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EPIDEMIOLOGICAL ASPECTS OF FUNGAL INFECTIONS (T CHILLER AND J BADDLEY, SECTION EDITORS)

Paracoccidioidomycosis: Latin America's Own Fungal Disorder

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Abstract Paracoccidioidomycosis (PCM) is a systemic, endemic fungal disorder restricted to Latin America (Mexico to Argentina); Brazil accounts for the largest number of cases. Imported cases diagnosed in North America, Europe and Asia represent patients who had previously lived in recognized endemic areas. *Paracoccidioides brasiliensis,* the etiologic agent, is a thermally dimorphic fungus that in patients and cultures at 37 ° C adopts a yeast form while at lower temperatures it behaves as a mold that bears the infectious conidia. PCM has a peculiar gender distribution with preference for adult males at a ratio of ≥11 to 1. PCM afflicts predominantly adult males engaged in agriculture. It is mostly a chronic disease with acute/subacute cases accounting for less than 15 % of all reports. Specific diagnosis is established late and although available therapy is usually successful in controlling the fungal infection, patients who survive usually develop residual fibrotic lesions that heavily impair their quality of life.

Keywords Paracoccidioidomycosis · South American Blastomycosis · *Paracoccidioides brasiliensis* · Endemic areas · Chronic · Acute/subacute paracoccidioidomycosis · Latency · Laboratory diagnosis · Management

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Introduction

Eco-Epidemiologic Considerations

Paracoccidioidomycosis (PCM), previously known as South American Blastomycosis, is a systemic fungal disorder endemic in rural areas of Latin America that is acquired through the respiratory route with the lungs constituting the primary target while all other organs (lymph nodes, mucous membranes, skin, adrenals, bone, other) represent secondary manifestations [1–4, 5•, 6•]. According to image studies, PCM simultaneously involves more than one organ or system and, as such, should be considered a systemic disorder [7].

PCM is diagnosed almost exclusively in Latin America with the exception of certain imported cases as described below. Brazil has the largest number of cases followed at a significant distance by Colombia, Venezuela, Ecuador, Bolivia and Argentina. Uruguay, Paraguay and the Central American countries report lesser or no (Nicaragua, Belize) cases. It is extremely rare in the Caribbean Islands where only single cases have been informed in Trinidad, Grenada and Guadalupe. It has not been informed in Chile, Surinam or Guyana [1, 4, 5•, 6•, 8].

Approximately one hundred paracoccidioidomycosis cases have been reported from countries outside of the recognized endemic areas, mostly in Europe, as this Continent receives a large number of immigrants from Latin America, [1, 4, 5•, 8]. In the last 2 years (2010–2012), eight cases have been reported outside the endemic area [9–12, 13•, 14–16]. These patients had all lived in the Latin American endemic regions up to 14 years prior to the appearance of clinical manifestations [4, 5•, 9, 12, 13•, 14, 15]. This prolonged dormant period confirms Borelli's postulates [17] indicating that the infection is aquired in a place different to the diagnostic site with an undetermined lapsus between the two events.

The search for *P. brasiliensis* in the environment has been extensive but unrewarding, although it has been noticed that environmental changes in endemic areas are a constant feature [8]. Rios-Gonçalves et al., examined 36 children diagnosed in the state of Rio de Janeiro, Brazil finding that 44 % came from rural counties where the abundant primary forests had been gradually removed [18]. In another report from Brazil in Belem Para State near the Amazon basin where paracoccidioidomycosis had been infrequent, 13 childhood cases were diagnosed in a period of a few years. These children had lived permanently around forested areas where intense human colonization resulted in gradual destruction of the original ecosystem [19]. In a similar manner, Bellissimo-Rodrigues et al. [20••] in their study of 1,000 patients, pinpointed an area with the highest number of juvenile PCM cases, all of whom had resided close to coffee plantations, thereby raising the possibility of aerosol infection through agriculture-related exposure. Support for this hypothesis has been aided by the fact that ITS sequences of DNA detected in environmental aerosol samples by PCR were homologous to those of *Paracoccidioides lutzii* demonstrating the presence of this species [21].

In endemic countries, patients' residence can be associated with areas characterized by a humid atmosphere (high rainfall indexes, presence of humid forests, and waterways) plus stable, mild temperatures (17 °C–24 °C) [8, 22]. Despite the above, the exact *P. brasiliensis* habitat remains largely unknown and has only been isolated from a very few different environments including isolation from soil and from certain peculiar substances such as animal forage, bat guano and penguin feces [23]. Barroso et al., analyzed the records of 91 acute/ subacute patients in whom infection was estimated to have occurred 1–2 years previously and through multiple regression analyses determined that weather variability was a significant factor. Construction of a good fitting model provided significant estimates of the effect of absolute air humidity, soil water storage and Southern Oscillation Index [24•]. These environmental factors explained 49 % of the incidence variance of the cases and suggested that such variables influenced the conditions favoring infection of humans [24•].

The armadillos *Dassypus novemcinctus* and, occasionally also *Cabassous centralis,* have been shown to harbor *P. brasiliensis* in their internal organs; however, isolation of the fungus in the habitat of armadillos has failed [25]. Paracoccidioidomycosis has also been confirmed—or suspected to exist—through molecular methods in other feral and domesticated animals [26–28]. Experimental studies have shown that *P. brasiliensis* may grow and produce infectious conidia in sandy and clay-like soils with high water- but low in aluminum-content.

Personal communications received from Brazilian clinical researchers (Flavio de Queiros-Tellez and Mario L. Silva-Vergara from the Hospital de Clinicas, Federal University of Parana, Curitiba, and at the Infectious Diseases Unit of the Internal Medicine Division, Triangulo Mineiro, Uberaba Federal University, respectively), indicate that PCM is observed less frequently at present than previously, to the extent that half the usual number of cases are being diagnosed in their centers. This finding is thought to be attributed to changing agricultural practices whereby there has been a gradual substitution of coffee plantations to sugar cane farmers and cattle pastures. The area occupied by sugar cane crops has significantly increased, especially in some areas of the state of Sao Paulo, in Brazil's Southern Region. As sugar cane plantations require burning plus wide pesticide, herbicides and fungicide use, *P. brasiliensis'* whereabouts, no matter how imprecise, would be subjected to significantly elevated soil temperatures with concomitant extinction of the accompanying saprophytic soil microorganisms [29].

