamerican Symposium, PAHO, Washington, 254. Scientific Publication. pp. 129–133. [n°].

Arantes, T.D., *et al*., 2015. *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*, A secret love affair. Revista do Instituto de Medicina Tropical de Sao Paulo 57 (19), 25–30. doi:10.1590/S0036-46652015000700006.

Aristizábal, B.H., *et al*., 2002. Experimental *Paracoccidioides brasiliensis* infection in mice: influence of the hormonal status of the host on tissue responses. Medical Mycology 40 (2), 169–178. doi:10.1080/mmy.40.2.169.178.

Austin, J.H.M., *et al*., 1996. Glossary of terms for CT of the lungs: Recommendations of the nomenclature committee of the Fleischner society. Radiology 200 (2), doi:10.1148/radiology.200.2.8685321.

Azulay, R.D., *et al*., 1988. Acute disseminated paracoccidioidomycosis septic shock. International Journal of Dermatology 27 (7), 510–511. doi:10.1111/j.1365-4362.1988.tb00934.x.

Bagagli, E., *et al*., 1998. Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus noveminctus*) captured in an endemic area of paracoccidioidomycosis. American Journal of Tropical Medicine and Hygiene 58 (4), 505–512. doi:10.4269/ajtmh.1998.58.505.

Bagagli, E., *et al*., 2006. Phylogenetic and evolutionary aspects of *Paracoccidioides brasiliensis* reveal a long coexistence with animal hosts that explain several biological features of the pathogen. Infection, Genetics and Evolution 6 (5), 344–351. doi:10.1016/j.meegid.2005.12.002.

Bagagli, E., *et al*., 2008. *Paracoccidioides brasiliensis*: Phylogenetic and ecological aspects. Mycopathologia 165, 197–207. doi:10.1007/s11046-007-9050-7.

Bellissimo-Rodrigues, F., *et al*., 2013. Endemic paracoccidioidomycosis: relationship between clinical presentation and patients' demographic features. Medical Mycology 51 (3), 313–318. doi:10.3109/13693786.2012.714529.

Benard, G., *et al*., 1994. Severe acute paracoccidioidomycosis in children. Pediatric Infectious Disease 13 (6), 510–515. doi:10.1097/00006454-199406000-00009.

Benard, G., *et al*., 1996. Cryptococcosis as an opportunistic infection in immunodeficiency secondary to paracoccidioidomycosis. Mycopathologia 133 (2), 65–69. doi:10.1007/BF00439115.

Benard, G., *et al*., 2005. Contribution to the natural history of paracoccidioidomycosis: Identification of the primary pulmonary infection in the severe acute form of the disease-a case report. Clinical Infectious Diseases 40 (1), e1–e4. doi:10.1086/426691.

Benard, G., 2008. An overview of the immunopathology of human paracoccidioidomycosis. Mycopathologia 165, 209–221. doi:10.1007/s11046-007-9065-0.

Benard, G., *et al*., 2010. Fatal acute respiratory distress syndrome in a patient with paracoccidioidomycosis: First case report. Medical Mycology 48, 542–545. doi:10.3109/13693780903330563.

Benard, G., *et al*., 2013. Chronic paracoccidioidomycosis of the intestine as single organ involvement points to an alternative pathogenesis of the mycosis. Mycopathologia 176, 353–357. doi:10.1007/s11046-013-9699-z.

Benard, G., Duarte, A.J.S.J., 2000. Paracoccidioidomycosis: A model for evaluation of the effects of human immunodeficiency virus infection on the natural history of endemic tropical diseases. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 31 (4), 1032–1039. doi:10.1086/318146.

Benard, G., Campos, A.F., *et al*., 2012a. Treatment of severe forms of paracoccidioidomycosis: Is there a role for corticosteroids? Medical Mycology 50 (August), 641–648. doi:10.3109/13693786.2011.654135.

Benard, G., Patzina, R.L., *et al*., 2012b. Fatal septic shock due to a disseminated chronic form of paracoccidioidomycosis in an aged woman. Medical Mycology 50 (4), 407–411. doi:10.3109/13693786.2011.630685.

Benard, G., Mendes-Giannini, M.J.S., 2019. Paracoccidioidmycosis. In: Cherry, J., Demmler-Harrison, G.J., Kaplan, S.L., Steinbach, W.J., Hotez, P.J. (Eds.), Feign and Cherry's Textbook of Pediatric Infectious Diseases, eighth ed. Elsevier, pp. 2056–2066.

Bialek, R., *et al*., 2000. Detection of *Paracoccidioides brasiliensis* in tissue samples by a nested PCR assay. Journal of Clinical Microbiology 2000, 2940–2942. doi:10.1128/jcm.38.8.2940-2942.2000.

Biselli, P.J.C., *et al*., 2001. IgE antibody response to the main antigenic component of *paracoccidioides brasiliensis* in patients with paracoccidioidomycosis. Medical Mycology 39 (6), 475–478. doi:10.1080/mmy.39.6.475.478.

Bittencourt, A.L., Freire-de-Andrade, J.A., Cendon-Filha, S.P., 1986. Paracoccidioidomycosis in a four-year-old boy. Mycopathologia 93, 55–59. doi:10.1007/BF00437015.

Bocca, A.L., *et al*., 2013. Paracoccidioidomycosis: Eco-epidemiology, taxonomy and clinical and therapeutic issues. Future Microbiology 8 (9), 1177–1191. doi:10.2217/fmb.13.68.

Boccalandro, I., Albuquerque, J.F., 1960. Icterícia e comprometimento hepático na blastomicose sulamericana. A propósito de 10 casos e revisão bibliográfica. Revista Paulista de Medicina 56, 350–366.

Boniche, C., *et al*., 2020. Immunotherapy against systemic fungal infections based on monoclonal antibodies. Journal of Fungi 29 (6), E31. doi:10.3390/jof6010031.

Borges, S.R.C., *et al*., 2014. Itraconazole vs. trimethoprim-sulfamethoxazole: A comparative cohort study of 200 patients with paracoccidioidomycosis. Medical Mycology 52 (3), 303–310. doi:10.1093/mmy/myt012.

Bozzi, A., *et al*., 2006. Interleukin-10 and tumor necrosis factor-α single nucleotide gene polymorphism frequency in paracoccidioidomycosis. Human Immunology 67 (11), 931–939. doi:10.1016/j.humimm.2006.07.014.

Braga, C.J.M., *et al*., 2009. *Paracoccidioides brasiliensis* vaccine formulations based on the gp43-derived P10 sequence and the Salmonella enterica FliC flagellin. Infection and Immunity 77 (4), 1700–1707. doi:10.1128/IAI.01470-08.

