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267 Paracoccidioidomycosis

Ángela Restrepo-Moreno, Ángela Ma. Tobón-Orozco, and Ángel González-Marín

SHORT VIEW SUMMARY

Definition

- Paracoccidioidomycosis is a Latin American endemic and systemic fungal disease characterized by two main clinical forms as follows: (1) acute/subacute observed in children, adolescents, and immunocompromised individuals and (2) chronic as seen in adults 30 years of age or older.
- The acute/subacute form is a serious disease that results in lymph node hypertrophy and hepatosplenomegaly accompanied by fever, weight loss, and generalized malaise. Multiple skin and mucosal lesions occur in half of the cases. The primary pulmonary lesions are not apparent clinically but can be demonstrated radiologically in all cases.
- The chronic form results in pulmonary infiltrates in greater than 90% of the cases, as well as skin and mucosal lesions. Adrenal gland abnormalities are common and may lead to insufficiency.

Epidemiology

- The habitat(s) of *Paracoccidioides* spp. remain unknown, but they are restricted to Latin American countries.
- The largest endemic areas are found in Brazil.
- Most patients are males who work (or have worked) in agriculture-related occupations within the endemic areas.

Microbiology

- Paracoccidioidomycosis is caused by species in the genus *Paracoccidioides,* encompassing five distinct phylogenetic species, namely, *P. brasiliensis, P. americana, P. restrepiensis,* and *P. venezueliensis* (formerly designated as S1, PS2, PS3, and PS4, respectively). A new species, significantly different from the above on genetic and molecular grounds has been found and designated *P. lutzii.*
- *Paracoccidioides* spp. are thermally dimorphic fungi, identified by mycelial growth at 22°C to 24°C and by multiple-budding yeast cells (pilot's wheel) at 36°C to 37°C.

Diagnosis

- Both direct microscopy and histopathology allow prompt diagnosis.
- Detection of antibodies and fungal antigens prove useful not only for diagnosis but also for follow-up therapy results.
- Specific diagnosis is usually late due to confusion with other more prevalent diseases (e.g., tuberculosis). Lack of physicians' awareness is a problem.
- Lung imaging studies are essential in adult patients. Healing by fibrosis creates serious complications.

Therapy (see Table 267.1)

- Itraconazole is the treatment of choice for most patients, given at 200 to 400 mg/day for 6 to 9 months, depending on the severity of the disease.
- Amphotericin B deoxycholate is reserved for the most serious cases, at 1 mg/kg/day until achieving a 2-g total dose. This should be followed by triazole oral medications.
- Alternative treatment can be given with trimethoprim-sulfamethoxazole (TMP-SMX), given as TMP, 160 to 240 mg, and SMX, 800 to 1200 mg per day for 12 to 24 months, depending on disease severity. Treatment is long lasting (close to 2 years) and can result in relapses.

Prevention

- Taken into consideration that the precise fungus habitat remains undefined, preventing contact with *P. brasiliensis* does not appear feasible.

DESCRIPTION OF THE PATHOGEN

Until 2006[1] the genus *Paracoccidioides* was thought to consist of a single species, *P. brasiliensis,* considered the sole etiologic agent of paracoccidioidomycosis.[2,3] At present however, molecular and genetic studies have revealed that this genus includes a complex of five distinct phylogenetic species (S1a, S1b, PS2, PS3, PS4)[4] that appear to be confined to distinct regions within the endemic zones.[5–7] Recently, the *P. brasiliensis* spp. complex has been formally renamed, with the proposed names *P. brasiliensis* for S1, *P. americana* for PS2, *P. restrepiensis* for PS3, and *P. venezueliensis* for PS4.[8] Additional studies suggest that yeast morphology slightly, but significantly, differs across all five *Paracoccidioides* spp., an observation that may indicate that these phylogenetic species could well be designated as formal taxonomic species.[8] In addition, genomic comparison has indicated that *P. brasiliensis* is related to the uncultivable pathogen *Lacazia loboi*.[9,10]

Of note, a highly divergent monophyletic species, *Paracoccidioides lutzii,* has been described.[11–13] Distinguishing *P. lutzii* is based on a distinctive genome and differences in the conidia, as well as on morphology and size of the daughter yeast cells.[14] So far, *P. lutzii* has only been identified in central, southwestern, and northwestern Brazil.[13,15] Species within the complex *P. brasiliensis* also appear confined to particular endemic regions.[16]

Species within the *Paracoccidioides* genus exhibit the phenomenon called dimorphism, implicating that they can give rise to colonies corresponding to a mold or to a yeast, with temperature being the only recognized factor known to date that is capable of triggering this change. The latter phenomenon takes place when the β-glucan predominating in the mold is replaced almost entirely by the yeast-phase cell wall polysaccharide α-1,3-glucan.[7,17]

As for the *Paracoccidioides* genus classification, the relevant species are assigned to the family Ajellomycetaceae, order Onygenales.[18,19] Genomic analyses support the existence of a sexual cycle in species of the *Paracoccidioides* genus, by detection of heterothallic groups (mating type 1-1 [MAT1-1] or MAT1-2), with gene expression in certain isolates but with no production of fruiting sexual bodies.[20,21]

Since 2009 the Broad Institute at the Massachusetts Institute of Technology (Cambridge, MA) has completed the whole-genome sequencing database for the *P. brasiliensis* spp. complex, which is publicly available in the Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra).

In cultures incubated at 35°C to 37°C the fungus grows in about 10 days, producing a soft, convoluted, tan-to-cream–colored colony, microscopically composed of yeast cells of varying size (4–40 μm), some adopting the characteristic multiple-budding formation (pilot's wheel). Isolated, round-to-oval yeast cells and short chains of blastoconidia are also observed, as well as large broken yeasts (Figs. 267.1 and 267.2).[2,3,14] Viable yeasts have refractive cell walls and prominent lipid vacuoles.[2,3,14]

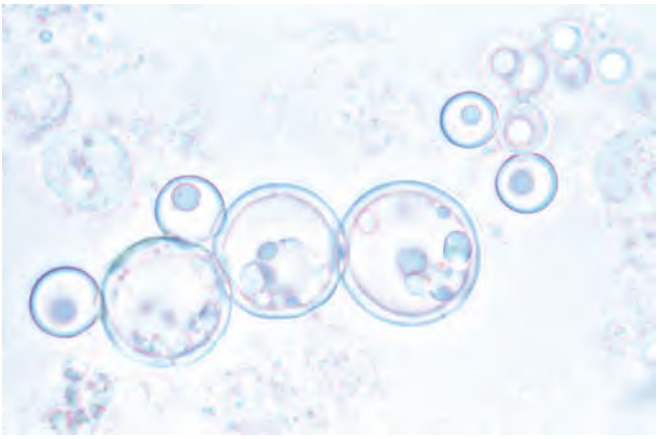

FIG. 267.1 ***Paracoccidioides brasiliensis.*** Multiple budding and variation in cell size are apparent (potassium hydroxide preparation from pus, ×1000).

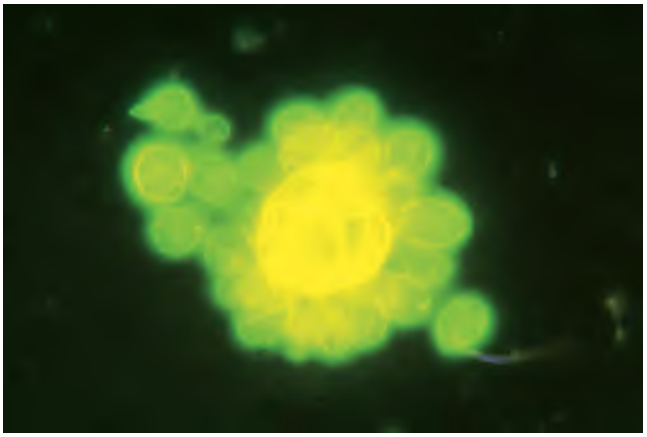

FIG. 267.2 ***Paracoccidioides brasiliensis.*** Multiple buds surrounding the mother cell can be observed in a wet mount from a 36°C culture (calcofluor white, ×1000).

P. brasiliensis has been repeatedly recovered from human clinical samples,[2,3] tissues of the nine-banded armadillo *(Dasypus novemcinctus)*[22] and, more rarely, from the northern naked-tailed armadillo *(Cabassous centralis).*[23] On occasion, *P. brasiliensis* has also been isolated from dogs and detected molecularly in other animal species.[24,25]

As indicated earlier, this fungus encompasses two morphotypes: a mold at temperatures less than 28°C and a yeast in mammalian tissues and cultures at 35°C to 37°C.[2,3] Both morphotypes require ample oxygen supply for their growth.[26] At temperatures less than 28°C, including 4°C if in liquid substrates, the fungus grows within 3 weeks and produces white, cotton-like colonies having short tufts of aerial mycelia.[2,3] Microscopically, only chlamydoconidia and thin septated hyphae are observed in media such as Sabouraud,[2,3] but in carbohydrate-reduced media, the fungus may produce conidia (size <5 μm),[2,27] especially if grown in sandy and claylike soils rich in water.[27,28] Conidia respond to temperature changes by germinating into hyphae at 20°C to 24°C or converting into yeasts, both in vitro (36°C)[27,28] and in infected mice; in the latter, conidia initiate a progressive pulmonary process ending in fibrosis and extrapulmonary dissemination.[2,3,29,30]

P. brasiliensis undergoes an intricate thermal transition process by switching from a mycelial saprobic form, probably in nature, to a virulent yeast form in infected mammal tissues.[1,3,27,29,30] This transition involves expression of an array of virulence factors (cell glucan type, proteolytic enzymes, melanins, fungal adherence factors, and others).[31–34] In addition, biofilm formation has been demonstrated and shown to correlate with increased expression of adhesins and hydrolytic and other types of enzymes.[35] All of the given factors facilitate *P. brasiliensis* interactions with its accidental mammalian host controlled by certain genes expressed during host adaptation.

ECOLOGY AND EPIDEMIOLOGY

Paracoccidioidomycosis, a noncontagious disease, is peculiarly restricted to Latin America, from Mexico (23° N) to Argentina (34° S), with Brazil accounting for the largest number of patients (>80%) and originating mainly from the States of São Paulo, Paraná, Rio Grande do Sul, Goiás, Rio de Janeiro, and Rondônia.[36,37] Venezuela, Colombia, Ecuador, Peru, Bolivia, and Argentina follow in rank with significantly fewer cases. Uruguay, Paraguay, and the Central American countries (Nicaragua, Belize) report few or no cases. The mycosis is extremely rare in the Caribbean Islands, with only single cases having been reported from Trinidad, Grenada, and Guadalupe. The mycosis is unknown in Chile, Surinam, and Guyana.[2,3,38–41] In addition, the mycosis appears to prefer a humid environment and surrounding woods.[3,38]

The main accidental hosts of *Paracoccidioides* spp. are men and armadillos, especially the northern nine-banded *Dasypus novemcinctus,* although other animals have also been implicated, presumably infected in rural or periurban environments.[13,39,42,43] As a rule, the primary infection is asymptomatic or gives rise to nonspecific symptoms that rarely progress to disease manifestations. No epidemic outbreaks or transmission between individuals have been reported.[39,44] Due to lack of knowledge on the precise habitats of the fungus, suspected to occur in rural settings,[44,45] and based on experimental animal infections,[46] it is accepted that the fungal infecting structures, the conidia (<5 μM), are inhaled and reach the lungs, where the infection is usually controlled by the immune cellular responses but may leave behind scarred focal points containing latent yeast cells that may reactivate years later, giving rise to full-blown disease.[3,38]

The disease is characterized by long periods of latency—30 or more years—as demonstrated by the nonautochthonous cases reported outside recognized endemic areas (North America, Europe, Asia).[47] In every case, however, the patient had previously resided in an endemic country, where the primary infection should have occurred, as exemplified by cases reported in Spain, France, Portugal, and Japan, among others.[48–50]

Areas of endemicity center around tropical and subtropical forests, where mild temperatures (17°C–24°C), high annual precipitations (2000–2999 mm), and abundant waterways are common, conditions that favor certain agricultural crops (e.g., coffee, tobacco).[2,3,38,51] Changes in traditional agricultural practices were implicated in the increased number of juvenile cases reported in Rio de Janeiro, Brazil.[52] Two large Brazilian studies emphasized the connection between agriculture-related jobs and paracoccidioidomycosis, pointing toward closer or repeated contacts with *P. brasiliensis* in soil.[39,53] Nonetheless, the fungus has been isolated only sporadically from the environment,[45] a fact that, together with the prolonged mycosis latency periods[47] and absence of outbreaks, has contributed to maintaining the uncertainty about the *P. brasiliensis* microniche. In an attempt at defining more precisely the fungus' environmental niche, Barrozo and coworkers[54,55] studied the role of weather conditions prevailing at the time of diagnosis of a number of juvenile patients, those with the shortest disease course. Absolute air humidity, soil, water storage, and presence of the Southern Oscillation Index influenced significantly the incidence, thereby suggesting their connection with acquisition of the infection. Imprecise knowledge of the fungal habitat has also hindered definition of the infection route, although clinical and experimental animal studies have ruled out traumatic implantation and pointed instead toward the aerosol route as the portal of entry.[2,3,44,45,56]

The incidence of paracoccidioidomycosis is difficult to estimate due to the noncompulsory report of cases and, in addition, to the influence of climate changes and also by human migration and occupation of large unexplored territories where medical care would not be available, as indicated by Martinez.[41] Nonetheless, the incidence of this mycosis was estimated for Brazil, the country with the largest number of patients—10 to 30 cases per 100,000 inhabitants per year.[40,41,57] However, variations were recorded, as indicated in the Itaipu Lake region, where 102 patients were diagnosed in a period of 18 months,[58] and in the State of Rondônia they had the closest to a "prolonged" outbreak, as 39.1 cases per 100,000 inhabitants were reported from 1997 to 2012.[59]

The age and gender distribution of clinical cases is unusual. The mycosis is rare in children (<2%) and teenagers (<9%), with the remaining patients being 30 years of age and older.[60,61,62,63] Several different series encompassing more than 5000 patients revealed that the male-to-female

ratio was 11 : 1.[64] This proportion contrasts with the infection rate as determined by a reactive paracoccidioidin skin test, which had a similar prevalence for both genders.[65] Of note, when the disease occurs in prepubertal patients, no major gender difference becomes apparent, as found with more than 100 children in whom the boy : girl ratio varied from 1.2:1.3 to 1.1:1.0.[38]