From the epidemiological point of view, paracoccidioidomycosis presents the following characteristics.

Age The disease is relatively uncommon in children and adolescents with approximately 2 % of all patients being less than 10 years of age and 8 % less than 20 years old [20••, 30, 31••, 32•, 33, 34]. If one counts children and adolescents (less than 14 years of age), less than 15 % of all cases will fall into this age bracket. PCM infection is most common in middle-age men (40–60 years) [20••, 30, 31••, 32•, 33, 34, 35•]. A slight deviation of this pattern is noticed in patients co-infected with HIV who are younger than the remaining adults [20••, 32•, 36•].

Gender In a recent review of over 5,000 paracoccidioidomycosis cases, Shankar et al. [32•] found that 91.8 % were males and only 8.2 % females. This series included children less than 10 years in whom no difference was noticed between gender in contradiction to the predominance of adult male patients. Although a slightly different prevalence by gender was seen in a series of one thousand cases from Brazil with 85.8 % males and 14.2 % females [20••], it is clear that a male predominance is the rule. It is known that the female hormone, 17-beta estradiol, hinders the conidium to yeast transition thus indicating the existence of hormonal influences, probably acting in cooperation with the stronger cellular immune responses present in females [32•].

Race It is diffcult to pin-point a racial trend in the endemic areas as mixed races are very common. Immigrants into endemic areas usually develop a severe disease indicative of their greater susceptibility to *P. brasiliensis* [1].

Occupation In the two largest series of patients that have been published [20••, 32•], approximately half of the

patients have or have had jobs centered in agriculture. Other occupations mentioned have been mansory and lumberjacking (12 % of the cases). Less frequent occupations such as mining, truck driving and industrial work, have also been mentioned [20••, 32•].

Other Factors Poor nutrition, smoking and alcoholism are important predisposing conditions [20••, 30, 31••, 34, 35•, 36•, 37]

Incidence Despite the measures being implemented in Brazil and with the exception of the States of Mato Grosso do Sul, Minas Gerais and São Paulo, paracoccidioidomycosis is not a reportable disease [20••]. Consequently, true incidence and prevalence rates remain unknown. In the Brazilian rural areas, annual incidence has been estimated to range from 3–4 new cases/1,000,000 inhabitants to 1–3 new cases/100,000 population. Such incidence is higher in males than in females [2, 20••] but in individuals less than 14 years of age, both genders are affected equally [2, 20••, 32•].

In Brazil, paracoccidioidomycosis is considered to be the third leading cause of death from a chronic infectious diseases and the corresponding mortality rate is estimated at 1.65 cases/1,000,000 population [36•, 37–40]. Santo reviewed 1,950 paracoccidioidomycosis death certificates and found that the largest number of these deaths occurred in men in the older age groups and among rural workers. The main causes associated with paracoccidioidomycosis as the underlying cause-of-death were pulmonary fibrosis, chronic lower respiratory tract diseases, and pneumonias [40]. Coutinho et al. [37] estimated an annual incidence rate of 1 to 3 per 100,000 inhabitants and based on 3,181 deaths an estimated annual mean mortality rate of 1.45 per million inhabitants. The authors concluded that paracoccidioidomycosis was of considerable burden but of low visibility as it represented the 8th most common cause of death from predominantly chronic or recurrent types of infection and parasitic diseases. It also had the highest mortality rate among the systemic mycoses. The majority of deaths occurred in males (84.7 %) and in the older age groups [38]. Bittencourt et al., analyzed 551 deaths of the mycosis in Parana's State, Brazil finding an average annual mortality rate of 3.48 per million inhabitants that represented not only the 5th cause of death among the predominantly chronic infectious diseases but also the highest mortality rate among the systemic mycoses [39]. In the setting of persons with HIV, the prevalence of this mycosis was 3.3 % and it was associated with a high mortality [41•, 42•]. According to the data from Brazil, paracoccidioidomycosis remains a major public health problem in Brazil emphasizing its status as a neglected disease [43•].

The Etiologic Agent

Paracoccidioides brasiliensis, the causative agent, is a thermally dimorphic fungus, characterized by two morphotypes, a mold at temperatures under 28 °C that frequently reveals chlamydoconidia and more rarely conidia (size <5 μm), propagules that transform into yeast cells under the influence of body temperature. In tissues and cultures at 35 °C–37 °C, this fungus grows as a round to oval yeast cell variable in size (4–40 μm) provided with prominent lipid vacuoles and that characteristically produces multiple budding daughter cells arranged in the form of a pilot's wheel; nonetheless, isolated cells, short chains of blastoconidia and large broken yeast cells are also seen [1, 4, 5•, 6•, 44]. When grown under nutritional deprivation, certain isolates produce conidia less than 5 μm capable of responding to temperature changes and transforming into either yeast cells at 36 °C or producing hyphae at 20 °C–24 °C [4, 5•]. Furthermore, conidia are infectious for mice and give rise to a chronic, disseminated process ending in lung fibrosis [1, 5•, 6•, 45]. When grown in vitro in sandy and clay-like soils rich in water but poor in aluminum contents, *P. brasiliensis* produces infectious conidia [46]. *P. brasiliensis* conidia and yeast cells have been shown to produce melanine-like compounds, probably involved in virulence [47–49]. M and Y forms are aerobic and require ample oxygen supply for their growth [1, 4, 5•, 6•, 44].

P. brasiliensis is only known in its asexual (anamorph) stage; nonetheless, molecular biology studies have allowed its classification in the phylum *Ascomycota,* order *Onygenales,* family *Onygenacea,* close to the *Histoplasma capsulatum, Blastomyces dermatitidis* and *Emmonsia parva* phylogenetic tree [50]. At present, the genus is considered to have three different phylogenetic species (clades) [51]. In addition and based on high polygenetic diversity and exclusive morphogenetic characteristics, a different species, *P. lutzi,* has been proposed [52]. Since 2009 the BROAD Institute has completed the database for *P. brasiliensis* genome (http://www.broad.mit.edu/annotation/genome/paracoccidioides_brasiliensis).