Brass, K., 1969. Observations on the pathologic anatomy, pathogenesis, and evolution of paracoccidioidomycosis. Mycopathologia et Mycologia Applicata 37 (2), 119–138.

Brito, T. de, Franco, M.F., 1994. Granulomatous inflammation. Revista do Instituto de Medicina Tropical de São Paulo 36 (2), 185–192.

Brummer, E., Castañeda, E., Restrepo, A., 1993. Paracoccidioidomycosis: An update. Clinical Microbiology Reviews 6, 89–117.

Buccheri, R., *et al*., 2015. Incubation period and early natural history events of the acute form of paracoccidioidomycosis: Lessons from patients with a single *paracoccidioides* spp. exposure. Mycopathologia 181 (5), 1–5. doi:10.1007/s11046-015-9976-0.

Bueno, R.A., *et al*., 2016. Antibodies against glycolipids enhance antifungal activity of macrophages and reduce fungal burden after infection with paracoccidioides brasiliensis. Frontiers in Microbiology 7 (February), doi:10.3389/fmicb.2016.00074.

Buissa-Filho, R., *et al*., 2008. The monoclonal antibody against the major diagnostic antigen of *Paracoccidioides brasiliensis* mediates immune protection in infected BALB/c mice challenged intratracheally with the fungus. Infection and Immunity 76 (7), 3321–3328. doi:10.1128/IAI.00349-08.

Bustamante-Simon, B., *et al*., 1985. Characteristics of the conidia produced by the mycelial form of *Paracoccidioides brasiliensis*. Medical Mycology 23 (6), 407–414. doi:10.1080/00362178585380601.
Campos, E.P., *et al*., 1986. Aggressive pulmonary paracoccidioidomycosis, stenosing endotracheitis and subacute cor pulmonale. Description of a case. Revista do Instituto de Medicina Tropical de São Paulo 28 (3), 185–189. doi:10.1590/S0036-46651986000300009.
Campos, E.P., Padovani, C.R., Caetano, A.M., 2008. Paracoccidioidomycosis: Radiologic and pulmonary study in 58 cases. Revista do Instituto de Medicina Tropical de São Paulo 33 (4), 267–276.
Campos, E.P., Bertioli, C.J., Barbosa, K.S., 1992. Linfonodo pulmonar na paracoccidioidomicose aguda infantil (relato de um caso). Revista da Sociedade Brasileira de Medicina Tropical 25 (3), 195–200.
Carmo, J.P.M., *et al*., 2006. TNF-α activates human monocytes for *Paracoccidioides brasiliensis* killing by an H2O2-dependent mechanism. Medical Mycology 44 (4), 363–368. doi:10.1080/13693780500536885.
Carvalho, F.M.C., *et al*., 2016. Polymorphisms on IFNG, IL12B and IL12RB1 genes and paracoccidioidomycosis in the Brazilian population. Infection, Genetics and Evolution 43, 245–251. doi:10.1016/j.meegid.2016.05.025.
Castro, C., *et al*., 1999. MRI of head and neck paracoccidioidomycosis. British Journal of Radiology 72, 717–722.
Cavalcante, R. de S., *et al*., 2014. Comparison between Itraconazole and cotrimoxazole in the treatment of paracoccidiodomycosis. PLoS Neglected Tropical Diseases 8 (4), e2793doi:10.1371/journal.pntd.0002793.
Chikamori, T., *et al*., 1984. Paracoccidioidomycosis in Japan. Report of a case. Revista do Instituto de Medicina Tropical de Sao Paulo 26 (5), 267–271. doi:10.1590/S0036-46651984000500007.
Cimerman, S., *et al*., 1997. Paracoccidioidomycosis in a boy infected with HIV. Mycoses 40 (9–10), 343–344. doi:10.1111/j.1439-0507.1997.tb00246.x.
Coelho, M.G., *et al*., 2016. Paracoccidioidomycosis mimicking sarcoidosis: A review of 8 cases. Mycopathologia 181 (1–2), 137–143. doi:10.1007/s11046-015-9938-6.
Conant, N.F., Howell, A., 1942. The similarity of the fungi causing South American blastomycosis (paracoccidioidomycosis) and North American blastomycosis (Gilchrist disease). Journal of Investigative Dermatology 5, 353–370.
Correa-de-Castro, B., *et al*., 2012. Case report: Unifocal bone paracoccidioidomycosis, Brazil. American Journal of Tropical Medicine and Hygiene 86 (3), 470–473. doi:10.4269/ajtmh.2012.11-0295.
Corredor, G.G., *et al*., 1999. Isolation of *Paracoccidioides brasiliensis* from the nine-banded armadillo Dasypus novemcinctus, in an endemic area for paracoccidioidomycosis in Colombia. Revista Iberoamericana de Micologia 16 (4), 216–220.
Costa, A.N., *et al*., 2013. The lung in paracoccidioidomycosis: New insights into old problems. Clinics 68 (4), 441–448. doi:10.6061/clinics/2013(04)02.
da Silva, M.B., *et al*., 2006. Melanin in the dimorphic fungal pathogen *Paracoccidioides brasiliensis*: Effects on phagocytosis, intracellular resistance and drug susceptibility. Microbes and Infection 8 (1), 197–205. doi:10.1016/j.micinf.2005.06.018.
de Almeida, J.N., *et al*., 2015. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for differentiation of the dimorphic fungal species *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*. Journal of Clinical Microbiology 53 (4), 1383–1386. doi:10.1128/JCM.02847-14.
de Almeida, J.N., Peçanha, P.M., Colombo, A.L., 2019. Paracoccidioidomycosis in immunocompromised patients: A literature review. Journal of Fungi 5 (1), 2. doi:10.3390/jof5010002.