The occupational distribution reveals a predilection for individuals who have been engaged in agriculture (>60%) or have had soil-related jobs with subsequent exposure to infected soil and dust, mainly in connection with coffee, cotton, tobacco, and sugar cane plantations.[3,39,51,60,63] Increased acreage of sugar cane fields in the southwestern region of Brazil may alter these data because this crop requires pesticide application and plant burning, a combination that may affect *Paracoccidioides* spp. saprobiotic life in nature.[66] Other occupations mentioned as relatively frequent are masonry and lumberjacking.[36,38,39]

The presence of *Paracoccidioides* spp. in soils or other types of habitats in Latin America, South America in particular, indicate that a large territory is to be taken as endemic and, furthermore, considering that the most prevalent occupation for the working population of such territory would be agriculture-related, including crops manipulation, *P. brasiliensis* airborne infection could represent a problem for newcomers. According to Martinez[41] the existence of unexplored territories, such as those along the Amazon River running through various South American countries, would attract migrants pursuing occupation of promising uninhabited land open to agriculture-related endeavors. This means that a naïve population from these diverse countries may well become exposed to *P. brasiliensis,* leading to infection, which may result in continued expansion of the endemic area, as already observed in north and center-west Brazil.[41] Nonetheless, progression of the infection to the disease stage and the clinical form that may become manifested in infected people would depend on various host factors: genetic background, demography, living conditions, presence of immunosuppression, and others. New endemic areas usually have medical care deficiencies, which would cause considerable social impact. Even if the corresponding clinical manifestations would not become apparent in a short period, *P. brasiliensis* latency poses a challenge, mainly considering that the fungus may become active with time, giving rise to a chronic disease that would cause suffering, disability to the patient, and leave sequelae even in the presence of specific treatment.[44]

Deaths due to paracoccidioidomycosis resulted from extensively disseminated lesions, respiratory and adrenal gland insufficiency, and several other complications, mainly those attributed to residual fibrosis. In Brazil the death toll was estimated as 3.48 per million inhabitants, thus making paracoccidioidomycosis the fifth most common cause of death among the chronic infectious diseases of the lower respiratory tract.[41,67] During the period 1998–2006, a Brazilian study was performed covering 35% of the country and representing 27% of the 5560 Brazilian municipalities, including the larger part of the endemic paracoccidioidomycosis area, consisting of an infected population estimated at 4.3 per 1 million inhabitants. The results analyzed 6732 hospitalization events attributable to paracoccidioidomycosis (82% male). The corresponding results estimated a global mortality of 1.4 per one million inhabitants, with 60% of such deaths attributed to the mycosis itself and the remaining to the fibrotic sequelae.[67,68] In two case series from different endemic Brazilian areas, lethality reached 6.1% and 7.6%, respectively. Nonetheless, at the end of treatment, when assessing patients who did not require hospitalization, lethality was zero.[68] Considering a 5% mortality rate as a mean value, it is possible to estimate the number of new annual cases of this mycosis in Brazil as 3360.[41]

Brazilian authors studying paracoccidioidomycosis, and assessing the number of new patients, observed the large group of cases with a prolonged follow-up and the medical-social characteristics of the mycosis as mentioned earlier, and this revealed the wide dimension of this mycosis as a public health problem in Brazil and emphasized this condition as a neglected disease.[41,67,68]

PATHOGENESIS AND THE IMMUNE RESPONSE

After the initial contact with *Paracoccidioides* spp., a *subclinical infection* may develop, which, if the host is unable to control it, becomes clinically apparent.[69] There are two main clinical presentations—the acute/subacute, or juvenile, and the chronic, or adult disease.[69,70] The former is characterized by marked involvement of the reticuloendothelial system and occurs in less than 15% of cases,[3,37,52,61,71–73] whereas the chronic or adult type of disease involves the lungs and characteristically results in extrapulmonary lesions. This is the predominant form occurring in approximately 90% of cases and probably represents endogenous reactivation after years of initial contact with the fungus.[3,15,37,38,53] In addition, a *residual form* is also recognized and is represented by fibrotic scarring occurring at the sites of previously active lesions.[71,73] A recent cluster analysis done in patients with concomitant lung, mucosal, and skin lesions has strongly supported the existence of two different sets of patients.[72–134] The first analysis includes patients with mucosal damage, odynophagia, and/or dysphagia, plus alveolointerstitial infiltrates, and the second one consists of patients exhibiting dermal lesions, dyspnea, and lung fibrosis. The former conditions would represent early stages of the infection, whereas the latter would correspond to a more chronic granulomatous and fibrosing process.[133–135]

Paracoccidioides spp. infection may become dormant but may be reactivated later under the influence of ill-defined conditions prevalent in rural settings, such as chronic alcoholism, malnutrition, and smoking.[40,67]

The pathogenesis of this mycosis depends partly on *Paracoccidioides* spp. virulence factors and their antigenic composition, on environmental conditions, and also on the efficiency of the host immune response.[74] Different in vitro, in vivo, in silico, and ex vivo experiments, as well as clinical studies, have demonstrated that specific and nonspecific defense mechanisms, both innate and adaptive, are crucial in building host resistance.[39] In consequence, this response is highly complex and multifactorial, but when a particular balance of the host-fungus interaction is achieved, the infection is controlled and progression to overt disease is halted; otherwise, individuals who have lost this balance develop clinically manifested paracoccidioidomycosis.[74] Thus the initial interaction of the microorganism with the first line of host defenses would determine the type of immune response to be developed (protective, deleterious), and, accordingly, would determine the disease outcome.[74]

Paracoccidioides spp. have several virulence factors. As an example, the fungus cell wall components—glycoproteins and polysaccharides—control the fungus morphologic transition from the conidia or mycelial morphotypes (26°C) to the parasitic yeast (37°C). This form switch is obligatory if the pathogen is to adapt and thrive inside the host in whom the infection is established.[75] Furthermore, on its surface *P. brasiliensis* expresses several adhesins, such as the glycoprotein gp43 plus the 19-kilodalton (kDa), 30-kDa, and 32-kDa proteins, all fulfilling similar functions, as well as a series of enzymes (malate synthase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, enolase). The adhesins allow the fungus to recognize the host extracellular matrix protein molecules (laminin, fibronectin, fibrinogen, plasminogen) that facilitate not only fungal adherence but also invasion and dissemination to other organs and systems.[76,77] This fungal pathogen also produces melanin and exhibits the capability to form biofilms in vitro and in vivo, factors that confer resistance to both a host's microbicidal mechanisms and to antifungal therapy.[35,78,79]

Innate Immune Responses

Once fungal propagules are inhaled, they interact with pulmonary epithelial cells; such interaction induces the activation of cell signaling molecules in these epithelial cells to produce cytokines and other molecules, thus initiating the immune response.[80,81] In vitro studies have shown that *P. brasiliensis* yeasts stimulate the lung's A549 epithelial cells to produce interleukins-6 (IL-6) and IL-8 by a process dependent on activation of p38 mitogen-activated protein kinase and extracellular signal-regulated kinase.[81]

Besides the lung epithelial cells, the first line of defense is represented by neutrophils, alveolar macrophages, dendritic cells, natural killer (NK) cells, complement, peptides, proinflammatory cytokines, and chemokines, all of which hinder fungal multiplication but are unable to destroy the invading microorganism.[44,74,82] Innate immune system cells detect fungal presence through pattern recognition receptors (PRRs) that interact

with conserved molecular structures present in the microorganisms but absent in the host, structures known as *pathogen-associated molecular patterns* (PAMPs). Among the PRRs that participate in the innate immune response against *P. brasiliensis*, Toll-like receptors (TLRs), mainly TLR2, TLR4, and TLR9; dectin-1; and the mannose receptor play important roles in the recognition of this fungal pathogen and activation of innate immune responses.[74,83–85] In addition, dectin-1 in association with the nucleotide-binding oligomerization domain-like receptor P3 (NLRP3), which activates the inflammasome, leads to maturation and secretion of IL-1β and IL-18, important proinflammatory cytokines connected with resistance against *P. brasiliensis*.[86,87]

Human neutrophils activated in vitro with granulocyte-macrophage colony-stimulating factor, IL-15, tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ), and infected with yeasts from a virulent *P. brasiliensis* strain, revealed that the indicated cytokines were able to induce increased expression of TLR2 and TLR4. On the other hand, in presence of the fungus, TLR2 expression increased, but the corresponding TLR4 decreased.[88] In addition, in vivo experimental studies using TLR2 knockout mice previously infected with *P. brasiliensis* revealed that a deficiency in this factor favored development of a less severe pulmonary infection compared with the infected TLR2-suffcient mice. This treatment increased KC (a chemokine that facilitates neutrophil chemotaxis), transforming growth factor-β (TGF-β), IL-6, IL-23, and IL-17. A Th17 cell profile was associated with decreased expansion of regulatory T cells (Tregs).[83] In addition, studies demonstrated that fungal lipid components modulated macrophage activity through TLR-dependent and -independent mechanisms.[83]

Various experimental studies have shown a dense neutrophilic infiltrate in the lungs of *P. brasiliensis*–infected animals that correlated with secretion of cytokines (TNF-α, IL-6, IL-1β) and chemokine (macrophage inhibitory protein 2 [MIP-2]), as well as with the expression of adherence molecules (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule 1 [VCAM-1]), and integrins, all of which facilitated the influx of such phagocytes.[89]

In addition, human neutrophils produce extracellular traps (NETs) against both *P. brasiliensis* conidia and yeast cell morphotypes, a process that appears to be dependent or independent of reactive oxygen species production but in correlation with the fungal morphotype used.[90] However, this mechanism was ineffective in killing the fungus.[90] In addition, Della Coletta and coworkers[91] demonstrated the presence of NETs in vivo in paracoccidioidomycosis patients with extrapulmonary lesions and also that the highly virulent *P. brasiliensis* strain 18 (Pb18) and the low-virulence Pb265 strain induced different NETs patterns in vitro.

Although neutrophils are considered important players in the innate immune response against *P. brasiliensis*, recent reports using a paracoccidioidomycosis experimental model suggested that neutrophils play a dual role in this mycosis, being essential during the early infection and detrimental during the chronic course of the disease.[92,93]

NK cells also participate in the innate immune response against *P. brasiliensis*. In vitro studies demonstrated that NK cells from paracoccidioidomycosis patients exhibited a decreased cytotoxic response compared with that from healthy individuals. In addition, NK cells were capable of recognizing and killing extracellular yeasts through a mechanism dependent on granules and independent of perforins, whereas cytotoxicity against the intracellular fungus depended on the latter.[94] Granulysin seemed a possible mediator of the granules-dependent mechanism because this molecule was detected in NK cells plus *P. brasiliensis* supernatants and was capable of killing the fungus in a dose-dependent manner.[94] In addition, patients with the mycosis had a low frequency of $CD56^+$ granulysin-producing cells when compared with healthy control subjects. Reports indicated that NK cells, once stimulated with recombinant IL-15, produced proinflammatory cytokines (IFN-γ, TNF-α) that played an immunomodulatory role in paracoccidioidomycosis.[9]

Macrophages represent the major cellular defense against *P. brasiliensis* and are extremely important, not only for infection control but also for induction and regulation of the inflammatory responses. When activated by IFN-γ, they ingest and kill both conidia and yeasts through expression of inducible nitric oxide synthase.[74,89] Nitric oxide, however, plays a dual role (resistance and susceptibility), depending on its expression degree.[89] Along the same lines, IFN-γ also induced the indolamine 2,3-dioxygenase, an enzyme that catalyzes tryptophan metabolism and is associated with inhibition of *P. brasiliensis* growth.[95] In addition, it has been reported that *P. brasiliensis* induced transcription of the Notch1 receptor on macrophages, thus increasing IL-6 production, the latter favoring fungal virulence.[96]

Eosinophils are commonly connected with the immune response during allergic and parasitic infections. In the case of paracoccidioidomycosis, eosinophils appear to play a detrimental effect; thus, in these patients, the severity of the disease is associated with eosinophilia.[97] More recently, a functional evaluation of eosinophils in paracoccidioidomycosis patients and control subjects revealed that eosinophils from both groups exhibited a similar ability to kill *P. brasiliensis*, although those from patients were less responsive to IL-5 stimulation.[98]

Dendritic cells (DCs) play an important role in bridging the innate and acquired immune responses. These cells bind, capture, kill, and process pathogens and then migrate to peripheral lymphoid organs to present the processed antigens to T cells, thus initiating the acquired immune response. *P. brasiliensis* infection inhibits prostaglandin E_2 production by DCs and subsequently their maturation and migration.[99] More recently, it was shown that DCs treated with a TLR9 agonist exhibited an increased fungicidal activity against *P. brasiliensis*.[85]

As expected, the host's immune response represents the summation of a series of biologic effects resulting from fungal interaction with different cells, molecules, and receptors geared at eliminating the microorganism. If this is not achieved, the specific or acquired immune response, cellular or humoral, will then be initiated.[74]

Acquired Immune Responses

During the development of the adaptive or specific immune response, dichotomy between the humoral and cellular immune responses develops, with an increase in the production of antibodies associated with an observed hypoproliferative response of the T cells.[100]

Cell-mediated immunity is crucial to defense. It is usually depressed at the disease's peak but can be restored with successful treatment. De Castro and coworkers[101] showed that *Paracoccidioides*-infected individuals are characterized by the presence of the Th1 immune response. Patients with the acute form of the disease had a mixed Th2/Th9 response, whereas those with the chronic form had Th17/Th22 profiles, as well as a substantial Th1 cell participation. Of note, a Th1/Th17 profile is associated with resistance to disease; moreover, a balance between Th17/Treg cells is necessary to control the severity of the fungal disease.[102]

Recently, it has been reported that patients with the acute form of the mycosis, compared with those having the chronic form, expressed high serum levels of IL-1β, IL-18, IL-33, soluble IL-33 receptor (sST2), and IL-37, plus a strong expression of the given cytokines in lymph node lesions.[103] Moreover, the antifungal treatment reduced the levels of these cytokines in all patients groups, indicating that these cytokines may be also associated with the activity and severity of mycosis.[103]