Mycologic Diagnosis

Direct Examination and Histopathology In clinical specimens *P. brasiliensis* appears as an oval to round yeast cell often provided with multiple peripheral buds (pilot wheel configuration) and has a thick refractile wall plus prominent intracytoplasmatic vacuoles. Often yeast cells appear in chains and have single buds; large bizarre yeast cells may also be observed. Several procedures are adequate to visualize fungal elements, including fresh or wet KOH

preparations, as well as calcofluor and immunofluorescence methods. Sensitivity of the direct examinations varies from 85 to 100 % depending on specimen, clinical manifestations and treatment status [1, 4, 5•, 6•, 37, 44]. Histopathologic preparations stained with H&E, Gomori methenamine-silver, Papanicolaou or periodic acid-Schiff are also quite useful as they reveal the multiple budding yeast elements, especially within granulomatous foci. Differentiation of *P. brasiliensis* from *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and even *Histoplasma capsulatum*, must be made [1, 4, 5•, 6•, 37, 53••, 54•].

Cultures Isolation of *P. brasiliensis* from clinical specimens requires a battery of selective and non-selective culture media (Sabouraud plus asparagine and thiamine; Mycocel; BHI plus glucose); several clinical samples should be sent for culturing. The addition of antibacterial drugs and mould inhibitors to the media has resulted in improved recovery rates, around 80 % [1, 4, 5•, 37, 53••, 54•]. Modified Sabouraud's (Mycocel agar) and yeast extract agars incubated at room temperature (19 °C–24 °C) are the best media for isolation. In bacteria-free specimens (e.g., tissue biopsies, CSF, bone marrow), media without antibiotics with incubation at 36 °C, can also be employed [1, 4, 5•, 6•, 53••]. At 18 °C–24 °C, growth is slow and takes 20–30 days. Microscopically, the mould shows only thin septate hyphae and chlamydospores (15–30 μm) and in media with no carbohydrates the mould may also produce conidia after ≥2 months of incubation. The mycelial form is not distinctive and dimorphism must be demonstrated [1, 4, 5•, 6•, 53••]. At this temperature, *P. brasiliensis* yeast form grows in 8–10 days as a cerebriform, cream-colored colony. Microscopically, oval to spherical yeast cells, 4 to 40 mm in diameter, are observed. The large mother yeast cell bearing multiple buds (pilot's wheel) is characteristic of this fungus [1, 4, 5•, 37, 53••, 54•].

Immunodiagnostic Tests Immune-based methods are useful not only for diagnosis but also for monitoring the patient's course. Immunodifussion (ID), Complement Fixation (CF) and ELISA are currently the tests of choice. The principal antigens used in these tests are derived from *P. brasiliensis* culture filtrates of mycelial- or yeast-phase broth cultures. The major diagnostic antigen found in these preparations is a 43 kDa glycoprotein. Cell wall antigens have proved less useful than culture-filtrate antigens, largely because wall antigens are dominated by cross-reactive galactomannan [53••, 54•, 55]. The ID is highly specific, and is positive in 65–100 % of cases of acute or chronic pulmonary infection, or disseminated paracoccidioidomycosis. The CF test is less specific than the ID test, and cross-reactions can occur in cases with histoplasmosis. However, CF titers of ≥1:8 are considered presumptive evidence of paracoccidioidomycosis [54•, 55]. Falling CF titers are often predictive of successful treatment, and high or fluctuating CF titers are suggestive of poor prognosis. Commercial mycelial-form culture filtrate antigen can be obtained for in-house use from IMMY. The CF test is performed with *P. brasiliensis* yeast-form culture-filtrate antigen but this reagent is not commercially available. Specific proteins and polysaccharide antigens have been identified, cloned and characterized by different laboratories to be used as targets in the production of monoclonal and polyclonal antibodies [56–61]. In addition, several laboratories have developed "in house" immunodiagnostic tests based on methods such as ELISA, inhibition ELISA, competition ELISA, Dot blot, Western blot [53••, 54•, 55].

Immunologically reactive components such as the 87, 70, 58, 52, 43, 27 and 20 kDa antigens have been purified and recognized by sera from patients with PCM [62]. The Gp43 is considered the immunodominant antigen and is recognized by all sera from the PCM patient. In addition, a 70-kDa glycoprotein was recognized by 96 % of the PCM patients and decreased significantly in patients undergoing antifungal therapy. Consequently, Gp43 and Ggp70 are markers of this mycosis [62, 63]. The use of cocktail antigens or antigens combination may also represent a valid strategy as shown by a promising diagnostic and follow-up assay that relies on the use of the 27-kDa recombinant antigen and the 87-kDa hsp [64•].

Antigen detection tests have some important advantages over antibody detection in the diagnosis of PCM, particularly in immunocompromised patients and in those previously exposed to *P. brasiliensis* who may have pre-existing antibody titers [57, 60, 61]; however, these methodologies are not yet available as regular laboratory tests.

Molecular Tests for Diagnosis

Molecular assays have been successfully used to detect *P. brasiliensis*; however, DNA-based methods have not yet been established as regular diagnostic tools for this fungal infection, nor is a PCR assay commercially available [53••, 54•, 55]. Conserved regions of the ribosomal RNA (rRNA) genes have been used as targets in a number of PCR-based detection assays: a note of caution, it is important to understand that amplification of conserved genes can result in products derived both from pathogenic- and genetically related nonpathogenic fungal species and, consequently, the identity of the amplicons detected using these conserved genes should be verified by direct sequencing [53••, 54•]. A real-time PCR that used as a target the ITS1 region of rDNA was developed to detect *P. brasiliensis* DNA in both cultures and in 10 patients' clinical specimens. Real-time PCR was positive in all the culture strains tested, as well as in clinical specimens, mainly sputum and biopsies, but was less useful in blood. Although this molecular test was evaluated with a low number of

patients, the authors reported 100 % sensitivity and specificity [65•]. Amplification and sequencing of *P. brasiliensis* ribosomal DNA regions, especially 5.8 and 28S subunits, as well as intergenic regions, have been successfully performed. PCR tests that used DNA extracted from culture have allowed for design of specific primers that enable discrimination between *P. brasiliensis* and other human pathogenic fungi [66].