de Almedia, S.M., *et al*., 2004. Central nervous system paracoccidioidomycosis: Clinical features and laboratorial findings. Journal of Infection 48 (2), 193–198. doi:10.1016/j.jinf.2003.08.012.
de Amorim, J., *et al*., 2013. DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis induces long-term protection in presence of regulatory T cells. Microbes and Infection 15 (3), 181–191. doi:10.1016/j.micinf.2012.11.007.
de Camargo, Z.P., 2008. Serology of paracoccidioidomycosis. Mycopathologia 165 (4–5), 289–302. doi:10.1007/s11046-007-9060-5.
de Castro, L.F., *et al*., 2013. Characterization of the immune response in human paracoccidioidomycosis. Journal of Infection 67, 470–485. doi:10.1016/j.jinf.2013.07.019.
de Castro, L.F., *et al*., 2018. NLRP3 inflammasome is involved in the recognition of *Paracoccidioides brasiliensis* by human dendritic cells and in the induction of Th17 cells. Journal of Infection 77 (2), 137–144. doi:10.1016/j.jinf.2018.03.004.
de Mattos Grosso, D., *et al*., 2003. Characterization of gp70 and Anti-gp70 monoclonal antibodies in *Paracoccidioides brasiliensis* pathogenesis. Infection and Immunity 71 (11), 6534–6542. doi:10.1128/IAI.71.11.6534-6542.2003.
de Melo Braga, G., Hessel, G., Pereira, R.M., 2013. Hepatic involvement in pediatric patients with paracoccidioidomycosis: A clinical and laboratory study. Mycopathologia 176 (3–4), 279–286. doi:10.1007/s11046-013-9682-8.
de Moraes-Vasconcelos, D., *et al*., 2005. *Paracoccidioides brasiliensis* disseminated disease in a patient with inherited deficiency in the 1 subunit of the interleukin (IL)-12/IL-23 receptor. Clinical Infectious Diseases 41 (4), 31–37. doi:10.1086/432119.
de Pina, D.R., *et al*., 2018. Paracoccidioidomycosis: Level of pulmonary sequelae in high resolution computed tomography images from patients of two endemic regions of Brazil. Quantitative Imaging in Medicine and Surgery 7 (3), 318–325. doi:10.21037/qims.2017.06.04.
del Negro, G., *et al*., 1980. limited adrenal reserve in paracoccidioidomycosis: Cortisol and aldosterone responses to 1–24 ACTH. Clinical Endocrinology 13 (6), 553–559. doi:10.1111/j.1365-2265.1980.tb03423.x.
Della Coletta, A.M., *et al*., 2015. Neutrophil extracellular traps identification in tegumentary lesions of patients with paracoccidioidomycosis and different patterns of NETs generation in vitro. PLoS Neglected Tropical Diseases 9 (9), e0004037. doi:10.1371/journal.pntd.0004037.
Demicheli, M.C., *et al*., 2006. *Paracoccidioides brasiliensis*: Attenuation of yeast cells by gamma irradiation. Mycoses 49 (3), 184–189. doi:10.1111/j.1439-0507.2006.01229.x.
Desjardins, C.A., *et al*., 2011. Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. PLoS Genetics 7 (10), e1002345doi:10.1371/journal.pgen.1002345.
Diaz, M., *et al*., 1992. A pan-american 5-year study of fluconazole therapy for deep mycoses in the immunocompetent host. Clinical Infectious Diseases 14 (Suppl. 1), 68–76. doi:10.1093/clinids/14.Supplement_1.S68.
do Valle, A.C.F., *et al*., 1993. Recovery of adrenal function after treatment of paracoccidioidomycosis. American Journal of Tropical Medicine and Hygiene 48 (5), 626–629. doi:10.4269/ajtmh.1993.48.626.
do Valle, A.C.F., *et al*., 1995. Clinical and endoscopic findings in the mucosae of the upper respiratory and digestive tracts in post-treatment follow-up of paracoccidioidomycosis patients. Revista do Instituto de Medicina Tropical de Sao Paulo 37 (5), 407–413. doi:10.1590/s0036-46651995000500005.
Doria, A.S., Taylor, G.A., 1997. Bony involvement in paracoccidioidomycosis. Pediatric Radiology 27, 67–69. doi:10.1007/s002470050067.
dos Santos, W.A., *et al*., 2003. Association between smoking and paracoccidioidomycosis: A case-control study in the State of Espírito Santo, Brazil. Cadernos de saúde pública / Ministério da Saúde, Fundação Oswaldo Cruz, Escola Nacional de Saúde Pública 19 (1), 245–253. doi:10.1590/S0102-311 $\times$ 2003000100027.
Ferreira, M.S., *et al*., 1990. Isolation and characterization of a *Paracoccidioides brasiliensis* strain from a dogfood probably contaminated with soil in uberlândia, Brazil. Medical Mycology 28 (3), 253–256. doi:10.1080/02681219080000311.
Fonseca, L.C., Mignone, C., 1976. Paracoccidioidomicose do intestino delgado, aspectos anatomo-clínicos e radiológicos de 125 casos. Revista do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo 31, 199–207.
Francesconi, F., *et al*., 2011. Long-term outcome of neuroparacoccidioidomycosis treatment. *Revista* da Sociedade Brasileira de Medicina Tropical 44 (1), 22–25. (S0037-86822011000100006 [pii]).
Franco, M., *et al*., 1987. Paracoccidioidomycosis: A recently proposed classification of its clinical forms. Revista da Sociedade Brasileira de Medicina Tropical 20 (2), 129–132. doi:10.1590/S0037-86821987000200012.
Franco, M., *et al*., 2000. A critical analysis of isolation of *Paracoccidioides brasiliensis* from soil. Medical Mycology 38 (3), 185–191. doi:10.1080/714030941.