The occurrence and severity of human paracoccidioidomycosis may also be associated with genetic factors, such as single nucleotide polymorphisms (SNPs) on cytokine-encoding genes. Carvalho and coworkers[104] investigated the association among these polymorphisms and the different clinical forms of the mycosis and found similar genotypic and allelic frequencies of the investigated SNPs among the clinical forms described. In another study two polymorphisms in the IL-4 gene were analyzed and showed a significant correlation between *Paracoccidioides* spp. infection and the RP2/RP2 genotype, whereas the RP1/RP1 genotype was linked to resistance.[105] Furthermore, patients with the RP2/RP2 genotype produced high levels of IL-4, whereas healthy individuals with the RP1/RP1 genotype produced low levels of this cytokine. Moreover, a higher frequency of classes I and II human leukocyte antigen alleles has been described in paracoccidioidomycosis patients.[4]

In addition, it has been indicated that *P. brasiliensis* is capable of modulating the immune response toward a permissive state, with the thymus playing a major role. In this sense, experimental studies using BALB/c mice showed that acute infection with *P. brasiliensis* yeast cells promoted thymic alterations leading to a defective repertoire of peripheral

T cells.[106] These results may represent new mechanisms by which *P. brasiliensis* subverts the host's immune response, favoring chronic infection as observed in humans.[106]

Granuloma formation, the hallmark of chronic disease, is considered the most evolved and effective biologic defense weapon against *P. brasiliensis*.[74,82,107] Granuloma formation is involved in the activation of T lymphocytes and effector cells, the latter represented mainly by macrophages and neutrophils. Several Th1 cytokines, especially IFN-γ and IL-12, are associated with resistance, whereas Th2 cytokines (IL-4, IL-10, TGF-α, TGF-β) are associated with increased host susceptibility, probably because they interfere with correct macrophage function.[107] In addition, oral paracoccidioidomycosis granulomas are predominantly populated by $CD163^+$ multinucleated giant cells,[108] with activated M2 macrophages representing the large majority among the inflammatory cells in these lesions, thus characterizing this response as Th2.[109]

Humoral Immune Responses

To date, and despite the strong polyclonal response represented by B lymphocytes associated with hypergammaglobulinemia and the elevated specific antibody titers observed in most patients, the protective nature of this particular immune response has not yet been demonstrated. In patients with the subacute form, specific antibodies of the immunoglobulin (Ig)A, IgG, and IgE subclasses are markedly increased.[74,110] In addition, these patients show eosinophilia and increased levels of TGF-β, a switching factor for IgA. Patients with severe adult-type disease also have elevated antibody titers, but those with a less severe mycosis show significantly lower antibody production.[110]

Of interest, B-1 cells appear to play a detrimental effect in *P. brasiliensis* infection as these cells migrate to the spleen and stimulate an increase in the Treg subpopulation.[111] Genetic differences in the genus *Paracoccidioides* could elicit distinct host immune responses. Lenhard-Vidal and coworkers[112] demonstrated that *P. brasiliensis* and *P. lutzii* antigens elicited different serum IgG responses in chronic paracoccidioidomycosis patients.

Immunoregulation, Immunomodulation, and Vaccines

The combination of an antifungal medication with an effective immunotherapeutic measure, such as an immunomodulator agent or a vaccine, would appear to be useful for treatment of most severe cases because reactivation of the host's immune response would permit shorter treatment periods with conventional medications and may also prevent relapses and fibrotic scarring.[113] Experimental studies are being performed along these lines with promising results when administering the gp43 protein, P10 peptide derived from gp43, and heat shock proteins (DNAhsp65), as well as other antigens (Pb27, rPb27, paracoccin, radioattenuated yeast cells).[113–122] By the same token, experimental studies in an animal model induced by inhalation of *P. brasiliensis* conidia have shown that dual treatment with the antifungal itraconazole (ITC) and the immunomodulator compound pentoxifylline significantly avoided fibrous scarring in infected versus control animals.[107] More recently, the use of a monoclonal antibody specific to neutrophils not only controlled the infection but also reduced the inflammatory response and the pulmonary fibrosis in an experimental model of paracoccidioidomycosis.[93] Moreover, the combination of the monoclonal antibody specific to neutrophils in combination with ITC reduced both disease extension and pulmonary fibrosis through downregulation of inflammatory and profibrotic genes.[123] These results open new avenues to devise therapeutic protocols that may benefit patients with this mycosis.

CLINICAL MANIFESTATIONS

Paracoccidioidomycosis has been divided into three categories—one lacking clinical manifestations and another two directly related to the disease processes and largely dependent on age and host immune status.[69]

1. Paracoccidioidomycosis infection

   This is considered to be a latent, clinically asymptomatic process lasting 1 month to years after the initial fungal contact[124] and that may later evolve to progressive, clinically manifested disease. This form is acquired when a healthy individual enters in contact with *Paracoccidioides* spp. The infection stage is diagnosed by a reactive intradermal test to specific antigens and also at necropsy by demonstrating the presence of fungal cells in tissues.[38,39,44,69]

2. Paracoccidioidomycosis disease

   A. *Acute/subacute disease (moderate or severe):* This is an overt process evidenced by involvement of multiple organs with lymph node, liver, and spleen hypertrophy and also by manifestation of skin lesions, the latter often multiple and widely distributed throughout the body. The lungs do not often reveal radiographic abnormalities, nor are clinical manifestations obvious, despite the fact that *P. brasiliensis* is frequently detected in respiratory specimens. This form is usually diagnosed in children, young adults, and acquired immunodeficiency syndrome (AIDS) patients, as well as in those with other immune alterations.[38,39,44,61,69]

   B. *Chronic progressive disease/adult form (mild, moderate, or severe):* This is the most common (90%) of the clinical presentations, diagnosed in older patients and characterized by important lung involvement and by frequent lesions in the mucosae, skin, adrenal glands, and other sites. Severe cases are defined by meeting three or more of the following criteria: (1) weight loss greater than 10% of the normal body weight; (2) intense pulmonary involvement; (3) involvement of other organs, such as adrenal glands, central nervous system (CNS), and bones; (4) presence of pseudotumoral (>2 cm diameter) lymph nodes in multiple chains, superficially or deeply located, with or without suppuration; and (5) high antibody titers.[38,39,44,69]

   Mild cases are those with weight loss less than 5% and involvement of a few organs, with no dysfunction. In both clinically manifested forms, constitutional symptoms (fever, asthenia, general malaise, weight loss) are regularly observed.[26,38,82,125]

3. Residual form

   This is characterized by the presence of sequelae originating in the previously infected fibrous tissues, mainly in the lung but also reported in adrenal glands and mucosae.[26,38,39,44]

CHARACTERISTICS OF THE LESIONS

Lungs

Lungs are the primary site of infection, but the corresponding clinical manifestations may be scarce; nonetheless, progression follows over time, and at diagnosis, symptoms (cough, expectoration, blood-tinged sputum, chest pain) and some degree of dyspnea are regularly noticed. Auscultation reveals minimal abnormalities compared with radiographic findings, with a clear dissociation between symptoms and imaging studies. The initial respiratory symptoms decrease or disappear fully after the onset of treatment, but dyspnea on heavy exertion might become worse and progress even with moderate or mild exertion. Often noticed are pulmonary function alterations and abnormalities in ventilation-perfusion.[26,38,82,124,125] Most (85%) patients exhibit the obstructive pattern that may be mild, moderate, or severe with no relation with the degree of involvement. Hypoxemia is a sequel occurring in approximately one-third of the cases.[126] These findings, however, are difficult to interpret because the coexistence with smoking habits, resulting in an obstructive pattern involving the small airways, usually accompanied by an increase in the dead space and in the alveolar-arterial oxygen gradient.[82,127]

Chest radiograph images reveal mostly interstitial infiltrates (64%), followed by mixed lesions consisting of nodular and alveolar infiltrates that are occasionally confluent, frequently bilateral, symmetrical, and located preferentially in the central and lower fields (Fig. 267.3).[3,82,127] High-resolution computed tomography demonstrates abnormal findings in greater than 90% of patients with chronic pulmonary disease, with the commonest abnormalities being interlobular septal thickening (88%), peribronchovascular interstitial thickening (78%), centrilobular opacities (63%), intralobular lines (59%), ground-glass opacities (34%), cavities (17%), and airspace consolidation (12%), with the "reversed halo sign" being observed in 10% of the cases.[82,127,128] (Fig. 267.3). Gallium-67 scans reveal pulmonary lesions in practically all patients, irrespective of the clinical forms, thus confirming the lungs as the main target.[70,82,127]

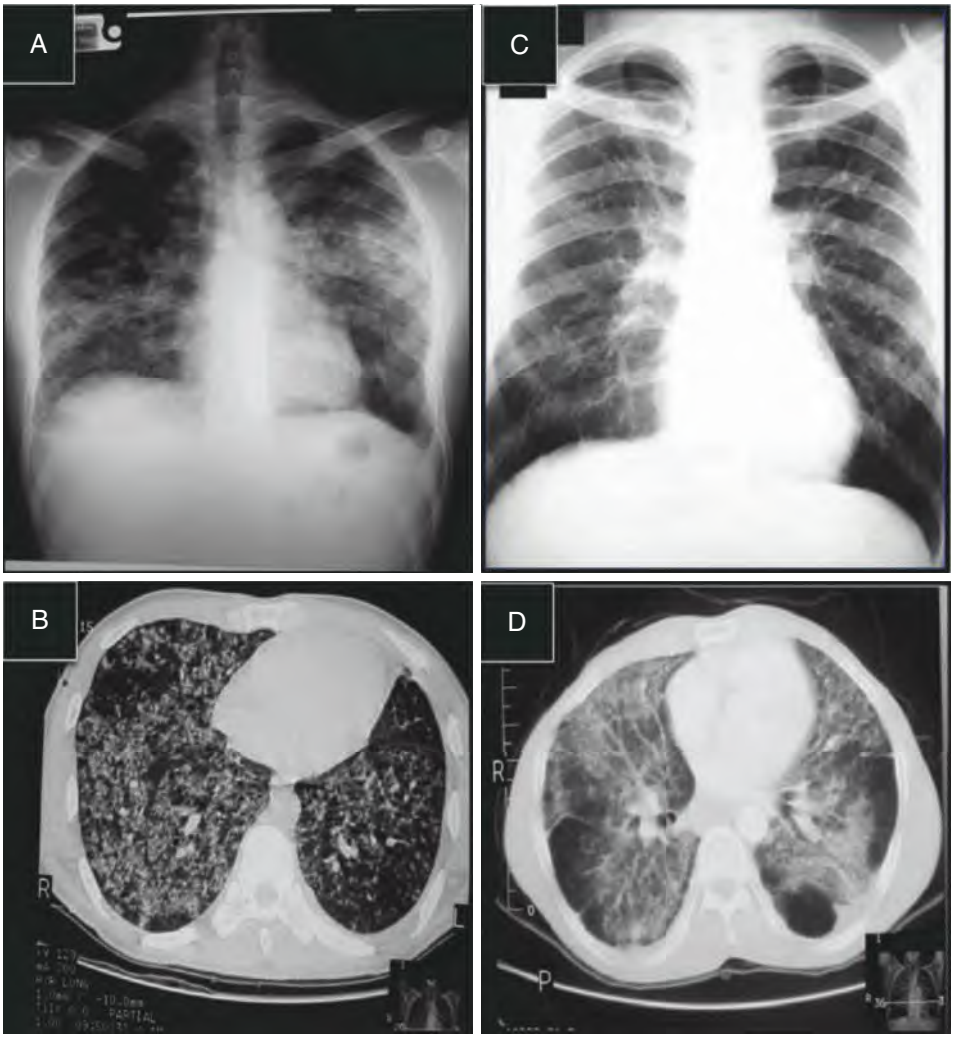

FIG. 267.3 Chest radiograph and chest high-resolution computed tomography (CT) scan in paracoccidioidomycosis patients before treatment. (A) and (C) Plain chest radiographs showing bilateral mixed alveolar and interstitial lesions in lower and central lung fields. (B) and (D) CT scan: The involved lungs show alveolar areas with the ground-glass image. Note bronchial dilatations and presence of bullae as sequelae.

At diagnosis, one-third of the patients with long-lasting disease have serious pulmonary sequelae, such as fibrosis (32%) and bullae (27%), and indirect signs of pulmonary hypertension with right ventricle enlargement and even cor pulmonale.[70,82,127]

A Brazilian study examined 1059 deaths caused by the mycosis and found that mortality could be attributed to pulmonary fibrosis, chronic lower airway diseases, and pneumonic processes, thus signaling the importance of the lung in paracoccidioidomycosis.[82,129] Awareness of the given abnormalities emphasizes early diagnosis and prompt initiation of specific treatment, thereby reducing associated morbidity and mortality.[67,82,127] Pleural involvement is detected in only 2% of cases on plain chest radiographs and is characterized by small effusions (<300 cc) and pleural thickening without effusion as observed in 60% of the autopsy cases.[130]

Mucosa

The oral mucosa is involved in 58.5% of the cases, with lesions regularly localized in the gingiva and the palate and, with extension, to the neighboring skin on the lips and nose. The larynx, pharynx, and gastrointestinal mucosa are also affected. Lesions are infiltrative, edematous, ulcerated, and have a granulomatous base, mulberry-like in appearance (Fig. 267.4). They are extremely painful and hinder the patient's food intake; upon healing, they produce fibrous scarring. Also, in the absence of proper dental care, tooth loss occurs regularly. Other symptoms, such as diarrhea, odynophagia, dysphagia, sialorrhea, and dysphonia may appear, depending on the location of the lesion.[125,131–136] Mucosal involvement is the principal difference with tuberculosis, as in the latter disease the oral presentation is rare, with a prevalence ranging from 0.1% to 0.4%.[137]

Skin

Cutaneous lesions are observed in patients with either the adult form (61.2%) or the acute/subacute presentation (50%). Skin abnormalities may represent hematogenous dissemination from the primary pulmonary focus or secondary extension from contiguous mucous membrane lesions. They tend to ulcerate and present a thick well-delimited external border with a granulomatous base, accompanied by infiltration of the neighboring tissues. In AIDS patients lesions are multiple and rather small, flat, covered by crusts, and with no inflammation in the surrounding tissues (Fig. 267.5).[131,135,136,138,139]

Lymph Nodes

All lymph node chains may be involved, with predilection for cervical, axillary, mediastinal, and mesenteric nodes. Patients with either of the two progressive forms present with clinically detectable hypertrophied lymph nodes. If mucosal or skin lesions exist, enlargement of the corresponding draining lymph nodes is noted, and they may form fistulas. Lymphatic hypertrophy can cause complications by compression of contiguous structures, such as bronchi, vessels, and the periampullary region.[a] These lesions are most easily detected by gallium-67 scans[70] and magnetic resonance imaging (MRI).[141]

[a]References 15, 36, 60, 82, 125, 133, 136, 140.