Gp43 is considered the immunodominant antigen for the diagnosis of PCM; thus, the gene coding for this molecule has been used as a molecular target to detect *P. brasiliensis* DNA Bialek et al., developed a nested PCR assay targeting the immunogenic gp43 gene and evaluated it the detection of *P. brasiliensis* DNA in lung homogenates from infected and uninfected mice, as well as from culture-positive lung homogenates, 91 % of which were positive [67]. A test based on the 5′ nuclease assay using a fluorescent probe derived from the sequence of the gene coding for the *gp43* antigen was developed to detect *P. brasiliensis*; sensitivity and specificity were 100 % and the assay could detect at least ten copies of this DNA sequence [68]. The loop-mediated isothermal amplification (LAMP) assay has been tested to detect the Gp43 gene of *P. brasiliensis* and positive results from DNA extracted from FFPE tissue samples of PCM patients using this methodology were reported by Endo et al. [69]. In addition, respiratory samples from PCM patients were evaluated by LAMP with sensitivity and specificity values of 61 % and 100 %, respectively [70]. LAMP methodology exhibits advantages in speed in developing the assay and also in simplicity when compared to the classic diagnostic methods as it does not require sophisticated equipment.

Clinical Manifestations

Paracoccidioidomycosis has been divided into four main clinical entities, two clinically progressive forms, one being an acute/subacute (juvenile) form and the second being a chronic (adult) form both of which are dependent, to some extent, on age and host immune status. The third is a subclinical, non-symptomatic form probably representing a somewhat recent contact with the fungus in its habitat. The fourth and last form is mostly characterized by fibrous scarring appearing at the site of formerly active lesions [1–4, 5•, 20••, 30, 32•, 33, 34, 35•].

In nearly all patients, the target organ is the lung albeit its involvement usually runs a silent course and may go unnoticed until the mycosis has advanced to the point of causing severe respiratory and cardiovascular symptoms [2–4, 5•, 35•, 37, 71•, 72••, 73]. Gallium 67 images show that the mycosis disseminates regularly to various extra-pulmonary organs [1, 4, 5•, 7, 35•, 37] as revealed by extensive organic involvement in most patients when using high-resolution computed tomography [7, 74•, 75•].

The Acute/Subacute Form It represents 15 % of all cases and is observed mainly in children and young adults ≥30 years of age [1, 5•, 18, 19, 76–80], as well as in those with cancer [81•, 82, 83], and immunosuppressive conditions including HIV co-infection [36•, 41•, 42•, 80]. It is characterized by hypertrophy of the lymphatic system with enlargement of several lymph node chains, especially those in the neck, hepatosplenomegaly and bone marrow involvement [18, 19, 34, 81•, 82–84]. Constitutional symptoms (fever, anorexia, weariness, poor general conditions) including undernourishment accompany this clinical form. The latter is usually a pre-existing condition. Respiratory symptoms are uncommon, as are mucosal and skin manifestations albeit the latter two may appear dispersed involving the trunk, the extremities and the face [1–4, 5•, 18, 19, 77]. These external manifestations contrast with those observed in the chronic, adult type disease [30, 35•, 37, 78]. Among the differential diagnosis, one should consider lymphoproliferative disorders, tuberculosis and malignancies [1, 5•, 37, 80, 82]. Establishment of the proper diagnosis requires an alert physician capable of requesting the appropriate mycological studies.

The Chronic Adult Form Approximately 90 % of all cases exhibit this form, one that is characterized by primary pulmonary involvement and secondary extra-pulmonary manifestations, the latter constituting the patient's principal reason to seek medical advice [1, 2, 4, 5•, 77–79]. This form occurs preferentially in adult patients, especially those 30–50 years old; its course is slow, taking years or even decades, to manifest and then to be diagnosed correctly as the chronic disease is often confused with tuberculosis, cancer, and other systemic illnesses [1–4, 5•, 77–79]. This form has important lung involvement but may, nonetheless, be rather asymptomatic with only dry or productive cough and some dyspnea. The X-ray images reveal alveolar, interstitial or mixed infiltrates in both lungs localized preferentially in the central and lower fields with certain respect for the apices. At diagnosis, lung fibrosis is a prominent abnormality (32 %) and is regularly accompanied by emphysematous areas and bullae; cavities are also regularly detected [1, 5•, 77–79]. Chest tomography reveals changes mainly in the lungs' periphery with ground glass infiltrates, small non-calcified nodes, cavities and septal lines indicative of fibrous scars [1–4, 5•, 77–80, 81•].

As many patients smoke regularly, the symptoms described are often attributed to this and not to the mycosis. Physical examination reveals scanty and non-specific findings even in cases with extensive pulmonary involvement by imaging. A clear dissociation occurs among

scarce respiratory symptoms, notable image abnormalities, and paucity of auscultatory findings [1–4, 5•, 78–80, 81•]. General complaints such as malaise, weariness, nocturnal diaphoresis and weight loss are recorded in most cases [1–4, 5•, 78, 79].

The oral mucosa is involved in 50 % of the cases with lesions localized on the gingiva, the palate and the lips; the nasal and less often, the anal mucosa, may also be affected. At times, lesions develop also in the gastrointestinal mucosa [1–4, 5•, 34, 78–80, 83, 84]. Not uncommonly, symptoms such as odino or dysphagia and diarrhea, are recorded and attributed to previous lesions in larynx, pharynx and the gastrointestinal tract [1–4, 5•, 34, 80, 81•, 82–84]. From the mucosa, lesions may extend to the skin where they tend to be granulomatous. Skin lesions are preferentially located in the face, thorax and limbs; their appearance is extremely variable and non-diagnostic [1–4, 5•, 78–80, 85]. In adult patients, hypertrophied lymph nodes may also be detected albeit not as frequently as in the juvenile form; they are located preferentially in the neck region; if in the abdomen, enlarged nodes may coalesce forming large masses that simulate a tumor [1–4, 5•, 78–80]. Adrenal gland involvement is also a classic sign with Addison syndrome being detected in ≥ 10 % of patients; at autopsy, a much higher (85 %) number reveal adrenal gland involvement [79, 86, 87].

The Residual Form (Sequelae) Fibrotic scarring tends to form at those sites where formerly active PCM lesions had existed [4,5]. Of note, bilateral lung fibrosis and bullae are already present at time of diagnosis in 32 % of the patients indicating that the disease process had been initiated in the past [65•]. Additionally, indirect signs of pulmonary hypertension and cor pulmonare detected in a few patients, corroborate the long undiagnosed course of this mycosis. At the end of specific treatment, such residual sequelae tend to increase up to 60 % [71•] greatly impairing the patient's well being [71•]. Fibrous sequelae may also account for the observed dysphagia and dysphonia, adrenal insufficiency and intestinal obstruction recorded for patients with prior active lesions at these sites [1, 3, 4, 5•, 71•].