Franco, M.F., *et al*., 1989. Paracoccidioidomycosis. Baillieres Clinical Tropical Medicine and Communicable Diseases 4 (185–220), 185–220.
Funari, M., *et al*., 1999. Chronic pulmonary paracoccidioidomycosis (South American blastomycosis): High-resolution CT findings in 41 patients. American Journal of Roentgenology 173 (1), 59–64. doi:10.2214/ajr.173.1.10397100.
Gasparetto, E.L., *et al*., 2003. Central nervous system paracoccidioidomycosis: Imaging findings in 17 cases. Journal of Computer Assisted Tomography 27, 12–17.
Gasparetto, E.L., *et al*., 2005. Reversed halo sign in pulmonary paracoccidioidomycosis. American Journal of Roentgenology 184 (6), 1932–1934. doi:10.2214/ajr.184.6.01841932.
Gegembauer, G., Araujo, L.M., Pereira, E.F., *et al*., 2014. Serology of Paracoccidioidomycosis Due to *Paracoccidioides Lutzii*. *PLoS* neglected tropical diseases. 8 (7), e2986. doi:10.1371/journal.pntd.0002986.
Giraldo, R., *et al*., 1976. Pathogenesis of Paracoccidioidomycosis: A model based on the study of 46 patients. Mycopathologia 58 (2), 63–70. doi:10.1007/BF00707174.
Goldani, L.Z., *et al*., 1991. HLA antigens in Brazilian patients with paracoccidioidomycosis. Mycopathologia 114 (2), 89–91. doi:10.1007/BF00436427.
Gomes, E., Wingeter, M.A., Svidzinski, T.I.E., 2008. Clinical-radiological dissociation in lung manifestations of paracoccidioidomycosis. Revista da Sociedade Brasileira de Medicina Tropical 41 (5), 454–458. doi:10.1590/S0037-86822008000500004.
Gomes, G.M.M., *et al*., 2000. PCR for diagnosis of paracoccidioidomycosis. Journal of Clinical Microbiology 38 (9), 3478–3480. doi:10.1128/jcm.38.9.3478-3480.2000.
Gómez, B.L., *et al*., 1998. Antigenemia in patients with paracoccidioidomycosis: Detection of the 87-kilodalton determinant during and after antifungal therapy. Journal of Clinical Microbiology 36 (11), 3309–3316. doi:10.1128/jcm.36.11.3309-3316.1998.
Grose, E., Tamsitt, J.R., 1965. *Paracoccidioides brasiliensis* recovered from the intestinal tract of three bats (Artibeus lituratus) in Colombia, S.A. Medical Mycology 4 (2), 124–125. doi:10.1080/00362176685190281.
Gryschek, R.C.B., *et al*., 2010. Paradoxical reaction to treatment in 2 patients with severe acute paracoccidioidomycosis: A previously unreported complication and its management with corticosteroids. Clinical Infectious Diseases 50 (10), 56–58. doi:10.1086/652290.
Hahn, R.C., *et al*., 2003. Disseminated paracoccidioidomycosis: Correlation between clinical and in vitro resistance to ketoconazole and trimethoprim sulphamethoxazole. Mycoses 46 (8), 324–329. doi:10.1046/j.1439-0507.2003.00901.x.
Holanda, R.A., *et al*., 2017. Recombinant vaccines of a $CD4^+$ T-cell epitope promote efficient control of *Paracoccidioides brasiliensis* burden by restraining primary organ infection. PLoS Neglected Tropical Diseases 11 (9), doi:10.1371/journal.pntd.0005927.
Horré, R., *et al*., 2002. A case of imported paracoccidioidomycosis in a German legionnaire. Medical Mycology 40 (2), 213–216. doi:10.1080/mmy.40.2.213.216.
Hrycyk, M.F., *et al*., 2018. Ecology of *Paracoccidioides brasiliensis*, *P. lutzii* and related species: Infection in armadillos, soil occurrence and mycological aspects. Medical mycology 56 (8), 950–952. doi:10.1093/mmy/myx142.
Iwai, L.K., *et al*., 2003. In silico prediction of peptides binding to multiple HLA-DR molecules accurately identifies immunodominant epitopes from gp43 of *Paracoccidioides brasiliensis* frequently recognized in primary peripheral blood mononuclear cell responses from sensitized ind. Molecular Medicine 9 (9–12), 209–219. [Available at].http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1430984&tool=pmcentrez&rendertype=abstract
Kamikawa, C.M., Mendes, R.P., Vicentini, A.P., 2017. Standardization and validation of Dot-ELISA assay for *Paracoccidioides brasiliensis* antibody detection. Journal of Venomous Animals and Toxins including Tropical Diseases 23 (1), 11. doi:10.1186/s40409-017-0101-3.
Kayser, M., *et al*., 2019. Chronic progressive pulmonary paracoccidioidomycosis in a female immigrant from Venezuela. Therapeutic Advances in Respiratory Disease 13. doi:10.1177/1753466619894913.
Lacaz, C.S., 1982. Evolução dos conhecimentos sobre a paracoccidioidomicose. Um pouco da história. In: Lacaz, C.S., Del Negro, G.D., Fiorillo, A.M. (Eds.), Paracoccidioidomicose - Blastocomicose Sul-Americana. São Paulo: Savier/Edusp, pp. 1–9.
Lacaz, C.S., Sampaio, S.A.P., 1958. Tratamento da blastomicose sulamericana com anfotericina B. Resultados preliminares. Revista Paulista de Medicinata 52, 443–450.
Lacaz, C.S., Porto, E., Martins, J.E.C., 1991. Paracoccidioidomicose, eighth ed. São Paulo: Savier.
Lacerda, G.B., Arce-Gomez, B., Filho, F.Q.T., 1988. Increased frequency of HLA-b40 in patients with paracoccidioidomycosis. Medical Mycology 26 (4), 253–256. doi:10.1080/02681218880000351.
Lemle, A., *et al*., 1983. Pulmonary function in paracoccidioidomycosis (South American blastomycosis). An analysis of the obstructive defect. Chest 83 (5), 827–828. doi:10.1378/chest.83.5.827.
Leo, K.I., *et al*., 2007. T-cell recognition of *Paracoccidioides brasiliensis* gp43-derived peptides in patients with paracoccidioidomycosis and healthy individuals. Clinical and Vaccine Immunology 14 (4), 474–476. doi:10.1128/CVI.00458-06.
Londero, A.T., *et al*., 1996. Paracoccidioidomycosis in Brazilian children. A critical review (1911–1994). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 70, 197–203.
Longhi, L.N.A., *et al*., 2012. Phenotypic and functional characterization of NK Cells in human immune response against the dimorphic fungus *Paracoccidioides brasiliensis*. Journal of Immunology 189 (2), 935–945. doi:10.4049/jimmunol.1102563.
Lopera, D.E., *et al*., 2015. Pentoxifylline immunomodulation in the treatment of experimental chronic pulmonary paracoccidioidomycosis. Fibrogenesis and Tissue Repair 8. doi:10.1186/s13069-015-0027-8.
Lozano, V.F., *et al*., 2011. Polymorphism analysis of the CTLA-4 gene in paracoccidioidomycosis patients. Memorias do Instituto Oswaldo Cruz 106 (2), 220–226. doi:10.1590/S0074-02762011000200017.
Magalhães, A., *et al*., 2012. Prophylactic and therapeutic vaccination using dendritic cells primed with peptide 10 derived from the 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis*. Clinical and Vaccine Immunology 19 (1), 23–29. doi:10.1128/CVI.05414-11.
Mamoni, R.L., *et al*., 2002. Enhanced production of specific IgG4, IgE, IgA and TGF-β in sera from patients with the juvenile form of paracoccidioidomycosis. Medical Mycology 40 (2), 153–159. doi:10.1080/mmy.40.2.153.159.
Marques, S.A., *et al*., 1985. Paracoccidioidomycosis: a comparative study of the evolutionary serologic, clinical and radiologic results for patients treated with ketoconazole or amphotericin B plus sulfonamides. Mycopathologia 89, 19–23. doi:10.1007/BF00437128.
Marques, S.A., 2002. Treatment of paracoccidioidomycosis with itraconazole. Annu Rev Biomed Sci. Special issue), 24–25.
Marques, S.A., *et al*., 2007. Paracoccidioidomicose: freqüência, morfologia e patogênese de lesões tegumentares. Anais Brasileiros de Dermatologia 82 (4), 411–417. doi:10.1590/s0365-05962007000500003.
Marques, S.A., Lastória, J.C., Marques, M.E.A., 2011. Paracoccidioidomycosis in a patient with cervical cancer. Anais Brasileiros de Dermatologia 86 (3), 587–588. doi:10.1590/S0365-05962011000300029.
Marques da Silva, S.H., *et al*., 2003. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. Journal of Clinical Microbiology 41 (8), 3675–3680. doi:10.1128/JCM.41.8.3675-3680.2003.
Marques Da Silva, S.H., *et al*., 2004. Monitoring gp43 antigenemia in paracoccidioidomycosis patients during therapy. Journal of Clinical Microbiology 42 (6), 2419–2424. doi:10.1128/JCM.42.6.2419-2424.2004.
Martinez, R., *et al*., 1979a. O comprometimento gastrintestinal na blastomicose sul-americana (paracoccidioidomicose). I. Estudo clínico, radiológico e histopatológico. Revista da Associação Médica Brasileira 25, 31–34.
Martinez, R., *et al*., 1979b. O comprometimento gastrintestinal na blastomicose sul-americana (paracoccidioidomicose) II. Estudo funcional do intestino delgado. Revista da Associação Médica Brasileira 25, 70–77.
Martinez, R., 2010. Paracoccidioidomycosis: the dimension of the problem of a neglected disease. Rev Soc Bras Med Trop. 43 (4), 480.
Martinez, R., 2015. Epidemiology of Paracoccidioidomycosis. Revista do Instituto de Medicina Tropical de São Paulo 57 (Suppl 1), 11–20. doi:10.1590/S0036-46652015000700004.
Martinez, R., 2017. New trends in paracoccidioidomycosis epidemiology. Journal of Fungi 3 (1), 1. doi:10.3390/jof3010001.