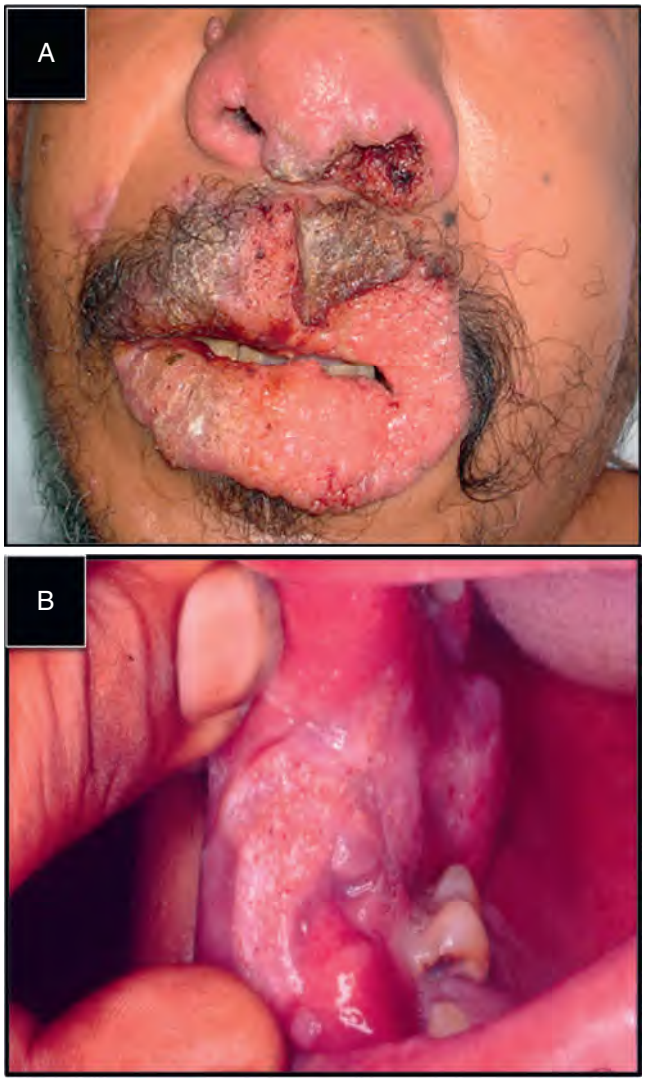

FIG. 267.4 Paracoccidioidomycosis patients. (A) Note edema, ulceration, and crust formation in lips and nostrils. This patient also had extensive lesions of the oral mucosae, as well hypertrophied lymph nodes. (B) Note typical "mulberry-like" lesions in the buccal mucosa of a patient with disseminated disease.

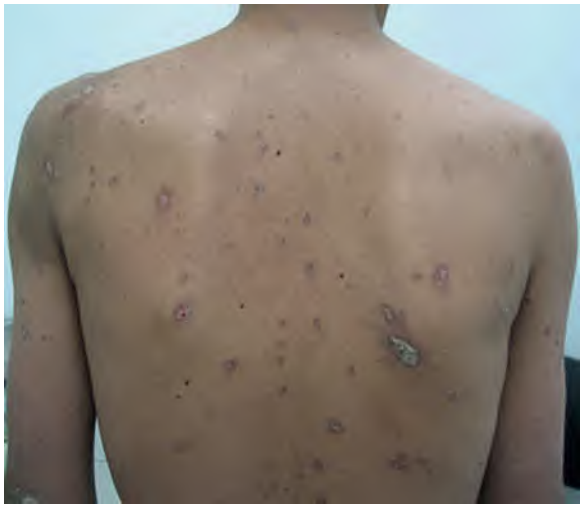

FIG. 267.5 Paracoccidioidomycosis and acquired immunodeficiency syndrome. Note multiple, small, polymorphic, and noninflammatory lesions disseminated throughout the back, arms, and neck areas.

Adrenal Glands

In endemic countries this mycosis is one of the main causes of adrenal gland insufficiency, with the glands considered to be the third most frequently affected organ. Involvement is evidenced by gland hypertrophy with damage to both the cortex and the medulla; in addition, 10% of the patients may present Addison syndrome. When cortisol is measured at basal and post–adrenocorticotropic hormone stimulation times, diminished adrenal glands reserve is detected in 48% of the assessed patients.[141,142] Autopsy studies, however, have shown that adrenal glands involvement may occur in up to 85% of cases. Prompt initiation of antifungal therapy may restore adrenal gland function.[26,39,70,125,143]

Other Lesions

Paracoccidioidomycosis also involves the CNS in 9.6% to 25.4% of the cases, with formation of cerebral meningeal lesions and spine involvement with spondylodiskitis. Lesions are more frequent in the cerebral hemispheres (69%), with multiple granulomas in 65% of the patients, characterized by nodular or annula, contrast-enhancing hypodense images.[144–146]

Other organs affected are liver, spleen, pancreas, and adrenal glands, thus simulating malignant neoplasia and abnormalities of vascular structures, bones, and bone marrow, each one producing its corresponding clinical manifestations.[38,125,132,134–136,141] Septic shock may present a challenging diagnosis.[147] Gallium scanning, computerized tomography, MRI, and necropsies have revealed unexpected lesions in all organs and systems, reflecting the mycosis' disseminated nature as revealed in a study of 26 patients, 69.2% of whom showed these abnormalities.[67,70,148]

PARACOCCIDIOIDOMYCOSIS IN HUMAN IMMUNODEFICIENCY VIRUS–INFECTED PATIENTS

The prevalence of paracoccidioidomycosis in immunocompromised patients with AIDS is relatively low and occurs more frequently in advanced AIDS patients. The study of Chiarella and coworkers[149] analyzed the function of CD4 and CD8 T cells in the immunity of mice infected with *P. brasiliensis* and showed that CD8 cells play an important role in pulmonary immunoprotection. In Brazil 4% to 5% of patients with paracoccidioidomycosis exhibit HIV infection as a comorbidity.[150] Use of the gp43 intradermal test has shown that 12% of HIV patients were reactive, demonstrating coinfection with *Paracoccidioides* spp. Nonetheless in rural areas of the same region, the prevalence of this coinfection was higher, at 47%.[151,152] Clinical and epidemiologic characteristics in this comorbid association differed concerning younger age at diagnosis (45.3 vs. 33.5 years), lesser involvement in agriculture-related occupations (59.4% vs. 27.5%), and a lower male : female ratio (9.6 : 1 vs. 4.3 : 1), as well as the time needed to establish the diagnosis (±30 days after initiation of symptoms).[151,153]

Skin lesions are extremely common (94%) compared with non-AIDS counterparts (38%). Other symptoms reported included cough and dyspnea in half of the patients, even in those with no radiographic abnormalities.[139,151,153] Also, these patients more often had fever, hepatosplenomegaly, and mucosal lesions in a manner resembling the subacute form of the mycosis, one that has the characteristics of an opportunistic disease.[139]

In patients with HIV, and according to the series, mortality varied between 3% and 53%, depending on the presence of alcoholism or tuberculosis. The HIV group with the stated complications had a higher relapse probability (30%) than those without these complications, both at 12 months (9.4% vs. 1.4%, respectively) and 24 months (18.5% vs. 3.3%, respectively) after treatment.[151,153,154]

At the time of diagnosis of paracoccidioidomycosis, most of the comorbid patients had overt AIDS (CD4 counts <200 cells/mL, viral copies >100,000/mL). Forty percent of them had received trimethoprim-sulfamethoxazole (TMP-SMX) treatment for pneumocystosis and toxoplasmosis prophylaxis.[151,153] This finding indicates that prevention of paracoccidioidomycosis with this medication had proved ineffective.

In these cases treatment follows the same schemes used for the non-HIV patients, except that maintenance therapy should be required up to the time when CD4 counts are consistently greater than 200 cells/mm^3 and viral copies become undetectable through administration of

TABLE 267.1 Antifungal Therapy for Paracoccidioidomycosis

ANTIFUNGAL PRESCRIBED (ADMINISTRATION ROUTE)	MINIMAL TREATMENT DURATION BASED ON ORGAN INVOLVEMENT[a]	DOSE	ADVERSE EFFECTS	RESPONSE (%)	RELAPSES (%)
TMP-SMX (PO/IV)	Minor: 12 mo Moderate: 18–24 mo	*Adults* TMP: 160–240 mg/day SMX: 800–1200 mg/day, divided into two doses per day *Children* TMP: 8–10 mg/kg/day, divided into two doses per day	Leukopenia Hypersensitivity reactions, such as rash	80	20
Amphotericin B deoxycholate (IV)[b]	Until patient improves and can be treated by the oral route and achieves a total dose of 2 g[c]	1 mg/kg/day	Nephrotoxicity Hypokalemia Nausea/vomiting Fever Anemia	70	25
Ketoconazole (PO)	9–12 mo	200–400 mg/day	Hormonal alterations Increase in hepatic enzymes Nausea/vomiting	90	11
Itraconazole (PO)	6–9 mo	*Adults* Loading dose 600 mg/day for 3 days; continue 200 mg/day *Children* (<30/kg and >5 yr) 5–10 mg/kg/day	Nausea/vomiting ↑Hepatic enzymes Drug interactions	94–98	3–5
Voriconazole (PO/IV)	6 months	Initial dose: 400 mg each 12 h for one day, then 200 mg each 12 h Diminish the dose to 50% if weight is <40 kg	Visual alterations ↑Hepatic enzymes Skin rash Photosensitivity Hallucinations Periostitis	88	No data

[a]Shown only with the aim of guiding therapy. Total duration must be defined in accordance with clinical and immune-based tests, as well with the mycosis clinical form.
[b]Other formulations can be used.
[c]Should always be continued with TMP-SMX or itraconazole oral therapy, once clinical improvement has been obtained.
IV, Intravenous; *PO,* orally; *TMP-SMX,* trimethoprim-sulfamethoxazole.

antiretroviral therapy (ART). In most cases this approach requires 18 to 24 months of treatment to curtail relapses.[151,153]

Initiation of ART may result in worsening of the mycosis' clinical symptoms because of the immune reconstitution inflammatory syndrome, one that can be treated successfully with corticosteroids.[155]

PARACOCCIDIOIDOMYCOSIS AND CANCER

Paracoccidioidomycosis can mimic neoplasia, such as colon cancer and CNS malignant tumors. Therefore, in endemic areas, the mycosis must be considered in the differential diagnosis of intraabdominal and cerebral tumors.[156] The cellular immune dysfunction known to occur in patients with active paracoccidioidomycosis may be connected to the occurrence of associated cancers. This has been postulated to be the result of the prolonged effect of fungal antigens on epithelial cells and mononuclear phagocytes, with the subsequent alterations of cellular surveillance hindering malignant transformation.[156] In 58% of the cases both diagnoses were established simultaneously.[156]

Paracoccidioidomycosis can be considered an opportunistic disease in patients undergoing chemotherapy or receiving high levels of corticosteroids. In such patients the mycosis presents in its subacute form.[156] The association between organ transplantation and paracoccidioidomycosis is rare, and in an unusual clinical presentation, a Brazilian kidney transplant recipient developed skin lesions and a cervical mass that on biopsy were indicative of paracoccidioidomycosis.[157]

DIFFERENTIAL DIAGNOSIS

The most important differential diagnosis is tuberculosis, a disease that may coexist with the mycosis in 8% to 13% of patients.[158] Other diseases that should be considered are cancer and neoplastic disorders (including lymphoma), histoplasmosis, leishmaniasis, leprosy, and syphilis. Only laboratory testing establishes the correct diagnosis.[36,38,136,156,159–161]

TREATMENT

Because of the systemic nature of the mycosis, consideration should be given to patient's general status, target organs and systems, and also to the form of presentation (subacute, chronic). Existence of comorbidities, such as tuberculosis, AIDS, and cancer, should be determined and managed accordingly. Treatment should include supportive general measures, such as bed rest, anemia correction, and suspension of tobacco use and alcohol intake, as well as balanced nutrition. Associated clinical complications should also be treated.[b]

Antifungal therapy is the mainstay for patient improvement, with three groups of medications being available: sulfonamides, amphotericin B, and azole derivatives (Table 267.1).

Sulfonamides

In some countries TMP-SMX is the preferred medication because of its low cost, facility for oral or parenteral use, plus being administered free of cost by governmental health systems. The rate of response is high, but treatment should be prolonged for 2 or more years, and the probability of relapse is high.[39,53,125,133,163]

Amphotericin B

This polyene is to be given intravenously and is reserved for patients with severe disease, for those who do not tolerate the oral route, for women in their first 12 weeks of pregnancy, and also for patients with fourfold increases in hepatic enzymes. Cumulative total dosages for the deoxycholate formulation vary from 1 to 2 g. Amphotericin B is not fungicidal in vivo, and all patients thus treated should also receive maintenance sulfonamide or azole therapy to avoid relapses. There has been little experience with the lipid formulations of amphotericin B, largely based on cost and limited availability.[39,125,133–135]

Azole Derivatives

Ketoconazole for oral administration has proven effective, but its hepatotoxicity and multiple interactions with other medications argue against its use. Among the triazoles, ITC is the preferred medication for treatment of patients with both minor and moderate clinical manifestations. It is also recommended for maintenance therapy in severe cases, once the

[b]References 39, 61, 67, 132, 133, 162.

initial amphotericin B course has been concluded. The advantages of ITC include shorter treatment periods (6 months), relapse rates lower than with other medications, and oral administration when renal function is impaired. The addition of corticosteroids has been advocated for certain patients.[164]

Voriconazole or fluconazole can be used successfully in patients with neuroparacoccidioidomycosis because both have better penetration into the CNS than other drugs.[133,162,165] Alternative triazoles have been incompletely evaluated in the treatment of paracoccidioidomycosis, although posaconazole and isavuconazole have appeared to be effective.[166]

Experimentally, the combined ITC and pentoxifylline therapy resulted in a significantly more rapid reduction of both granulomatous inflammation and pulmonary fibrosis,[167] but extrapolation to human disease has not been attempted.

Another strategy used to decrease fibrosis is by augmenting the host's cellular immune responses. DNA vaccines based on heat shock proteins, such as Hsp65 from *Mycobacterium leprae,* have shown prophylactic and therapeutic effects in experimental models of diseases, including tuberculosis, leishmaniasis, and paracoccidioidomycosis. In the last of these, decrease in fungal burden and changes in the granulomatous inflammatory reaction were noticed in vaccinated animals, with smaller and more mature granulomas and a reduction in fibrosis formation.[168,169] Until now, none of the experimental strategies just described has been evaluated in a clinical trial.