Management

The antifungal medications presently available for paracoccidioidomycosis treatment are sulfonamides, amphotericin B, and the various azole derivatives, most of which are active against *P. brasiliensis* [1–3, 5•, 37, 72••, 73, 88, 89••].

Sulfonamides PCM is the only mycosis amenable to sulfonamide treatment. Various derivatives have been used but presently the combination trimethoprim-sulfametoxazol is regularly prescribed in Brazil for the ambulatory treatment of minor and moderate forms of the mycosis. Costs are low, it is administered orally and side effects can be controlled. In addition it is available free of cost in Brazil. Initial response is high (70 %) but the relapse rate is important (25 %) with development of resistance in about 15 % of the cases. Duration of therapy is long (over 2 years) with non-compliance being a frequent problem [1–3, 37, 88, 89••].

Amphotericin B (Deoxycholate and Liposomal Presentations) The former preparation is no longer used in the USA; nonetheless, in Latin America countries, this is the compound regularly used because of cost as the liposomal preparations, although more powerful, are too expensive for most health institutions. The need for intravenous administration and the well-known kidney complications limit the more expanded use of this polyene, and it is mainly reserved for treatment of severely ill patients. Once the total prescribed dose is attained with AMB, oral treatment with trimethoprim/sulfametoxazol, itraconazol or other azoles, is then given [1–3, 35•, 37, 72••, 89••].

Azole Derivatives In the case of paracoccidioidomycosis, these orally administered compounds have revolutionized treatment and with the exception of fluconazol—ineffective due to high relapse rates plus need for high doses and prolonged treatment periods—the remaining compounds are effective against *P. brasiliensis*. Ketoconazole attains marked improvement in 90 % of the patients but its use has been presently limited because of treatment failure (8 %) and high relapse rates (11 %), as well as its associated adverse effects (hormonal, gastric, hepatic). Additionally, ketoconazole has important interactions with several medications [1–4, 5•, 35•, 37, 72••, 88, 89••]. Itraconazole is considered the best therapeutic option due to its effectiveness in 98 % of the cases and also because it presents a low relapse rate (3 %). Adverse effects are minor but problems exist in absorption depending on a gastric acid pH, erratic serum levels and frequent interactions with other medications that also influence proper absorption [4, 5•, 35•, 37, 72••, 88, 89••]. Voriconazole was evaluated in a comparative study with itraconazole in 35 Brazilian patients with a satisfactory response rate: 88.6 % with voriconazole versus 94.4 % with itraconazole, with no relapses after 8 weeks of follow-up [90]. Recently, it has been proposed that for severe paracoccidioidomycosis involving inflammatory reactions, treatment should consist not only of antifungal medications but also of corticosteroids [91]

Conclusions

Paracoccidioidomycosis (PCM) is one of Latin America's most prevalent systemic mycosis that has a significant

burden. Unfortunately, its agent, *Paracoccidioides brasiliensis,* has rarely been identified in nature and consequently measures to control exposure cannot be implemented. Low-income rural workers are the most at risk and they have limited access to the health system. Additionally, diagnostic tools are not widely available in medical centers in Latin America; consequently, by the time patients are diagnosed with this fungal infection, many are already severely ill. The disease is progressive and severe in children and adolescents (acute/subacute juvenile form), as well as in adult patients (chronic type form). Effective treatment regimens are available to control the fungal infection but, nonetheless, most patients develop fibrotic sequelae that may severely hamper respiratory and adrenal function and permanently alter the patient's well being.