Martinez, R., Moya, M.J., 1992. The relationship between paracoccidioidomycosis and alcoholism. Rev Saúde Pública 26 (1), 12–16.
Martinez, R., Moya, M.J., 2009. Primary complex of paracoccidioidomycosis and hypereosinophilia. Jornal brasileiro de pneumologia: Publicacao oficial da Sociedade Brasileira de Pneumologia e Tisilogia 35 (12), 1259–1262. doi:10.1590/s1806-37132009001200016.
Martinez, R., Belluci, A.D., Fiorillo, A.M., 1988. A tomografia computadorizada na avaliação do comprometimento abdominal na paracoccidioidomicose. Revista da Sociedade Brasileira de Medicina Tropical 21 (2), 47–50.
Matute, D.R., *et al.*, 2006. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. Molecular Biology and Evolution 23 (1), 65–73. doi:10.1093/molbev/msj008.
Mayorga, O., *et al.*, 2012. The role of adjuvants in therapeutic protection against paracoccidioidomycosis after immunization with the P10 peptide. Frontiers in Microbiology 3. doi:10.3389/fmicb.2012.00154.
Mcewen, J.G., *et al.*, 1987. Experimental murine paracoccidiodomycosis induced by the inhalation of conidia. Medical Mycology 25 (3), 165–175. doi:10.1080/02681218780000231.
Melo, I.S., Londero, A.T., 1983. Spontaneously resolving pulmonary lesions in paracoccidioidomycosis – Case report and review. Mycopathologia 82, 57–59. doi:10.1007/BF00436947.
Mendes, R.P., 1994. The gamut of clinical manifestations. In: Franco, M., Lacaz, C.S., Restrepo-Moreno, A., del Negro, G. (Eds.), Paracoccidioidomycosis. CRC Press, pp. 233–258.
Mendonça, M.S., *et al.*, 2015. High interleukin-4 expression and interleukin-4 gene polymorphisms are associated with susceptibility to human paracoccidioidomycosis. Memorias do Instituto Oswaldo Cruz 110 (6), 781–785. doi:10.1590/0074-02760150197.
Migliari, D.A., *et al.*, 1998. Periodontal aspects of the juvenile form of paracoccidioidomycosis. Revista do Instituto de Medicina Tropical de Sao Paulo 40 (1), 15–18. doi:10.1590/S0036-46651998000100004.
Monsignore, L.M., *et al.*, 2012. Radiologic findings of osteoarticular infection in paracoccidioidomycosis. Skeletal Radiology 41 (2), 203–208. doi:10.1007/s00256-011-1214-3.
Montoya de Restrepo, F., Restrepo, M., Restrepo, A., 1983. Blood groups and HLA antigens in paracoccidioidomycosis. Sabouraudia 21 (1), 35–39.
Moreira, A.P.V., 2008. Paracoccidioidomicose: histórico, etiologia, epidemiologia, patogênese, formas clínicas, diagnóstico laboratorial e antígenos. BEPA - Boletim Epidemiológico Paulista 5 (51), 11–24.
Morejón, K.M.L., *et al.*, 2009. Paracoccidioidomycosis in patients infected with and not infected with human immunodeficiency virus: A case-control study. American Journal of Tropical Medicine and Hygiene 80 (3), 359–366. doi:10.4269/ajtmh.2009.80.359.
Moscardi-Bacchi, M., *et al.*, 1989. In situ localization of t lymphocyte subsets in human paracoccidioidomycosis. Medical Mycology 27 (3), 149–158. doi:10.1080/02681218980000211.
Motoyama, A.B., *et al.*, 2000. Molecular identification of *Paracoccidioides brasiliensis* by PCR amplification of ribosomal DNA. Journal of Clinical Microbiology 38 (8), 3106–3109. doi:10.1128/jcm.38.8.3106-3109.2000.
Naranjo, M., *et al.*, 1990. Treatment of paracoccidioidomycosis with itraconazole. Medical Mycology 28 (1), 67–76.
Negroni, R., *et al.*, 1987. Oral treatment of paracoccidioidomycosis and histoplasmosis with itraconazole in humans. Reviews of Infectious Diseases 9 (1), 47–50. doi:10.1093/clinids/9.supplement_1.s47.
Nery, A.F., *et al.*, 2017. Therapeutic response in adult patients with nonsevere chronic paracoccidioidomycosis treated with sulfamethoxazole-trimethoprim: A retrospective study. American Journal of Tropical Medicine and Hygiene 97 (2), 556–562. doi:10.4269/ajtmh.16-0255.
Neves, A.R., *et al.*, 2003. Negative immunodiffusion test results obtained with sera of paracoccidioidomycosis patients may be related to low-avidity immunoglobulin G2 antibodies directed against carbohydrate epitopes. Clinical and Diagnostic Laboratory Immunology 10 (5), 802–807. doi:10.1128/CDLI.10.5.802-807.2003.
Neves, J.S., Bogliolo, L., 1951. Researches on the etiological agents of the American blastomycosis. I Morphology and systematics of the Lutz´ disease agent. Mycopathologia et Mycologia Applicata 5, 133–142.
Nogueira, M.G.D.S., *et al.*, 2006. Laboratory evolutive aspects of children under paracoccidioidomycosis treatment. Revista da Sociedade Brasileira de Medicina Tropical 39 (5), 478–483. doi:10.1590/S0037-86822006000500011.
Peçanha, P.M., *et al.*, 2016. Amphotericin B lipid complex in the treatment of severe paracoccidioidomycosis: A case series. International Journal of Antimicrobial Agents 48 (4), 428–430. doi:10.1016/j.ijantimicag.2016.06.011.
Peçanha, P.M., *et al.*, 2017. Paracoccidioidomycosis: Epidemiological and clinical aspects in 546 cases studied in the state of Espírito Santo, Brazil. American Journal of Tropical Medicine and Hygiene 97 (3), 836–844. doi:10.4269/ajtmh.16-0790.
Pereira, G.H., *et al.*, 2010. Bone marrow involvement in a patient with paracoccidioidomycosis: A rare presentation of juvenile form. Mycopathologia 170 (4), 259–261. doi:10.1007/s11046-010-9314-5.
Pereira, R.M., *et al.*, 2004. Paracoccidioidomycosis in children: Clinical presentation, follow-up and outcome. Revista do Instituto de Medicina Tropical de Sao Paulo 46 (3), 127–131. doi:10.1590/s0036-46652004000300002.
Pereira, R.M., Guerra-Júnior, G., Tresoldi, A.T., 2006. Adrenal function in 23 children with paracoccidioidomycosis. Revista do Instituto de Medicina Tropical de Sao Paulo 48 (6), 333–336. doi:10.1590/S0036-46652006000600006.
Pina, A., 2006. Neutrophil role in pulmonary paracoccidioidomycosis depends on the resistance pattern of hosts. Journal of Leukocyte Biology 79 (6), 1202–1213. doi:10.1189/jlb.0106052.
Pinto, A.R., *et al.*, 2000. DNA-based vaccination against murine paracoccidioidomycosis using the gp43 gene from *Paracoccidioides brasiliensis*. Vaccine 18, 3050–3058. doi:10.1016/S0264-410X(00)00074-8.
Pollak, L., 1971. Aleuriospores of *Paracoccidioides brasiliensis*. Mycopathologia et Mycologia Applicata 45 (3), 217–219.
Prado, M., *et al.*, 2009. Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: A review from 1996 to 2006. Memorias do Instituto Oswaldo Cruz 104 (3), 513–521. doi:10.1590/S0074-02762009000300019.
Puccia, R., *et al.*, 1986. Exocellular components of *Paracoccidioides brasiliensis*: Identification of a specific antigen. Infection and Immunity 53 (1), 199–206.
Queiroz-Telles, F., Colombo, A.L., Nucci, M., 1992. Comparative efficacy of cotrimoxazole and itraconazole in the treatment of paracoccidioidomycosis 38th interscience conference on antimicrobial agents and chemotherapy. Clinical Infectious Diseases 14, S68–S76.
Ramos, C.D., Londero, A.T., Gal, M.C., 1981. Pulmonary paracoccidioidomycosis in a nine year old girl. Mycopathologia 74 (1), 15–18.
Resende, L.S.R., *et al.*, 2006. Infiltrative myelopathy by paracoccidioidomycosis. A review and report of nine cases with emphasis on bone marrow morphology. Histopathology 48 (4), 377–386. doi:10.1111/j.1365-2559.2006.02354.x.
Restrepo, A., *et al.*, 1976. The gamut of paracoccidioidomycosis. American Journal of Medicine 61, 33–42.
Restrepo, A., *et al.*, 1987. Itraconazole in the treatment of paracoccidioidomycosis: A preliminary report. Clinical Infectious Diseases - Reviews of Infectious Diseases 9 (1), S51–S56. doi:10.1093/clinids/9.supplement_1.s51.
Restrepo, A., 2000. Morphological aspects of *Paracoccidioides brasiliensis* in lymph nodes: Implications for the prolonged latency of paracoccidioidomycosis? Medical Mycology 38 (4), 317–322. doi:10.1080/mmy.38.4.317.322.
Restrepo, A., Trujillo, M., Gomez, I., 1989. Inapparent lung involvement in patients with the subacute juvenile type of paracoccidioidomycosis. Revista do Instituto de Medicina Tropical de Sao Paulo 31 (1), 18–22. doi:10.1590/S0036-46651989000100004.
Restrepo, A.M., 1985. The ecology of *Paracoccidioides brasiliensis*: A puzzle still unsolved. Sabouraudia 23 (5), 323–334. doi:10.1080/00362178585380481.
Ribeiro, D.O., 1940. Nova terapêutica para a blastomicose. Revista Paulista de Medicina 12, 36–54.