Despite efficacious antifungal therapy, latent foci with viable *Paracoccidioides* spp. fungal cells, may remain latent in tissues, requiring appropriate cellular immune responses to contain fungal proliferation.

Immune Stimulation

Patients treated with a combination of β-glucan and an antifungal agent exhibited better improvement in terms of clinical manifestations and showed, additionally, an increase in both the number of $CD4^+$ lymphocytes and TNF-α.[170]

Criteria of Recovery

1. Clinical: When the signs and symptoms of disease are no longer present and normalization of the erythrocytes' sedimentation rate is observed.[126]
2. Mycologic: There are negative results when attempts at detecting *Paracoccidioides* spp. in clinical samples are negative in those sites where it had been previously detected.[126]
3. Radiologic: Evidence is apparent when the lung's radiologic pattern remains stable after treatment, for instance, the same scarring lesions are found on five different radiographs taken every 3 months in the course of 1 year.[126]
4. Immunologic: The serum levels of specific antibodies decrease after the onset of treatment, and these levels become stabilized at a very low concentration on the complement fixation test.[126] Detection of antigens as a control measure may be required in special patients.[126,171]

Relapse is defined as the reappearance of the mycosis in patients who have remained in a state of clinical, radiologic, and serologic recovery for 2 years after discontinuation of antifungal treatment.[126]

LABORATORY DIAGNOSIS

Diagnostic tests are oriented toward visualization of the causative fungus in clinical samples or its isolation in culture.[3,15,53,143,159–161] Measurement of antibodies or detection of antigens in patients' body fluids also constitute valid indirect diagnostic criteria.[159,161,171–173]

P. brasiliensis visualization is not difficult to attain, and procedures such as fresh preparations, wet potassium hydroxide (KOH) mounts, calcofluor whitestaining, plus histopathologic studies have proven adequate.[15,38,39] It should be stated, however, that prompt diagnosis is hindered by the lack of awareness on the part of physicians.

Direct Examination

The sensitivity of direct examinations, mostly KOH preparations, varies from 85% to 100%, depending on the test, specimen, clinical manifestations, and patients' treatment status. In specimens such as sputum, exudates, bronchial washings, and pus, a simple wet mount suffices to reveal *P. brasiliensis* in greater than 85% of the cases.[c] If results are negative, repeated samples should be collected, mucous specimens digested, and samples concentrated by centrifugation. The relatively large size of the fungal yeasts, their translucent walls, and their multiple budding facilitate diagnosis.[d] On occasion, however, single buds and small yeast cells may be confused with other fungi, requiring more extensive observations. Direct *P. brasiliensis* DNA detection by the polymerase chain reaction (PCR) in exudates, tissues, and strain cultures appears promising.[174,175] On the other hand, the value of this procedure is questionable in sera.[176] Mention has been made of cytology as an appropriate diagnostic procedure.[177]

Histopathology

Biopsy is very often diagnostic, with the Grocott-Gomori silver stain being recommended. If the typical multiple-budding yeasts are not abundant, distinction from other fungi (e.g., *Blastomyces dermatitidis, Histoplasma capsulatum, Cryptococcus neoformans*) is necessary.[3,15,125,143] Frequently, with hematoxylin and eosin stains, infected tissues reveal a mixed inflammatory reaction characterized by the presence of granulomas centered on the yeasts, some of which had been phagocytized (Fig. 267.6). The granuloma is further characterized by the presence of neutrophils, mononuclear cells, and epithelioid cells, as well as multinucleated giant cells, with the latter constituting the hallmark of granulomatous inflammation. All these cells appear arranged concentrically around the yeasts in an effort to confine the infectious process.[3,26,30,38,107] Skin and oral lesions reveal pseudoepitheliomatous hyperplasia and intraepithelial microabscesses.[3,38,53,107,108] In the juvenile form, where cutaneous lesions are common and usually multiple,[61] tissue reactions are diffuse and phagocytosis is sparse, with lymph nodes exhibiting hyperplastic germinal centers and increased numbers of plasmocytes, a demonstration of the host's unsuccessful efforts to cope with the invading fungus.[61,107–109,143]

Cultures

Isolation of *P. brasiliensis* in culture proves active disease, but positive cultures are obtained only in 85% of cases and require 20 to 30 days for growth to occur.[e] Various culture media, such as Mycosel agar (BBL [Beckton Dickenson; Franklin Lakes, NJ]) and Sabouraud agar plus antibiotics and the fungal inhibitor cycloheximide, should be used and incubated preferably at room temperature (20°C–25°C).[26,38] Colonies thus obtained require temperature-mediated transformation to the yeast form for confirmation. Direct incubation of samples at 37°C carries the risk of contamination, especially in specimens rich in normal flora, for instance, respiratory secretions. Primary isolation often requires processing multiple samples (e.g., sputum) and using a variety of culture media.[26,38]

Immune-Based Diagnostic Tests

The laboratory diagnosis of paracoccidioidomycosis is achieved through several types of immune-based tests that have specific uses and limitations.[159–161] Antibody and antigen detections are valuable adjuncts to histopathology and culture. More recently, detection assays have used as antigens isolated fractions derived from purified fungal proteins, such as gp43, gp70, and the 87-kDa heat shock protein, thus improving reproducibility and facilitating monitoring antigen clearance during antifungal treatment.[171–173,178]

These tests aim to detect not only anti–*P. brasiliensis* antibodies in serum, cerebrospinal fluid (CSF), and bronchoalveolar lavage fluid[3,26,44,159–161,179] but also to determine the presence of fungal antigens in tissues and body fluids.[171–173,178] As such, they are useful not only for diagnosis but also for follow-up studies.[159,178] The simplest of the antibody detection methods, the agar gel immunodiffusion test, demonstrates circulating antibodies in greater than 90% of active patients, indicating that, if reactive, this test strongly supports the diagnosis; however, antibodies can also be detected years after apparently successful therapy.[3,26,159–161] In Brazil 15% of the healthy population residing in an endemic region was shown to have circulating anti–*P. brasiliensis* antibodies indicative of subclinical infections.[179] Another useful

[c]References 3, 15, 26, 44, 53, 159, 161.
[d]References 3, 15, 44, 47, 53, 159, 161.
[e]References 3, 15, 44, 53, 160, 161.

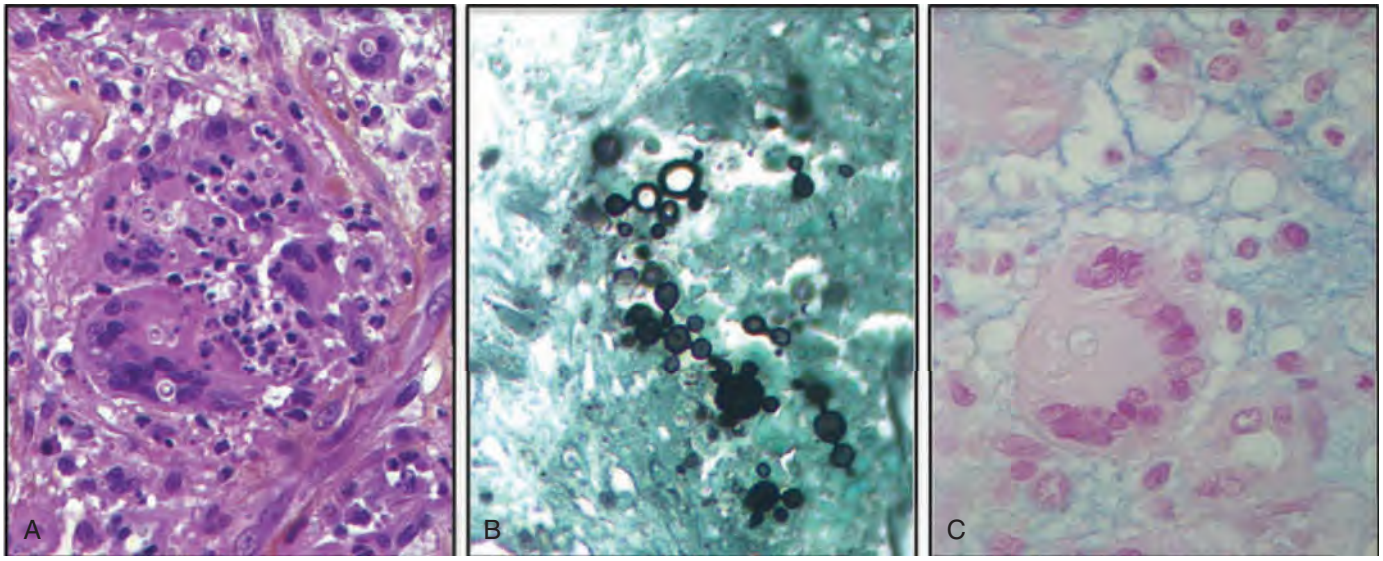

FIG. 267.6 Paracoccidioidomycosis histology. (A) Yeast cells in tissue exhibiting multiple budding and surrounding mononuclear infiltration (hemotoxylin and eosin, ×400). (B) Typical multiple-budding yeast cells (black staining) in a tissue sample (Grocott-Gomori methenamine silver, ×400). (C) Multiple-budding yeast cells inside a multinucleated giant cell in tissue (periodic acid–Schiff, ×400).

procedure, albeit a cumbersome one, is the complement fixation test because its quantitative nature allows a more precise evaluation of the patient's response to treatment. In this test, and in contrast to the immunodiffusion procedure, cross-reactions with *Histoplasma capsulatum* antigens are important.[3,39] Other tests, such as immunofluorescence, counterimmunoelectrophoresis, dot blot test, enzyme-linked immunosorbent assay, and immunoblotting, are also used.[159,161] Improvements in serodiagnosis include the detection of antibodies against chemically characterized or recombinant *P. brasiliensis* antigens, notably gp43, pb27, gp70, and the 87-kDa heat shock protein, with a combination of two recombinant products resulting in increased sensitivity (92%) and specificity (88%).[171–173] Of interest, the gp43 antigen used in diagnostic tests has excellent accuracy in patients infected with *P. brasiliensis* but has low sensitivity in diagnosing *P. lutzii* infections.[126] More recently, use of subtractive phage display with synthetic peptides was applied to the diagnosis of this mycosis with valuable results.[180]

Antigen detection in blood may be preferred for early diagnosis in immunocompromised individuals or when antibody detection appears inconclusive; greater than 60% of the active patients react in the antigenemia test.[44,171,178] Monitoring circulating antigens is also important as a criteria of recovery, whereas parallel testing for antibody levels in the same patients has revealed that titers are rather unpredictable and may persist in those who have cleared all active signs and symptoms of the mycosis.[171,178]

Molecular Testing

Several molecular diagnostic methods, such as PCR, nested PCR, PCR-enzyme immunoassay, real-time (RT) PCR, and loop-mediated isothermal amplification have been successfully used to detect specific *P. brasiliensis* DNA sequences.[172,174,175,181] The preferred target sequences have been the gp43 protein and the ribosomal RNA genes, which have been amply evaluated by means of DNA from cultures, sputum, CSF, and blood from paracoccidioidomycosis patients, as well as from tissues obtained from infected mice.[172,174,175,181] The PCR appeared to be specific and sensitive due to its low detection limit (1.1 pg/μL of DNA) in clinical specimens but not in sera.[176,181] There is no commercially available PCR kit, nor is there a molecular method established for the diagnosis of this mycosis. Nonetheless, a RT-PCR using species-specific primers and a probe that amplifies the Pb27 protein gene is under patent application.[182]

Skin Tests

Paracoccidioidin skin testing is not reliable for diagnosis, mainly because availability of standardized products that meet public health standards do not exist. In addition, it is known that at the time of diagnosis, an important number of active cases (<35%) are proven nonreactive, rendering the test unsatisfactory.[65] Cross-reactions with the histoplasmin intradermal test occur, although the use of the purified gp43 antigen appears to be more specific.[183]

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268 Uncommon Fungi and Related Species

Duane R. Hospenthal

SHORT VIEW SUMMARY

SCEDOSPORIUM APIOSPERMUM (*PSEUDALLESCHERIA BOYDII*) SPECIES COMPLEX

Definition
- Infection of the lungs, bones and joints, or central nervous system (CNS); may be disseminated.
- Also causes mycetoma (see Chapter 261).

Epidemiology
- Typically occurs in the immunocompromised or following trauma.
- CNS infection in immunocompetent persons after near drowning.
- Organism can be found in soil and fresh water, especially stagnant or polluted.

Microbiology
- *Scedosporium apiospermum, Scedosporium boydii* (formerly *Pseudallescheria boydii*), and *Scedosporium aurantiacum* are the most common species infecting humans.
- Identification is typically made by DNA sequencing. Formerly, identification was based on microscopic structures of organism in culture.

Diagnosis
- Diagnosis is made by culture recovery from infected site.
- Because *S. apiospermum* complex species may colonize airways, sputum cultures may not reflect infection.

Therapy
- Voriconazole is likely the most effective agent.

LOMENTOSPORA (SCEDOSPORIUM) PROLIFICANS

Definition
- Disseminated infection and bone and joint infections are most common.
- *Lomentospora* (formerly *Scedosporium*) *prolificans* may cause onychomycosis and infections of the eye and wounds.

Epidemiology
- Disseminated infection commonly occurs in the severely immunocompromised.
- Localized infection occurs in immunocompetent individuals after trauma.
- The organism can be found in soil and colonizing the respiratory tract, especially in patients with cystic fibrosis.

Microbiology
- Identification is made by culture.

Diagnosis
- Diagnosis is made by culture recovery from the infected site.
- Because *L. prolificans* may colonize airways, sputum cultures may not reflect infection.

Therapy
- No effective therapy. Consider voriconazole with amphotericin B.

DARK-WALLED FUNGI (*BIPOLARIS, EXOPHIALA, EXSEROHILUM, PHIALOPHORA, OCHROCONIS, CURVULARIA,* OTHERS)

Definition
- This infection involves fungi that have melanin in their cell walls and may appear dark walled in tissue.
- Infection is often termed "phaeohyphomycosis" and typically presents as localized skin and soft tissue infections, CNS infections, or allergic sinusitis.
- Dark-walled fungi that cause chromoblastomycosis (see Chapter 260) and mycetoma (see Chapter 261) are not included in the agents of phaeohyphomycosis.