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References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Lacaz C, Porto E, Martins J, et al. Paracoccidioidomicose. In: Lacaz C, Porto E, Martins J, et al., editors. Tratado de micologia médica lacaz. 9th ed. Sao Paulo: Sarvier; 2002. p. 639–729.
2. Wanke B, Aidê MA. Chapter 6—paracoccidioidomycosis. J Bras Pneumol. 2009;35:1245–9.
3. Ameen M, Talhari C, Talhari S. Advances in paracoccidioidomycosis. Clin Exp Dermatol. 2010;35:576–80.
4. Restrepo A, Tobon AM. Paracoccidioides brasiliensis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's principles and practice of infectious diseases. 7th ed. Philadelphia: Elsevier; 2010. p. 3357–63.
5. • Restrepo A, Gónzalez A, Agudelo CA. Paracoccidioidomycosis. In: Dismukes W, Kauffman C, Pappas P, Sobel J, editors. Essentials of medical mycology. 2nd ed. New York: Springer; 2011. p. 367–85. *This chapter is a comprehensive record of the mycosis, its etiologic agent, the host–fungus interaction and treatment approach. Adequate for "starters".*
6. • Cano LE, González A, Lopera D, et al. Pulmonary paracoccidioidomycosis: clinical, immunological and histopathological aspects. In: Malcolm-Irusen E, editor. Lung diseases: selected state of the art reviews. Rijeka: InTech; 2012. p. 359–92. *An updated review dealing specially with the host–fungus tissue interactions in an animal model that focuses in detailed tissue changes as a result of the fungus invasive capacities*.
7. Yamaga LY, Benard G, Hironaka FH, et al. The role of gallium-67 scan in defining the extent of disease in an endemic deep mycosis, paracoccidioidomycosis: a predominantly multifocal disease. Eur J Nucl Med Mol Imaging. 2003;30:888–94.
8. Restrepo A, McEwen JG, Castaneda E. The habitat of Paracoccidioides brasiliensis: how far from solving the riddle? Med Mycol. 2001;39:233–41.
9. Laccourreye O, Mirghani H, Brasnu D, et al. Imported acute and isolated glottic paracoccidioidomycosis. Ann Otol Rhinol Laryngol. 2010;119:89–92.
10. Ramírez-Olivencia G, Ramírez-Rubio O, González PR, et al. Paracoccidioidomycosis in a Spanish missionary. J Travel Med. 2010;17:139–40.
11. Botas-Velasco M, Jover-Díaz F, Ortiz de la Tabla-Duccase V, et al. Imported paracoccidioidomycosis in Spain. Enferm Infecc Microbiol Clin. 2010;28:259–60.
12. Onda H, Komine M, Murata S, et al. Letter: imported paracoccidioidomycosis in Japan. Dermatol Online J. 2011;17:11.
13. • Buitrago MJ, Bernal-Martínez L, Castelli MV, et al. Histoplasmosis and paracoccidioidomycosis in a non-endemic area: a review of cases and diagnosis. J Travel Med. 2011;18:26–33. *An interesting and well-planned review of two "foreign" mycoses diagnosed in non-endemic settings. Of especial interest to European physicians caring for Latin American immigrants*.
14. Kurai H, Ohmagari N, Ito K, et al. A case of oral paracoccidioidomycosis suspected to be pharyngeal cancer. Med Mycol J. 2012;53:49–52.
15. Yoshimura Y, Tachikawa N, Ooslawa T, et al. A case of paracoccidioidomycosis with severe adrenal insufficiency. Kansenshogaku Zasshi. 2012;86:291–4.
16. Armas M, Ruivo C, Alves R, et al. Pulmonary paracoccidioidomycosis: a case report with high-resolution computed tomography findings. Rev Port Pneumol. 2012;18:190–3.
17. Borelli D. Some ecological aspects of Paracoccidioidomycosis [Scient Publ. 254]. Presented at the 1st Pan American Symposium. Medellín, Colombia; October 25–27, 1971.
18. Goncalves AJ, Londero AT, Terra GM, et al. Paracoccidioidomycosis in children in the state of Rio de Janeiro (Brazil). Geographic distribution and the study of a "reservarea". Rev Inst Med Trop Sao Paulo. 1998;40:11–3.
19. Fonseca ER, Pardal PP, Severo LC. Paracoccidioidomycosis in children in Belem, Para. Rev Soc Bras Med Trop. 1999;32:31–3.
20. •• Bellissimo-Rodrigues F, Machado AA, Martinez R. Paracoccidioidomycosis: epidemiological features of a 1,000-cases series from a hyperendemic area on the Southeast of Brazil. AmJTrop Med Hyg. 2011;85:546–50. *This article analyzes the largest series of paracoccidioidomycosis patients, one thousand, ever analyzed with emphasis on epidemiological and clinical aspects*.
21. Arantes TD, Theodoro RC, Da Graça Macoris SA, et al. Detection of Paracoccidioides spp. in environmental aerosol samples. Med Mycol. 2012. doi:10.3109/13693786.2012.698444.
22. Calle D, Rosero DS, Orozco LC, et al. Paracoccidioidomycosis in Colombia: an ecological study. Epidemiol Infect. 2001;126:309–15.
23. Franco M, Bagagli E, Scapolio S, et al. A critical analysis of isolation of Paracoccidioides brasiliensis from soil. Med Mycol. 2000;38:185–91.
24. • Barrozo LV, Benard G, Silva ME, et al. First description of a cluster of acute/subacute paracoccidioidomycosis cases and its association with a climatic anomaly. PLoS Negl Trop Dis. 2010;4:e643. *An important approach when searching for connections among climate, incidence and possible clustering of juvenile patients in a hyperendemic Brazilian area*.
25. Bagaggli E, Franco M, Bosco Sde M, et al. High frequency of Paracoccidioides brasiliensis infection in armadillos (Dasypus novemcinctus): an ecological study. Med Mycol. 2003;41:217–23.
26. Ricci G, Mota FT, Wakamatsu A, et al. Canine paracoccidioidomycosis. Med Mycol. 2004;42:379–83.