Richini-Pereira, V.B., *et al*., 2008. Molecular detection of *Paracoccidioides brasiliensis* in road-killed wild animals. Medical Mycology 46 (1), 35–40. doi:10.1080/13693780701553002.
Rittner, G.M., *et al*., 2012. Therapeutic DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis. PLoS Neglected Tropical Diseases 6 (2), doi:10.1371/journal.pntd.0001519.
Rocha-Silva, F., *et al*., 2018. Paracoccidioidomycosis: Detection of *Paracoccidioides brasiliensis´* genome in biological samples by quantitative chain reaction polymerase (qPCR). Microbial Pathogenesis 121, 359–362. doi:10.1016/j.micpath.2018.05.039.
Romaneli, M.T.D.N., *et al*., 2019. Acute-subacute paracoccidioidomycosis: A paediatric cohort of 141 patients, exploring clinical characteristics, laboratorial analysis and developing a non-survival predictor. Mycoses 62 (11), 999–1005. doi:10.1111/myc.12984.
Rosa Junior, M., *et al*., 2019. Paracoccidioidomycosis of the central nervous system: CT and MR imaging findings. American Journal of Neuroradiology 40 (10), 1681–1688. doi:10.3174/ajnr.A6203.
Rossi, S.A., *et al*., 2019. Vaccine development to systemic mycoses by thermally dimorphic fungi. Current Tropical Medicine Reports 6, 64–75. doi:10.1007/s40475-019-00179-w.
Ruiz e Resende, L.S., *et al*., 2015. Paracoccidioidomycosis in patients with lymphoma and review of published literature. Mycopathologia 179, 285–291. doi:10.1007/s11046-014-9851-4.
Sadahiro, A., Roque, A.C., Shikanai-Yassuda, M.A., 2007. Generic human leukocyte antigen class II (DRB1 and DQB1) alleles in patients with paracoccidioidomycosis. Medical Mycology 45, 35–40.
Salina, M.A., *et al*., 1998. Detection of circulating *paracoccidioides brasiliensis* antigen in urine of paracoccidioidomycosis patients before and during treatment. Journal of Clinical Microbiology 36 (6), 1723–1728.
San-Blas, G., NiÑo-Vega, G., Iturriaga, T., 2002. *Paracoccidioides brasiliensis* and paracoccidioidomycosis: Molecular approaches to morphogenesis, diagnosis, epidemiology, taxonomy and genetics. Medical Mycology 40 (3), 225–242. doi:10.1080/mmy.40.3.225.242.
Sandoval, M., *et al*., 1996. Antigen distribution in mucocutaneous biopsies of human paracoccidiomycosis. International Journal of Surgical Pathology 3 (3), 181–187. doi:10.1177/106689699500300306.
Sant'Anna, G.D., *et al*., 1999. Laryngeal manifestations of paracoccidioidomycosis (South American blastomycosis). Archives of Otolaryngology – Head and Neck Surgery 125 (12), 1375–1378. doi:10.1001/archotol.125.12.1375.
Santos, J.W., Michel, G.T., Londero, A.T., 1997. Paracoccidioidoma: Case record and review. Mycopathologia 137 (2), 83–85.
Schimke, L.F., *et al*., 2017. Paracoccidioidomycosis associated with a heterozygous STAT4 mutation and impaired IFN-γ immunity. Journal of Infectious Diseases 16 (12), 1623–1634. doi:10.1093/infdis/jix522.
Severo, L.C., *et al*., 1979. The primary pulmonary lymph node complex in paracoccidioidomycosis. Mycopathologia 67, 115–118. doi:10.1007/BF00440683.
Shankar, J., *et al*., 2011. Hormones and the resistance of women to paracoccidioidomycosis. Clinical Microbiology Reviews 24 (2), 296–313. doi:10.1128/CMR.00062-10.
Shikanai-Yasuda, M.A., *et al*., 1992a. Comprometimento da medula óssea e eosinofilia na paracoccidioidomicose. Revista do Instituto de Medicina Tropical de São Paulo 34 (2), 85–90. doi:10.1590/s0036-46651992000200002.
Shikanai-Yasuda, M.A., *et al*., 1992b. Immunodeficiency secondary to juvenile paracoccidioidomycosis: Associated infections. Mycopathologia 120, 23–28. doi:10.1007/BF00578498.
Shikanai-Yasuda, M.A., *et al*., 1995. Paracoccidioidomycosis in a renal transplant recipient. Medical Mycology 33 (6), 411–414. doi:10.1080/02681219580000781.
Shikanai-Yasuda, M.A., *et al*., 2002. Randomized trial with itraconazole, ketoconazole and sulfadiazine in paracoccidioidomycosis. Medical Mycology 40 (4), 411–417. doi:10.1080/mmy.40.4.411.417.
Shikanai-Yasuda, M.A., *et al*., 2008. Neoplasia and paracoccidioidomycosis. Mycopathologia 165 (4–5), 303–312. doi:10.1007/s11046-007-9047-2.
Shikanai-Yasuda, M.A., 2015. Paracoccidioidomycosis treatment. Revista do Instituto de Medicina Tropical de Sao Paulo 57 (Suppl 19), 31–37. doi:10.1590/S0036-46652015000700007.
Shikanai-Yasuda, M.A., *et al*., 2017. Brazilian guidelines for the clinical management of paracoccidioidomycosis. Revista da Sociedade Brasileira de Medicina Tropical 50 (5), 715–740. doi:10.1590/0037-8682-0230-2017.
Silletti, R.P., Glezerov, V., Schwartz, I.S., 1996. Pulmonary paracoccidioidomycosis misdiagnosed as Pneumocystis pneumonia in an immunocompromised host. Journal of Clinical Microbiology 34 (9), 2328–2330.
Silva, L.B.R., *et al*., 2017. Dendritic cells primed with *Paracoccidioides brasiliensis* peptide P10 Are therapeutic in immunosuppressed mice with paracoccidioidomycosis. Frontiers in Microbiology 8.doi:10.3389/fmicb.2017.01057.
Silva, L.B.R., *et al*., 2019. Experimental therapy of Paracoccidioidomycosis using p10-primed monocyte-derived dendritic cells isolated from infected mice. Frontiers in Microbiology 10.doi:10.3389/fmicb.2019.01727.
Singer-Vermfs, L.M., *et al*., 2008. Pathogenicity and immunogenicity of *Paracoccidioides brasiliensis* isolates in the human disease and in an experimental murine model. Clinical & Experimental Immunology 97 (1), 113–119. doi:10.1111/j.1365-2249.1994.tb06588.x.
Slevogt, H., *et al*., 2004. Lymphadenopathy in a pregnant woman from Brazil. Lancet 363, 1282. doi:10.1016/S0140-6736(04)16001-7.
Souza, A.C.O., Taborda, C.P., 2020. Epidemiology of dimorphic fungi. Reference Module in Life Sciences. doi:10.1016/b978-0-12-809633-8.12056-4.
Souza, A.S., *et al*., 2006. High-resolution CT findings of 77 patients with untreated pulmonary paracoccidioidomycosis. American Journal of Roentgenology 187 (5), 1248–1252. doi:10.2214/AJR.05.1065.
Sperandio, F.F., *et al*., 2015. Resistance to *P. brasiliensis* experimental infection of inbred mice is associated with an efficient neutrophil mobilization and activation by mediators of inflammation. Mediators of Inflammation 2015.doi:10.1155/2015/430525.
Sylvestre, T.F., *et al*., 2018. Serological proteomic biomarkers to identify *Paracoccidioides* species and risk of relapse. PLoS One 13 (8), e0202804doi:10.1371/journal.pone.0202804.
Tabón, A.M., *et al*., 1995. Seguimiento post-terapia en pacientes con paracoccidioidomicosis tratados con itraconazol. Revista Colombiana de Neumología 7, 74–78.
Taborda, C.P., *et al*., 1998. Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis* which induces a Th-1 response protective against fungal infection in BALB/c mice. Infection and Immunity 66 (2), 786–793.
Taborda, C.P., *et al*., 2015. Paracoccidioidomycosis: Challenges in the development of a vaccine against an endemic mycosis in the Americas I Paracoccidioidomicose: Desafios no desenvolvimento de uma vacina contra micose endêmica nas Américas. Revista do Instituto de Medicina Tropical de Sao Paulo 57 (Suppl. 19), 21–24. doi:10.1590/S0036-46652015000700005.
Taborda, C.P., *et al*., 2018. *Paracoccidioides* spp. and *Histoplasma capsulatum*: Current and new perspectives for diagnosis and treatment. Current Topics in Medicinal Chemistry 18 (15), 1333–1348. doi:10.2174/1568026618666181002112231.
Taborda, C.P., Uran, J.M.E., Travassos, L.R., 2016. Paracoccidioidomycosis: An endemic mycosis in the Americas. Medical Mycology. 254–273. doi:10.1201/b18707-14.
Teixeira, F., Gayotto, L.C., de Brito, T., 1978. Morphological patterns of the liver in South American blastomycosis. Histopathology 2 (4), 231–237. doi:10.1111/j.1365-2559.1978.tb01716.x.
Teixeira, M.D.M., *et al*., 2014. *Paracoccidioides lutzii* sp. nov.: Biological and clinical implications. Medical Mycology 52 (1), 19–28. doi:10.3109/13693786.2013.794311.
Telles, F.Q., *et al*., 2007. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. Clinical Infectious Diseases 45, 1462–1469. doi:10.1086/522973.
Tendrich, M., *et al*., 1991. Computed tomography and ultrasonography of the adrenal glands in paracoccidioidomycosis. Comparison with cortisol and aldosterone responses to ACTH stimulation. American Journal of Tropical Medicine and Hygiene 44 (1), 83–92. doi:10.4269/ajtmh.1991.44.83.