Epidemiology
- Infection is commonly acquired from minor trauma or inhalation.
- Fungi are found in soil, organic material, plants, and air.
- They may be spread through contaminated products (e.g., injectable steroids).

Microbiology
- The most common agents of phaeohyphomycosis are *Alternaria, Bipolaris, Cladophialophora, Curvularia, Exophiala, Exserohilum, Ochroconis,* and *Wangiella.*

Diagnosis
- Diagnosis is made by recovery of these organisms in culture from the site of infection.
- Cell walls may appear dark brown or golden on histopathology (hematoxylin and eosin). Use of a Fontana-Masson stain may allow easier identification of these fungi.

Therapy
- Surgical débridement of lesions or colonizing fungi in the case of allergic fungal sinusitis.
- Amphotericin B in life-threatening infections.
- Voriconazole and itraconazole are typically effective but prolonged therapy is needed.

FUSARIUM SPP.

Definition
- Can cause disseminated infection in immunocompromised patients.
- Common cause of keratitis and other eye infections in contact lens wearers and following trauma.
- Skin and soft tissue infection after trauma, onychomycosis; can cause mycetoma.

Epidemiology
- Common plant pathogens; found in soil and organic debris.
- Have been recovered in hospital water supplies.

Microbiology
- The most common species infecting humans belong to one of three species complexes: *Fusarium solani, Fusarium oxysporum,* or *Fusarium fujikuroi,* although the number of species identified as causing infection is increasing as molecular methods of identification have supplanted morphology.
- *Fusarium* produces banana- (or crescent)-shaped multicellular macroconidia in culture.

Diagnosis
- Recovery of the fungus from culture of an otherwise sterile site.
- One of the few molds that are commonly recovered from blood culture.

Therapy
- Optimum therapy is not known.
- Recovery from neutropenia is essential in response to therapy of disseminated infection.
- Amphotericin B or voriconazole is suggested.

TRICHOSPORON SPP.

Definition
- Most commonly presents as disseminated infection.

Epidemiology
- Typically an infection of the immunocompromised; may be associated with central venous catheter.
- Fungi found colonizing the skin, gastrointestinal, respiratory, or genital tract.
- Found in soil and water.
- Breakthrough infections in patients receiving echinocandins.

Microbiology
- Most common pathogen is *Trichosporon asahii.*

SHORT VIEW SUMMARY—cont'd

- *Trichosporon* are identified by their unique ability to produce septate hyphae, arthroconidia, and budding yeast.
- Appears yeastlike on initial culture.

Diagnosis
- By recovery of the organism from the blood or lesion biopsy.

Therapy
- Azole antifungal (voriconazole, itraconazole, isavuconazole, posaconazole, or fluconazole).

MALASSEZIA FURFUR

Definition
- Catheter-related bloodstream infection.
- Also causes pityriasis versicolor (see Chapter 266).

Epidemiology
- Typically associated with parenteral lipid infusion.
- Commonly reported in neonates.

Microbiology
- May be difficult to grow from positive blood cultures without lipid supplementation.

Diagnosis
- Recovery of fungus from blood culture.

Therapy
- Catheter removal and discontinuation of parenteral lipids.
- Susceptible to most antifungals (voriconazole, fluconazole, or amphotericin B).
- Limiting use of parenteral lipids.

OTHER UNCOMMON YEASTS

Definition
- Other less common yeasts may also cause catheter-related bloodstream infection.

Epidemiology
- Typically associated with use of central venous catheters and immunocompromise.

Microbiology
- Include *Magnusiomyces capitatus* (formerly called *Saprochaete capitata* and *Blastoschizomyces capitatus*), *Pichia anomala, Rhodotorula* spp., and *Saccharomyces cerevisiae.*

Diagnosis
- Made by recovery of the yeast in blood culture.

Therapy
- Removal of central venous catheter.
- Antifungals based on recovered yeast.

TALAROMYCES (PENICILLIUM) MARNEFFEI

Definition
- Acute disseminated infection of persons infected with human immunodeficiency virus (HIV) in Southeast Asia.
- Infection similar to acute disseminated histoplasmosis in patients with acquired immunodeficiency syndrome.
- Rarely, localized infection in apparently immunocompetent persons.

Epidemiology
- Limited to Southeast Asia. More common in rainy season, in young adult males with HIV infection, typically with low CD4 cell counts.

Microbiology
- Typical *Penicillium*-like structures on microscopic examination of culture.
- Cultures produce red pigment that diffuses into the agar.

Diagnosis
- Typically made from recovery of the organism in blood.
- May also be recovered in culture of skin lesions, lymph nodes, or bone marrow aspirates.
- Serologic testing may be available in endemic regions.

Therapy
- Amphotericin B in life-threatening presentations.
- Itraconazole or voriconazole in initial therapy (not life-threatening).
- Itraconazole secondary prophylaxis in HIV-infected patients.

LACAZIA LOBOI

Definition
- Chronic nodular or keloidal skin infection, commonly of the ears or face.

Epidemiology
- Limited to Central and South America.
- Infection also found in dolphins.

Microbiology
- Has not been recovered in culture.
- Identified as closely related to *Paracoccidioides brasiliensis* by molecular techniques.

Diagnosis
- Based on clinical presentation and finding typical structures on histopathology.
- Globose (yeast) cells end-to-end in short "strings."

Therapy
- Surgical removal.

AGENTS OF ADIASPIROMYCOSIS (*EMMONSIA* SPP.)

Definition
- Disease secondary to host immune response to nonreplicating fungal conidia, termed "adiaspores."
- Chiefly a pulmonary disease; may range from asymptomatic to rapidly progressing respiratory failure and occasionally death.
- May also present as ocular nodules.

Epidemiology
- Seen with occupational inhalation of dusts in men, average age 40.
- Outbreak in Brazilian children in association with diving and freshwater sponges.

Microbiology
- Secondary to *Emmonsia* spp., usually *Emmonsia crescens.*
- Dimorphic fungi closely related to *Blastomyces dermatitidis.*

Diagnosis
- Identification is limited to observation of typical structures on histopathology showing adiaspores, up to 500 μm in diameter, nondividing, and surrounded by granulomata composed of epithelioid and giant cells.

Therapy
- Corticosteroids appear to be useful.

EMERGOMYCES AFRICANUS

Definition
- Disseminated infection most commonly afflicting HIV-infected persons.

Epidemiology
- Severely immunocompromised persons.
- Largest report from South Africa.

Microbiology
- Thermally dimorphic fungus of soil.

Diagnosis
- Recovery of the dimorphic fungus from skin biopsy or blood culture.

Therapy
- Responses to amphotericin B and triazole antifungals have been reported.

PROTOTHECA SPP.

Definition
- Localized skin or subcutaneous infection caused by algal pathogen.
- Rare reports of disseminated or deep infection.

Epidemiology
- Likely cause infection after traumatic inoculation.
- Organisms colonize skin, gastrointestinal and respiratory tracts.

Microbiology
- Disease is typically due to *Prototheca wickerhamii* or *Prototheca zopfii.*
- Unicellular algae lack chlorophyll.
- *Prototheca* spp. grow on fungal culture media with yeastlike colonial morphology.
- Microscopic appearance in tissue is diagnostic.

Diagnosis
- Recovery of algae in culture is diagnostic.
- Yeast biochemical panels commonly identify *Prototheca.*

Therapy
- Surgical excision with amphotericin B or itraconazole.

PYTHIUM SPP.

Definition
- Vascular infections in persons with iron overload, such as thalassemia, or ocular infections following trauma.

SHORT VIEW SUMMARY—cont'd

- Skin and subcutaneous and disseminated infection possible.

Epidemiology
- Worldwide distribution, but most commonly reported from Thailand.
- Risk factors: thalassemia-hemoglobinopathy syndrome and trauma.
- Presumed association with work in swampy (e.g., rice paddy) environment.
- High morbidity (eye or limb loss) and mortality.

Microbiology
- Fungus-like protist, *Pythium* grows rapidly as mold on fungal culture.

Diagnosis
- Recovery of the organism from culture is diagnostic.
- Local serologic testing may be available.

Therapy
- Can perform surgical resection or amputation.
- No satisfactory medical therapy is known.
- Doxycycline, minocycline, tigecycline, linezolid, and macrolides have activity in vitro.

RHINOSPORIDIUM SEEBERI

Definition
- Localized polypoidal infection, chiefly of the nose, upper airway, and conjunctiva.

Epidemiology
- Worldwide distribution, but most commonly reported from southern India and Sri Lanka.

Microbiology
- Has not been grown in culture.
- Identified as a protist by molecular methods.

Diagnosis
- Made by microscopic observation of typical structures: thick-walled cysts (100–350 μm) filled with numerous spores.

Therapy
- Surgical excision with electrocautery of lesion base.

SCEDOSPORIUM APIOSPERMUM (PSEUDALLESCHERIA BOYDII) SPECIES COMPLEX

In humans, infection with *Scedosporium apiospermum, Scedosporium boydii* (formerly *Pseudallescheria boydii*) *and Scedosporium aurantiacum* (collectively, the *S. apiospermum* species complex)[1,2] can produce two distinct diseases: mycetoma and scedosporiosis (pseudallescheriasis). Mycetoma is a chronic subcutaneous infection characterized by the production of grains (see Chapter 261), whereas scedosporiosis includes all other infections caused by *Scedosporium* spp. The most common sites of *Scedosporium* infections are lung, bone, joints, and the central nervous system (CNS).[3,4] Sinusitis, keratitis, endophthalmitis, skin and soft tissue infections, prostatitis, and endocarditis have also been described. These fungi are found in soil and fresh water, especially stagnant or polluted water, throughout the world. Disease is acquired after inhalation of this organism into the lungs or paranasal sinuses or after traumatic inoculation through the skin. There are more than a dozen reported cases of *Scedosporium*-related pneumonia after near drowning in contaminated water. Although colonization is more common than infection with this organism, an invasive pulmonary disease similar to invasive pulmonary aspergillosis occurs, usually in immunocompromised patients. Local trauma is the most common cause of eye, soft tissue, and osteoarticular infections in previously healthy persons. CNS infection is seen in both immunocompromised and healthy individuals.[5] Infections in immunocompetent patients usually have subacute to chronic courses, whereas those in immunocompromised patients are frequently acute and severe.

Scedosporium can colonize bronchiectatic lungs, including those of patients with cystic fibrosis, or intermittently obstructed paranasal sinuses. Masses of hyphae (fungus balls) have been found in lung cavities.[6] *Scedosporium* has also been reported as a cause of allergic bronchopulmonary disease (similar to allergic bronchopulmonary aspergillosis),[7] pleural space infection, lung abscess, pneumonia (including aspiration pneumonia), and invasive sinusitis.[8] As with invasive pulmonary aspergillosis, invasive pulmonary scedosporiosis most commonly occurs in patients with prolonged neutropenia, those receiving prolonged high-dose corticosteroid therapy, or those who have undergone allogeneic bone marrow transplantation.[9] Invasive pulmonary disease with associated dissemination appears common[10] and has also occurred in patients with acquired immunodeficiency syndrome (AIDS) and after solid-organ transplantation.[11] Pulmonary disease in severely immunocompromised patients usually manifests with fever, cough, pleuritic pain, and often hemoptysis. Chest radiography may show areas of nodularity, alveolar infiltrates, consolidation, or cavitation.[6] The classic signs of invasive pulmonary aspergillosis, the halo or air crescent signs, may also be seen in invasive pulmonary scedosporiosis. Disseminated disease that manifests with only painful cutaneous nodules or endophthalmitis has also been described in immunocompromised patients.[12,13] Invasive pulmonary disease with extension to the vertebrae has been described in a patient without apparent immunocompromise.[14]

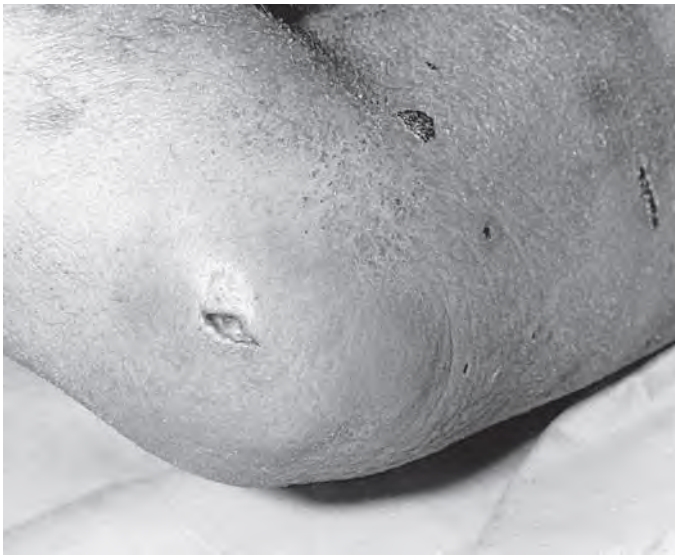

FIG. 268.1 *Scedosporium* (formerly *Pseudallescheria*) *boydii* olecranon bursitis in a corticosteroid-treated patient who fell on his elbow in the garden. Photograph shows the incision site over the subcutaneous abscess, which began in the bursa.

Localized disease—including infections of the eye, bone, cutaneous tissue, subcutaneous tissue (Fig. 268.1), and osteoarticular tissue—may be seen both with and without immunocompromise. Infection is commonly initiated through traumatic implantation of the fungus from soil or water. Surgery, intravenous drug injection, and repeated corticosteroid injections have less frequently been associated with localized infections.[15] Osteoarticular infection in immunocompetent patients often manifests as a painful, swollen joint with overlying erythema after penetrating injury. In occasional patients, weeks to even years may pass between antecedent trauma and the development of septic arthritis.[16,17]

Brain abscesses may result from a known or unsuspected lung lesion in immunocompromised patients, including those with AIDS.[5,18] CNS infection appears to be disproportionately prevalent among patients infected with *Scedosporium* spp., in comparison with many other mycoses. For example, of 23 solid-organ transplant recipients with *Scedosporium* spp. infections, 11 (48%) had CNS involvement.[11] Cerebral abscesses are usually multiple; in immunocompetent hosts, they are often reported in association with near drownings in polluted water, such as ponds, pig troughs, and roadside ditches.[5] CNS infection from contiguous spread of sinusitis[19] and after penetrating trauma[20] has also been described. Indolent, severe neutrophilic meningitis has been reported occasionally, usually in patients with intravenous drug abuse or human immunodeficiency virus (HIV) infection. Cerebrospinal fluid culture

and smear have yielded negative results; the diagnosis was made at autopsy. The first described human case of *Scedosporium* spp. was meningitis that was probably iatrogenic after lumbar puncture for the administration of anesthesia.[21]

Isolation of *Scedosporium* spp. from normally sterile sites is diagnostic. Only rarely are *Scedosporium* spp. cultured from blood.[4] Growth of the organism from sputum, bronchoalveolar lavage, draining wounds, or paranasal sinus aspirates is less convincing evidence of infection, unless it is accompanied by hyphae on smear or biopsy. Histologically, *Scedosporium* spp. resemble *Aspergillus* spp., with dichotomously branching septate hyphae seen in tissue, although branching off to the side at a 60- to 70-degree angle, instead of Y-shaped dichotomous branching, is more common in scedosporiosis.[22] Air-containing spaces, such as a paranasal sinus, can permit sporulation. In addition to hyphae and conidia, distinctive coremia or an ascocarp may indicate *Scedosporium* as the mold. In neutropenic patients, blood vessel invasion and thrombosis are usual. The fungus grows well in standard mycologic media. After a few days, the mold colony takes on a tan color and has sporulating structures that are quite different from those of *Aspergillus.* Cultures can produce either asexual conidia or the sexual reproductive structure, cleistothecia. Identification is usually by DNA sequencing, but matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is being used increasingly for identification of the mold.