27. Richini-Pereira VB, Bosco Sde M, Griese J, et al. Molecular detection of Paracoccidioides brasiliensis in road-killed wild animals. Med Mycol. 2008;46:35–40.
28. Oliveira GG, Navarro IT, Freire RL. Serological survey of Paracoccidioidomycosis in sheep. Mycopathologia. 2012;173:63–8.
29. Queiroz-Telles F. Influence of alternating coffee and sugar cane agriculture in the incidence of paracoccidioidomycosis. Proceedings X International Congress on Paracoccidioidomycosis: a centennial celebration. Medellín, Colombia, August 7–10, 2008. p. 129.
30. Paniago AM, Aguiar JI, Aguiar ES, et al. Paracoccidioidomycosis: a clinical and epidemiological study of 422 cases observed in Mato Grosso do Sul. Rev Soc Bras Med Trop. 2003;36:455–9.
31. •• Colombo AL, Tobón A, Restrepo A, et al. Epidemiology of endemic systemic fungal infections in Latin America. Med Mycol. 2011;49:785–98. *A concise and well organized article on the importance of endemic systemic mycoses in Latin America*.
32. • Shankar J, Restrepo A, Clemons KV, et al. Hormones and the resistance of women to paracoccidioidomycosis. Clin Microbiol Rev. 2011;24:296–313. *An update on the hormonal influences occurring in this mycosis that also serves to present the disease and its most important epidemiological, clinical and immune-related aspects*.
33. Loth EA, Castro SV, Silva JR, et al. Occurrence of 102 cases of paracoccidioidomycosis in 18 months in the Itaipu Lake region, Western Paraná. Rev Soc Bras Med Trop. 2011;44:636–7.
34. Brazão-Silva MT, Andrade MF, Franco T, et al. Paracoccidioidomycosis: a series of 66 patients with oral lesions from an endemic area. Mycoses. 2011;54:e189–95.
35. • Queiroz-Telles F, Escuissato DL. Pulmonary paracoccidioidomycosis. Semin Respir Crit Care Med. 2011;32:764–74. *An update on the primary manifestations of the mycosis*.
36. • Bellissimo-Rodrigues F, Vitali LH, Martinez R. Serological diagnosis of paracoccidioidomycosis in HIV-coinfected patients. Mem Inst Oswaldo Cruz. 2010;105:904–7. *The unexplained paucity of the dual HIV-paracoccidioidomycosis occurrence is approached in this apparently diagnosis-geared paper.*
37. Nucci M, Colombo AL, Queiroz-Telles F. Paracoccidioidomycosis. Curr Fungal Infect Rep. 2009;3:15–20.
38. Coutinho ZF, Silva D, Lazera M, et al. Paracoccidioidomycosis mortality in Brazil (1980–1995). Cad Saude Publica. 2002;18:1441–54.
39. Bittencourt JI, de Oliveira RM, Coutinho ZF. Paracoccidioidomycosis mortality in the State of Parana, Brazil, 1980/1998. Cad Saude Publica. 2005;21:1856–64.
40. Santo AH. Paracoccidioidomycosis-related mortality trend, state of Sao Paulo, Brazil: a study using multiple causes of death. Rev Panam Salud Publica. 2008;23:313–24.
41. • Ribeiro LC, Hahn RC, Favalessa OC, et al. Systemic mycosis: factors associated with death among patients infected with the human immunodeficiency virus, Cuiabá, State of Mato Grosso, Brazil, 2005–2008. Rev Soc Bras Med Trop. 2009;42:698–705. *A solid analysis on the influence of paracoccidioidomycosis in the death toll of HIV-infected patients*.
42. • Prado M, Silva MB, Laurenti R, et al. Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006. Mem Inst Oswaldo Cruz. 2009;104:513–21. *This paper analyzes the importance of the mycosis as the cause of death in this particular patient population*.
43. • Martines R. Paracoccidioidomycosis: the dimension of the problem of a neglected disease. Rev Soc Bras Med Trop. 2010;43:480. *A solid editorial written by the most experienced paracoccidioidomycosis physician in Brazil who calls the disease a neglected one*.
44. San-Blas G, Niño-Vega G, Iturriaga T. Paracoccidioides brasiliensis and paracoccidioidomycosis: molecular approaches to morphogenesis, diagnosis, epidemiology, taxonomy and genetics. Med Mycol. 2002;40:225–42.
45. Cock AM, Cano LE, Vélez D, et al. Fibrotic sequelae in pulmonary paracoccidioidomycosis: histopathological aspects in BALB/c mice infected with viable and non-viable Paracoccidioides brasiliensis propagules. Rev Inst Med Trop Sao Paulo. 2000;42:59–66.
46. Terçarioli GR, Bagagli E, Reis GM, et al. Ecological study of Paracoccidioides brasiliensis in soil: growth ability, conidia production and molecular detection. BMC Microbiol. 2007;7:92.
47. Gomez BL, Nosanchuk JD, Díez S, et al. Detection of melanin-like pigments in the dimorphic fungal pathogen Paracoccidioides brasiliensis in vitro and during infection. Infect Immun. 2001;69:5760–7.
48. Urán ME, Nosanchuk JD, Restrepo A, et al. Detection of antibodies against Paracoccidioides brasiliensis melanin in in vitro and in vivo studies during infection. Clin Vaccine Immunol. 2011;18:1680–8.
49. Silva MB, Thomaz L, Marques AF, et al. Resistance of melanized yeast cells of Paracoccidioides brasiliensis to antimicrobial oxidants and inhibition of phagocytosis using carbohydrates and monoclonal antibody to CD18. Mem Inst Oswaldo Cruz. 2009;104:644–8.
50. Bialek R, Ibrecevic A, Fothergill A, et al. Small subunit ribosomal DNA sequences shows Paracoccidioides brasiliensis closely related to Blastomyces dermatitidis. J Clin Microbiol. 2000;38:3190–3.
51. Matute DR, Sepúlveda VE, Quesada LM, et al. Microsatellite analysis of three phylogenetic species of Paracoccidioides brasiliensis. J Clin Microbiol. 2006;44:2153–7.
52. Teixeira MM, Theodoro RC, de Carvalho MJ, et al. Phylogenetic analysis reveals a high level of speciation in the Paracoccidioides genus. Mol Phylogenet Evol. 2009;52:273–83.
53. •• Teles FR, Martins ML. Laboratorial diagnosis of paracoccidioidomycosis and new insights for the future of fungal diagnosis. Talanta. 2011;85:2254–64. *A comprehensive review on the subject of diagnosis.*
54. • Brandt ME, Gómez BL, Warnock D. Histoplasma, Blastomyces, Coccidioides, and other dimorphic fungi causing systemic mycoses. In: Versalovic J, Warnock D, editors. Manual of clinical microbiology. 10th ed. Washington: ASM press; 2011. p. 1902–18. *The first in the reading list of articles for non-mycology experienced laboratory personnel.*
55. Lindsley MD, Warnock DW, Morrison CJ. Serological and molecular diagnosis of fungal infection. In: Rose NR, Hamilton RG, Detrick B, editors. Manual clinical laboratory immunology. Washington: ASM Press; 2006. p. 569–605.
56. Figueroa JI, Hamilton AJ, Allen MH, et al. Isolation and partial characterization of a Paracoccidioides brasiliensis 58 kDa extracellular glycoprotein which is recognized by human immune sera. Trans R Soc Trop Med Hyg. 1995;89:566–72.
57. Gómez BL, Figueroa JI, Hamilton AJ, et al. Use of monoclonal antibodies in diagnosis of Paracoccidioidomycosis: new strategies for detection of circulating antigens. J Clin Microbiol. 1997;35:3278–83.
58. Ortiz BL, Díez S, Urán ME, et al. Use of the 27-kilodalton recombinant protein from Paracoccidioides brasiliensis in serodiagnosis of paracoccidioidomycosis. Clin Diagn Lab Immunol. 1998;5:826–30.
59. Díez S, Gómez BL, Restrepo A, Hay RJ, et al. Paracoccidioides brasiliensis 87-kilodalton antigen, a heat shock protein useful in diagnosis: characterization, purification, and detection in biopsy material via immunohistochemistry. J Clin Microbiol. 2002;40:359–65.
60. Marques da Silva SH, Colombo AL, Blotta MH, et al. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. J Clin Microbiol. 2003;41:3675–80.