Theodoro, R.C., *et al*., 2012. Genus *Paracoccidioides*: Species recognition and biogeographic aspects. PLoS One 7 (5), doi:10.1371/journal.pone.0037694.
Thomaz, L., *et al*., 2014. Monoclonal antibodies to heat shock protein 60 induce a protective immune response against experimental *Paracoccidioides lutzii*. Microbes and Infection 16 (9), 788–795. doi:10.1016/j.micinf.2014.08.004.
Thompson, G.R., *et al*., 2016. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clinical Infectious Diseases 63 (3), 356–362. doi:10.1093/cid/ciw305.
Tobón, A.M., *et al*., 2003. Residual pulmonary abnormalities in adult patients with chronic paracoccidioidomycosis: Prolonged follow-up after itraconazole therapy. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 37 (7), 898–904. doi:10.1086/377538.
Travassos, L.R., Taborda, C.P., 2012. Paracoccidioidomycosis vaccine. Human Vaccines and Immunotherapeutics 8 (10), 1450–1453. doi:10.4161/hv.21283.
Travassos, L.R., Taborda, C.P., Iwai, L.K., Cunha-Neto, E., Puccia, R., 2004. The gp43 from *Paracoccidioides brasiliensis*: A major diagnostic antigen and vaccine candidate. In: Domer, J.E., Kobayashi, G.S. (Eds.), The Mycota XII, Human Fungal Pathogens. Berlin-Heildeberg: Springer-Verlag, pp. 279–296.
Tuder, R.M., *et al*., 1985. Pathology of the human pulmonary paracoccidioidomycosis. Mycopathologia 92 (3), 179–188. doi:10.1007/BF00437631.
Turissini, D.A., *et al*., 2017. Species boundaries in the human pathogen *Paracoccidioides*. Fungal Genetics and Biology 106, 9–25. doi:10.1016/j.fgb.2017.05.007.
Uribe, F., *et al*., 1987. Histopathology of cutaneous and mucosal lesions in human paracoccidioidomycosis. Revista do Instituto de Medicina Tropical de São Paulo 29 (2), 90–96. doi:10.1590/S0036-46651987000200005.
Valle, A.C.F., *et al*., 1992. Aspectos radiológicos torácicos na paracoccidioidomicose. Revista do Instituto de Medicina Tropical de São Paulo 34 (2), 107–115. doi:10.1590/s0036-46651992000200005.
Vidal, M.S.M., *et al*., 2014. Serological diagnosis of paracoccidioidomycosis: High rate of inter-laboratorial variability among medical mycology reference centers. PLoS Neglected Tropical Diseases 8 (9), 4–9. doi:10.1371/journal.pntd.0003174.
Weber, S.A.T., *et al*., 2006. Dysphonia and laryngeal sequelae in paracoccidioidomycosis patients: A morphological and phoniatric study. Medical Mycology 44 (3), 219–225. doi:10.1080/13693780500340320.
Woyciechowsky, T.G., *et al*., 2011. Paracoccidioidomycosis induced by immunosuppressive drugs in a patient with rheumatoid arthritis and bone sarcoma: Case report and review of the literature. Mycopathologia 172, 77–81. doi:10.1007/s11046-011-9403-0.
Xidieh, C.F., *et al*., 1999. Influence of the genetic background on the pattern of lesions developed by resistant and susceptible mice infected with *Paracoccidioides brasiiensis*. Medical Microbiology and Immunology 188, 41–49. doi:10.1007/s004300050103.
Yamaga, L.Y.I., *et al*., 2003. The role of gallium-67 scan in defining the extent of disease in an endemic deep mycosis, paracoccidioidomycosis: A predominantly multifocal disease. European Journal of Nuclear Medicine and Molecular Imaging 30, 888–894. doi:10.1007/s00259-003-1172-7.
Yarzábal, L., *et al*., 1980. Demonstration and quantification of ige antibodies against *Paracoccidioides brasiliensis* in paracoccidioidomycosis. International Archives of Allergy and Immunology 62, 346–351. doi:10.1159/000232533.