Effective therapy of scedosporiosis remains elusive. In vitro and clinical resistance to amphotericin B, as well as breakthrough infections, have been reported repeatedly. Surgical débridement has been an important adjunct in treatment of soft tissue, bone, joint, and pleural and paranasal sinuses, although it is not curative by itself. Intraarticular instillation of amphotericin B may have contributed to success of treatment in a few patients. The rate of mortality with brain abscess has traditionally been noted to exceed 75%.[5] Susceptibility to azoles and echinocandins in vitro has proven variable.[3,23–25,26,27] Voriconazole has the best activity in vitro and is often considered the drug of choice, but reducing the level of immunosuppression is vital.[28,29,30,31] Response to posaconazole was achieved in a single reported case of brain abscess.[32] Voriconazole is approved by the US Food and Drug Administration (FDA) for patients with *Scedosporium* spp. infection refractory to other approved antifungal agents and for patients who cannot tolerate those agents. This indication was based on success reported in 15 of 24 patients treated with this agent (including 6 of 10 with CNS infection). Because of poor response to the only approved agent, amphotericin B, most experts believe that voriconazole is the drug of choice in the treatment of scedosporiosis.

LOMENTOSPORA (FORMERLY SCEDOSPORIUM) PROLIFICANS

Lomentospora prolificans (formerly *Scedosporium prolificans* or *Scedosporium inflatum*), a fungus found in soil, was first described in 1984 as an agent of human disease.[33] Since that time at least 161 additional cases have been reported. Infection can occur in both immunocompromised and immunocompetent patients. Patients with intact immunity most frequently have focal infections (usually osteoarticular) associated with trauma, whereas immunocompromised persons most frequently have disseminated disease, associated with malignancy.[34] In one review, only 34 of 162 patients (21%) were noted to have no underlying disease; 72 of the 162 patients (44%) had disseminated infection.[34]

In immunocompetent patients, infection is usually localized and associated with trauma, including surgery.[34–36] These cases have included infections of bone and joints (Fig. 268.2), eye, or wounds and onychomycosis. Immunocompromised patients, commonly those undergoing cytoreductive chemotherapy or bone marrow transplantation, present with fungemia and fever with neutropenia.[9,10,37,38] Skin lesions, myalgia, endophthalmitis, endocarditis,[39] and pulmonary infiltrates have been described in this setting.[34,40] Skin lesions have been described as a papular rash, later becoming necrotic. Disseminated disease without neutropenia has been described in patients who have undergone lung and kidney transplantation.[41] Fatal localized CNS infection was reported in a child with acute leukemia who had received six intrathecal chemotherapeutic injections.[42] *L. prolificans* has also been recovered from the external ear and sputum of patients without apparent disease. Sputum colonization has been observed in patients with AIDS and cystic fibrosis and in those who have undergone liver or lung transplantation.[36,43–46]

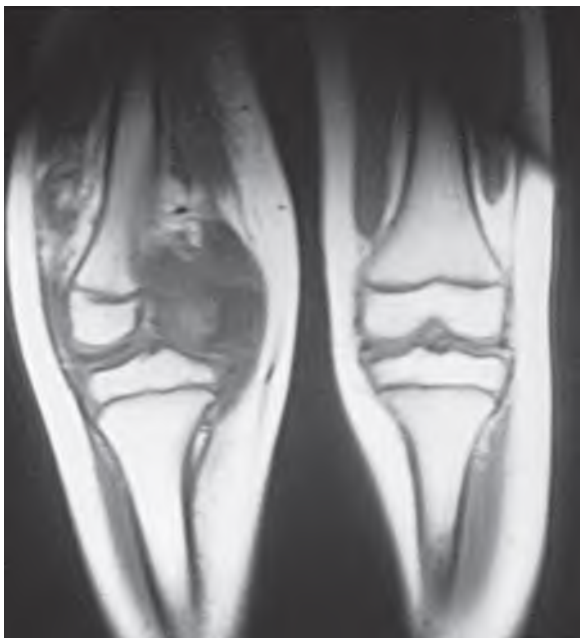

FIG. 268.2 *Lomentospora* (formerly *Scedosporium) prolificans* septic arthritis of the knee in an otherwise healthy 12-year-old boy. This condition developed in association with a splinter obtained during a playground fall. Magnetic resonance imaging depicts osteomyelitis of the medial condyle.

Diagnosis is most commonly established by the recovery of the organism from culture of infected sites, including skin biopsy samples. Disseminated disease in immunocompromised patients is usually diagnosed through blood culture.[34,38] Identification of *L. prolificans* is based chiefly on DNA sequencing or the morphologic characteristics of the asexual structures produced by the mold in culture.[47,48]

No antifungal therapy currently available is effective in treating these infections. *L. prolificans* appears to be intrinsically resistant to most antifungals.[2,3,27,31] Successful therapy of joint infections has, however, been reported with the use of surgical débridement with or without intraarticular amphotericin B. Disseminated infection is usually resistant to antifungal agents and carries a high mortality rate.[34,37] Survival was reported in one patient with disseminated disease and neutropenia who received granulocyte colony-stimulating factor (G-CSF) and amphotericin B, followed by itraconazole.[38] In one animal model, liposomal amphotericin B with the addition of G-CSF improved survival.[49] Of the currently available antifungal agents, voriconazole appears most promising in vitro, with better activity than amphotericin B, itraconazole, or posaconazole.[2,27] Unfortunately, current dosing regimens of voriconazole are not associated with serum concentrations at which the drug appears to be effective in vitro. One report described a 44% response rate (16 of 36 patients) with voriconazole.[28] The investigational azole, albaconazole (UR-9825), appears more active than voriconazole in vitro and has shown potential in one animal model.[27,50] Because of the in vitro and in vivo resistance of *L. prolificans* to currently available agents, the effect of combining agents has been examined. In laboratory studies, synergy has been shown through the use of combinations of amphotericin B with pentamidine[51] and of terbinafine with voriconazole, itraconazole, or miconazole.[52,53] Clinical support for this in vitro synergy is limited, although anecdotal experiences have been reported with voriconazole and terbinafine.[54,55]

DARK-WALLED FUNGI AND AGENTS OF PHAEOHYPHOMYCOSIS

Phaeohyphomycosis is a loosely defined term used to group infections caused by molds (and a few yeasts) that produce dark cell walls. Also described as *dematiaceous,* these are a diverse group of fungi found in

TABLE 268.1 Cutaneous and Subcutaneous Infections Caused by Dark-Walled Fungi

DISEASE	LESIONS	PATHOLOGIC FEATURES	ORGANISMS[a]
Chromoblastomycosis	Scaly, friable, often verrucous nodules, commonly pruritic	Muriform cells (golden brown cells with cross walls in more than one plane)	*Fonsecaea* spp., *Cladophialophora carrionii, Phialophora verrucosa, Rhinocladiella aquaspersa*
Mycetoma (eumycetoma, eumycotic mycetoma)	Nodular with draining sinuses, areas of healing	Grains composed of septate hyphae	*Madurella mycetomatis, Falciformispora* (formerly *Leptosphaeria*) *senegalensis, Trematosphaeria* (formerly *Madurella*) *grisea*
Subcutaneous phaeohyphomycosis	Painless, subcutaneous nodules	Septate hyphae (pseudohyphae or yeasts may also be apparent)	*Curvularia* (formerly *Bipolaris*) spp., *Exophiala* spp., *Exserohilum* spp., *Phaeoacremonium* spp., *Phialophora*

[a]The most common causes are listed. See individual chapters for more complete listings (Chapters 260 and 261).

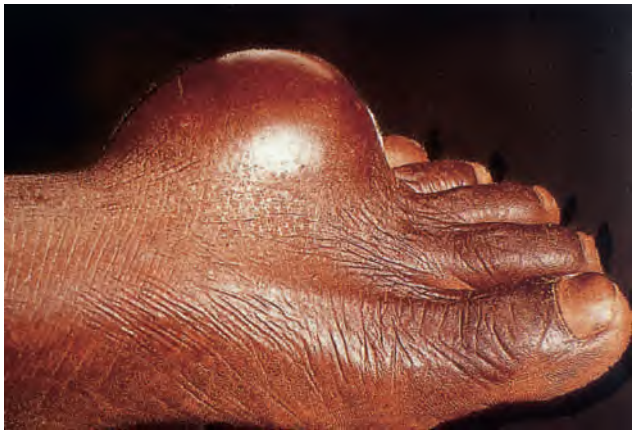

FIG. 268.3 Phaeohyphomycosis manifesting as a cyst. *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

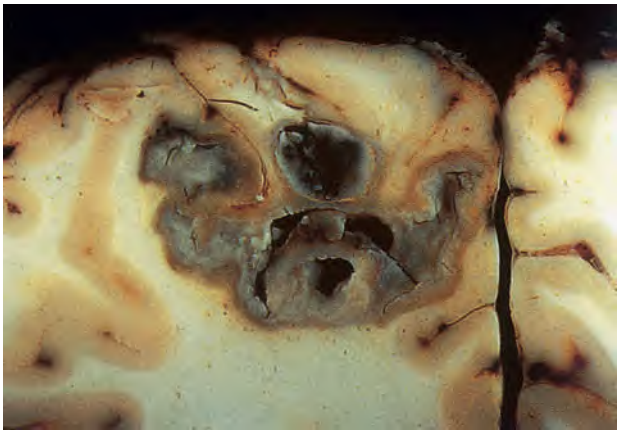

FIG. 268.4 Phaeohyphomycosis of the brain caused by *Cladophialophora* sp. *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

the soil and air and growing on plants and in organic debris. The number of genera and species of fungi causing phaeohyphomycosis is quite large.[56] Frequent changes in species names have compounded the difficulty in comparing similar cases from the literature. Chromoblastomycosis (see Chapter 260) and mycetoma (see Chapter 261) are distinct infections that include dark-walled fungi as etiologic agents that are generally not included in this loose classification (Table 268.1). The syndromes most commonly produced by the dark-walled fungi include cutaneous and subcutaneous disease (other than chromomycosis or mycetoma), brain abscesses, and sinusitis. Fungemia[57] and disseminated disease[58] have more commonly been described in immunocompromised individuals. Meningitis, pneumonia, prosthetic valve endocarditis, contamination of saline-filled breast implants, infections in peritoneal dialysis and central venous catheters, osteomyelitis, and septic arthritis have also been reported. For most clinical purposes, it is preferable to describe disease by the type of infection and species name, such as "*Cladophialophora bantiana* brain abscess," and to reserve the term *phaeohyphomycosis* for cases in which no culture data exist or in which recovered fungi have not yet been identified.

Subcutaneous phaeohyphomycosis typically begins as a single red nodule, usually on the extremities. In an immunocompetent person, an indolent, painless expansion in the skin and subcutaneous tissue occurs, sometimes with cyst formation (Fig. 268.3). More rapid local progression and, in rare cases, extension to the brain can occur in immunosuppressed patients. A history of minor trauma is often present, or a splinter is found in the resected lesion. The fungi causing subcutaneous phaeohyphomycosis are extraordinarily diverse, but species of *Exophiala, Exserohilum, Phialophora,* and *Curvularia* (which includes most clinical species formerly in the genus *Bipolaris*)[59] are particularly common.

Brain abscess is one of the best-described syndromes produced by the dark-walled fungi.[60,61] Disease manifests with headache of indolent onset, low-grade or no fever, and development of focal neurologic signs. There is rarely a history of exposure to dust or mold, no obvious pulmonary portal, and no evidence of dissemination outside the CNS.

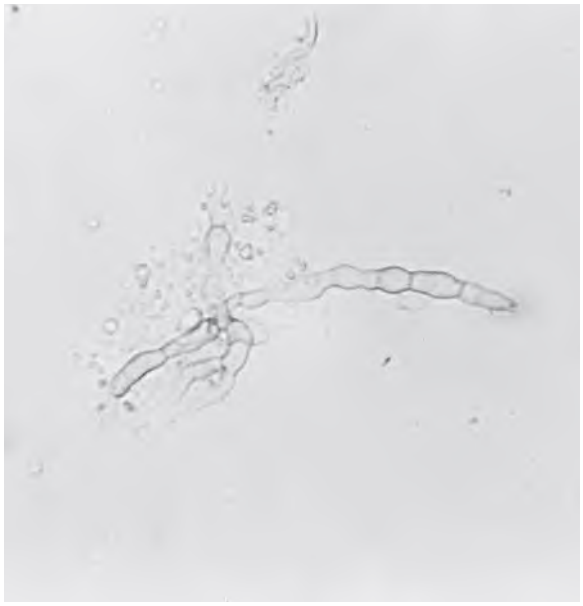

FIG. 268.5 Weakly pigmented, segmented hyphae of *Cladophialophora bantiana* in the wet mount of pus from the base of the brain.