61. da Silva SH, Grosso Dde M, Lopes JD, et al. Detection of Paracoccidioides brasiliensis gp70 circulating antigen and follow-up of patients undergoing antimycotic therapy. J Clin Microbiol. 2004;42:4480–6.
62. Camargo ZP, Unterkircher C, Travassos LR. Identification of antigenic polypeptides of Paracoccidioides brasiliensis by immunoblotting. J Med Vet Mycol. 1989;27:407–12.
63. Giannini MJ, Bueno JP, Shikanai-Yasuda MA, et al. Antibody response to the 43 kDa glycoprotein of Paracoccidioides brasiliensis as a marker for the evaluation of patients under treatment. AmJTrop Med Hyg. 1990;43:200–6.
64. • Díez S, Gómez BL, McEwen JG, et al. Combined use of Paracoccidioides brasiliensis recombinant 27-kilodalton and purified 87-kilodalton antigens in an enzyme-linked immunosorbent assay for serodiagnosis of paracoccidioidomycosis. J Clin Microbiol. 2003;41:1536–42. *More than one recombinant or purified antigen should be used for specific diagnosis*.
65. • Buitrago MJ, Merino P, Puente S, et al. Utility of real-time PCR for the detection of Paracoccidioides brasiliensis DNA in the diagnosis of imported paracoccidioidomycosis. Med Mycol. 2009;47:879–82. *The door to the use of molecular tests for diagnosis*.
66. Motoyama AB, Venancio EJ, Brandão GO, et al. Molecular identification of Paracoccidioides brasiliensis by PCR amplification of ribosomal DNA. J Clin Microbiol. 2000;38:3106–9.
67. Bialek R, Ibricevic A, Aepinus C, et al. Detection of Paracoccidioides brasiliensis in tissue samples by a nested PCR assay. J Clin Microbiol. 2000;38:2940–2.
68. Semighini CP, de Camargo ZP, Puccia R, et al. Molecular identification of Paracoccidioides brasiliensis by 5′ nuclease assay. Diagn Microbiol Infect Dis. 2002;44:383–6.
69. Endo S, Komori T, Ricci G, et al. Detection of gp43 of Paracoccidioides brasiliensis by the loop-mediated isothermal amplification (LAMP) method. FEMS Microbiol Lett. 2004;234(1):93–7.
70. Tatibana BT, Sano A, Uno J, et al. Detection of Paracoccidioides brasiliensis gp43 gene in sputa by loop-mediated isothermal amplification method. J Clin Lab Anal. 2009;23:139–43.
71. • Tobón AM, Agudelo CA, Osorio ML, et al. Residual pulmonary abnormalities in adult patients with chronic paracoccidioidomycosis: prolonged follow-up after itraconazole therapy. Clin Infect Dis. 2003;37:898–904. *Be aware of the fibrotic sequela in this mycosis*.
72. •• Restrepo A, Benard G, de Castro CC, et al. Pulmonary paracoccidioidomycosis. Semin Respir Crit Care Med. 2008;29:182–97. *A comprehensive review to understanding the pivotal role of the lungs in this mycosis*.
73. Restrepo A, Tobón A, Agudelo C. Paracoccidioidomycosis. In: Hospenthal DR, Rinaldi MG, editors. Diagnosis and treatment of human mycoses. Totowa: Humana Press; 2008. p. 331–42.
74. • Marchiori E, Valiante PM, Mano CM, et al. Paracoccidioidomycosis: high-resolution computed tomography-pathologic correlation. Eur J Radiol. 2011;77:80–4. *Evidence for the large damage caused by the etiologic agent*.
75. • Barreto MM, Marchiori E, Amorim VB, et al. Thoracic paracoccidioidomycosis: radiographic and CT findings. RadioGraphics. 2012;32:71–84. *A correlation between image techniques that reveals the severity and frequency of the lung damage*.
76. Grossklaus Dde A, Tadano T, Breder SA, et al. Acute disseminated paracoccidioidomycosis in a 3 year-old child. Braz J Infect Dis. 2009;13:242–4.
77. Carneiro CR, Miranda BG, Camilo Neto C, et al. Juvenile aracoccidioidomycosis in urban area: report of two cases. Braz J Infect Dis. 2010;14:77–80.
78. Nascimento CR, Delanina WF, Soares CT. Paracoccidioidomycosis: sarcoid-like form in childhood. An Bras Dermatol. 2012;87:486–7.
79. Pereira GH, Santos AQ, Park M, et al. Bone marrow involvement in a patient with paracoccidioidomycosis: a rare presentation of juvenile form. Mycopathologia. 2010;170:259–61.
80. Marques SA, Camargo RM, Abbade LP, et al. Paracoccidioidomycosis: an unusual presentation in a young girl disclosing an unnoted HIV-infection. Med Mycol. 2010;48:182–7.
81. • Rodrigues GS, Severo CB, Oliveira FM, et al. Association between paracoccidioidomycosis and cancer. J Bras Pneumol. 2010;36:356–62. *This association is not too frequent but must be taken into consideration*.
82. Marques SA, Lastória JC, Marques ME. Paracoccidioidomycosis in a patient with cervical cancer. An Bras Dermatol. 2011;86:587–8.
83. Woyciechowsky TG, Dalcin DC, dos Santos JW, et al. Paracoccidioidomycosis induced by immunosuppressive drugs in a patient with rheumatoid arthritis and bone sarcoma: case report and review of the literature. Mycopathologia. 2011;172:77–81.
84. Goldani LZ. Gastrointestinal paracoccidioidomycosis: an overview. J Clin Gastroenterol. 2011;45:87–91.
85. Araújo SA, Espindola BM, Pedroso ER. Cutaneous disseminated paracoccidioidomycosis. AmJTrop Med Hyg. 2012;86:1.
86. Oñate JM, Tobon AM, Restrepo A. Insuficiencia suprarrenal secundaria a paracoccidioidomicosis. Biomedica. 2002;22:280–6.
87. Tobón AM, Agudelo CA, Restrepo CA, et al. Adrenal function status in patients with paracoccidioidomycosis after prolonged post-therapy follow-up. AmJTrop Med Hyg. 2010;83:111–4.
88. Shikanai-Yasuda MA, Benard G, Higaki Y, et al. Randomized trial with itraconazole, ketoconazole and sulfadiazine in paracoccidioidomycosis. Med Mycol. 2002;40:411–7.
89. •• Shikanai-Yasuda MA, Telles Filho FQ, Mendes RP, et al. Guidelines in paracoccidioidomycosis. Rev Soc Bras Med Trop. 2006;39:297–310. *A classic in treatment approaches*.
90. Queiros-Tellez F, Goldani LZ, Schamm HT, et al. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. Clin Infect Dis. 2007;45:1462–9.
91. Benard G, Campos AF, Netto LC, et al. Treatment of severe forms of paracoccidioidomycosis: is there a role for corticosteroids? Med Mycol. 2012;50:641–8.