Further Reading

Boniche, C., Rossi, S.A., Kischkel, B., *et al*., 2020. Immunotherapy against systemic fungal infections based on monoclonal antibodies. Journal of Fungi 6 (1), doi:10.3390/jof6010031.
Shikanai-Yasuda, M.A., Mendes, R.P., Colombo, A.L., *et al*., 2017. Brazilian guidelines for the clinical management of paracoccidioidomycosis. Revista da Sociedade Brasileira de Medicina Tropical 50 (5), 715–740. doi:10.1590/0037-8682-0230-2017.
Souza, A.C.O., Taborda, C.P., 2017. Epidemiology of dimorphic fungi. Reference Module in Life Sciences. Elsevier. doi:10.1016/B978-0-12-809633-8.12056-4. (ISBN: 978-0-12-809633-8).
Taborda, C.P., Buccheri, R., Benard, G., *et al*., 2018. *Paracoccidioides* spp. and Histoplasma capsulatum: Current and new perspectives for diagnosis and treatment. Current Topics in Medicinal Chemistry 18 (15), 1333–1348. doi:10.2174/1568026618666181002112231.
Travassos, L.R., Taborda, C.P., 2017. Linear epitopes of *Paracoccidioides brasiliensis* and other fungal agents of human systemic mycoses as vaccine candidates. Front Immunol. 8, 224. doi:10.3389/fimmu.2017.00224.
Turissini, D.A., Gomez, O.M., Teixeira, M.M., McEwen, J.G., Matute, D.R., 2017. Species boundaries in the human pathogen *Paracoccidioides*. Fungal Genetics and Biology 106, 9–25. doi:10.1016/j.fgb.2017.05.007.