Affected male patients have outnumbered female patients 3:1, the median age of diagnosis is 38 years, and most patients have been immunocompetent.[61,62–63] Abscesses may be single or multiple and, on computed tomography or magnetic resonance imaging, are well localized within the cerebral cortex (Fig. 268.4).[64] Purulent meningitis, with or without brain abscess, may also be observed (Fig. 268.5).[65] Hematoxylin and

eosin (H&E) staining reveals abscesses to have purulent centers with surrounding granulomatous reaction, and organisms appear as septate hyphae with golden brown cell walls. As in other forms of infections with the dark-walled fungi, hyphae are commonly irregular in diameter, and yeastlike cells are seen with some species. The species most commonly causing these infections is *C. bantiana* (previously named *Xylohypha bantiana, Cladosporium bantianum,* and *Cladosporium trichoides*), but disease is also caused by *Rhinocladiella* (formerly *Ramichloridium*) *mackenziei, Ochroconis gallopava* (formerly known as *Dactylaria constricta* var. *gallopava*), *Exophiala (Wangiella) dermatitidis, Curvularia* (formerly *Bipolaris*) *spicifera, Curvularia* (formerly *Bipolaris*) *hawaiiensis, Chaetomium* spp., and, even more rarely, other phaeohyphomycetes.[61,63,66] *R. mackenziei* infections are reported chiefly from the Middle East and India,[67,68] and *E. dermatitidis* cases predominate in the Far East. *Fonsecaea monophora* (formerly classified with *Fonsecaea pedrosoi*) has been recognized as an etiologic agent of CNS phaeohyphomycosis.[69]

Iatrogenic meningitis and other infections related to epidural injections of corticosteroids have been reported in two recent outbreaks traced to environmental contamination at compounding pharmacies. The first report that involved infection with *E. dermatitidis* resulted in four cases of meningitis and one case of sacroiliitis in 2002.[70] The second and more recent outbreak included meningitis due to *Exserohilum rostratum*. Between September and December of 2012, 590 cases of infection and 37 deaths were confirmed among the 13,534 people potentially exposed to the contaminated lots of methylprednisolone.[71] Early cases usually presented with meningitis, some with stroke from invasion of arteries in the basilar meninges.[72] Later cases more often presented as more localized disease, depending on the site injected with steroid. Lumbar injections were most common, leading to epidural abscess with increased lumbar pain and some with cauda equina syndrome. Indolent septic arthritis occurred in the sacroiliac joint or rarely in injected peripheral joints. Although hyphae were commonly found in surgical specimens, cultures were only positive for *E. rostratum* in 14% and polymerase chain reaction in 29%.[73] Amphotericin B seemed the most effective treatment but was followed by several months of voriconazole therapy. The optimal course of treatment and prevention of relapse is not yet known.

Twenty-one surgical site infections with *Bipolaris* (currently *Curvularia*) spp. were reported after cardiothoracic surgery in 10 hospitals during 2008–2013. Most infections were in the mediastinum or sternal wound. Delayed wound closure was the most frequent predisposing factor.[74]

Allergic fungal sinusitis may be caused by a wide variety of fungi, although the dark-walled fungi (usually *Bipolaris, Exserohilum, Curvularia,* or *Alternaria* spp.) and *Aspergillus* spp. are the most common causes.[75] By definition, disease is allergic and confined to the lumen of the paranasal sinuses. Patients present with an indolent onset of sinus pain or painless proptosis. A history of seasonal or allergic rhinitis is common, and there may be a history of nasal polyps. On computed tomography or magnetic resonance imaging, one or more paranasal sinuses appear full of fluid, with outward pressure on the thinner bony sinus walls, such as the lamina papyracea, medial maxillary wall, or midline sphenoidal septum. Maxillary and ethmoid sinuses are usually involved, but sphenoid and frontal sinuses may be diseased (Fig. 268.6). Surgical débridement of the paranasal sinus removes dark, inspissated mucus; histopathologic examination reveals that this mucus has eosinophils with Charcot-Leyden crystals (degenerated eosinophils) and scattered septate hyphae.[76] The walls of the hyphae may not appear as dark as those seen in brain abscess. Irregular diameter and bulbous swellings may help distinguish these hyphae from *Aspergillus,* but culture is essential for diagnosis. The most serious sequela of allergic fungal sinusitis is brain invasion, which, when it occurs, usually does so in immunocompromised hosts (Fig. 268.7). Extension from the ethmoid or frontal sinus into the frontal lobe of the brain can be clinically silent. Erosion into the frontal lobe, clivus, pterygoid space, or middle fossa occurs but is rare. Sudden blindness can result from compression of the optic nerve posterior to the orbital fissure. Compression of the orbit by lateral bulging of the lamina papyracea does not decrease visual acuity but does cause proptosis.

Diagnosis of these infections requires observation of the fungi invading tissue or recovery of the fungi in culture from an otherwise sterile site.

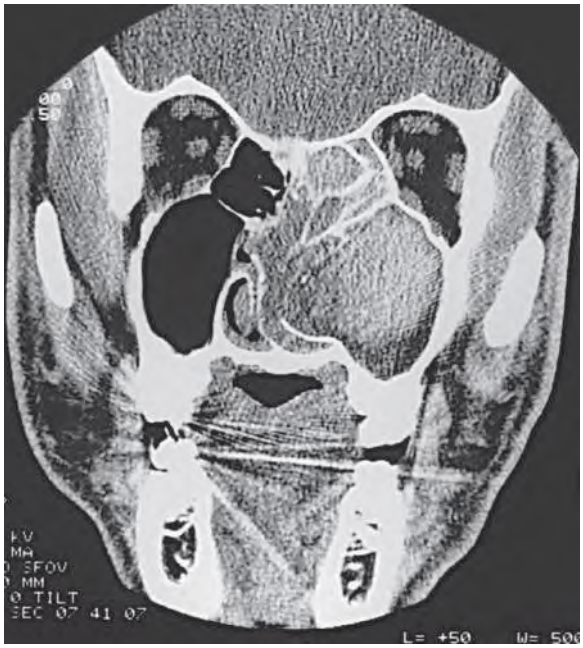

FIG. 268.6 **Computed tomographic scan showing outward-bulging mass in the maxillary and ethmoid sinuses of a patient with allergic fungal sinusitis.**

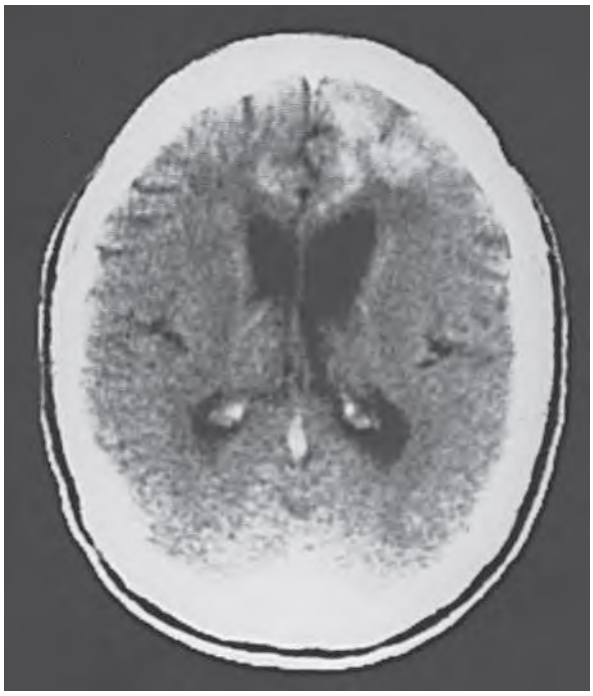

FIG. 268.7 **T2-weighted magnetic resonance image depicting extension into the frontal lobe of a patient with *Curvularia* (formerly *Bipolaris*) *hawaiiensis* sinusitis.**

Typical pathology is necessary for the diagnosis of allergic fungal sinusitis. In disease outside the CNS, these organisms may not always appear dark walled on standard histopathologic stains. Cell wall melanin may be visible as a brownish-yellow color on H&E stain (Fig. 268.8). If melanin is not evident on fresh preparations of H&E stain, it can be stained by the Fontana-Masson method, which better enables diagnosis, especially if culture results are negative or if culture is not performed.[77] Fontana-Masson stain is, however, not 100% specific for the dark-walled fungi because the cell walls of some *Aspergillus* and other fungi with hyaline hyphae have been shown to stain dark with this method.[78]

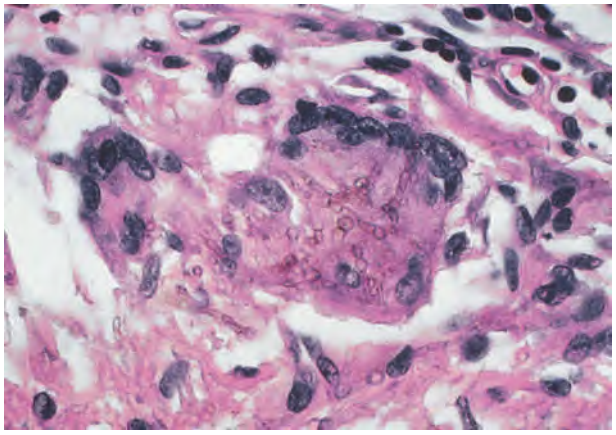

FIG. 268.8 Cutaneous phaeohyphomycosis caused by *Exophiala jeanselmei.* Note the brown hyphae. (Hematoxylin and eosin stain.) *(From Chandler FW, Watts JC. Phaeohyphomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

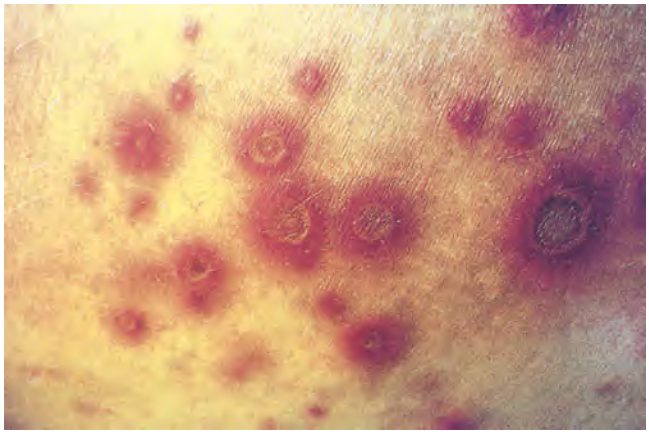

FIG. 268.9 Cutaneous lesions of disseminated infection caused by *Fusarium moniliforme.* *(From Beneke ES, Rogers AL.* Medical Mycology and Human Mycoses. *Belmont, CA: Star Publishing Company; 1996.)*

Surgical débridement is essential to the cure of most of the infections caused by the dark-walled fungi. Good surgical curettage often suffices in treating allergic sinusitis if the cranial cavity has not been invaded. Amphotericin B is probably the drug of choice for life-threatening infection, including CNS infection. Itraconazole has been used frequently with success in infections that are not life threatening.[79,80] To help prevent further recurrence in patients who have recurrent allergic fungal sinusitis, long-term itraconazole therapy may be used after repeated surgical drainage. In vitro, in response to most of these fungi, voriconazole commonly produces minimal inhibitory concentrations (MICs) that are similar to or lower than those seen with itraconazole, which makes this new drug a potentially useful therapeutic agent. Posaconazole and caspofungin have also been shown to have in vitro activity against many of these fungi. Posaconazole has been reported to produce a good clinical response in single cases of CNS and disseminated infection caused by *R. mackenziei* and *Exophiala spinifera,* respectively.[81,82] Response of a skin and soft tissue infection to terbinafine, after poor response to amphotericin B and itraconazole, has also been reported.[83]

FUSARIUM SPP.

Species in the genus *Fusarium* are common in soil and organic debris and are frequently the cause of disease in plants. Current taxonomy divides the species causing infections in humans into seven species complexes, called *Fusarium solani, Fusarium oxysporum, Fusarium fujikuroi, Fusarium dimerum, Fusarium chlamydosporum, Fusarium incaratum-equiseti,* and the complex including *Fusarium sporotrichioides.* The first three species complexes in the list are those most commonly encountered clinically. *Acremonium falciforme* has been moved to the *F. solani* spp. complex and renamed *Fusarium falciforme.* The most common manifestations of fusariosis are keratitis[84] and onychomycosis.[85] Infection of soft tissue, including mycetoma, can occur after traumatic inoculation in the healthy host. Inhalation into the lung or paranasal sinus or minor trauma to the skin can lead to fusariosis in immunocompromised patients.[86,87]

Rare cases of dissemination have been described in the clinical setting of severe burns,[88] trauma,[89] and heat stroke.[90] Most commonly, however, fusariosis occurs in patients with acute leukemia (70%–80% of cases)[91,92] and prolonged neutropenia (>90% of cases).[91,93] In one review of 43 patients, the median duration of neutropenia was more than 3 weeks.[91] Fusariosis is also increasingly reported in patients receiving allogeneic hematopoietic stem cell transplants.[94] The portal of entry in most of these cases of disseminated infection is not known. Inhalation, ingestion, and entry through skin trauma have been suggested. Sinusitis has preceded dissemination in a few reports. Hematogenous spread has been attributed to indwelling intravascular catheters.[95,96] Onychomycosis with paronychia has appeared to be a source of disseminated infection in some patients.[91,97] Water has been suggested as a source of these infections; the fungus was found in one hospital water supply system and in several water sources at a dialysis center.[98,99]

FIG. 268.10 Multiple necrotic skin lesions in a neutropenic patient with hematogenously disseminated fusariosis.

Infection commonly manifests with fever and myalgia unresponsive to broad-spectrum antibacterial antibiotics during periods of profound neutropenia. Disseminated fusariosis has been recognized in patients who have been receiving empirical or prophylactic antifungal therapy.[92,94,100–103] Skin lesions occur in 60% to 80% of infections, usually appearing as multiple papules or deep-set, painful nodules (Fig. 268.9). They may initially be flat (macular) with a central pallor, but later they become raised, erythematous, and necrotic (Fig. 268.10).[86,91,93] Lesions are most common on the extremities but have been reported on the trunk and face as well. In profoundly neutropenic patients, this infection can progress rapidly to death, in a manner similar to that in invasive aspergillosis. Skin lesions, denoting dissemination, can occur within a day of the onset of fever. In patients whose neutrophil levels recover, the infection can progress slowly over weeks until death or can become controlled and eventually cured.

Recovery of the fungus from blood and skin lesion biopsy are the two most common and effective ways to diagnose this infection. In contrast to aspergillosis, in which blood culture results are nearly always negative, fusariosis is accompanied by positive blood culture results about 50% of the time (48 of 98 patients in one review[91]). The septate hyphae of *Fusarium* spp. have both acute and right-angle branching and usually appear similar to those of *Aspergillus* on histopathology. Hyphae are often difficult to visualize with routine H&E staining but are easily identified when tissue is stained with Gomori methenamine

silver or periodic acid–Schiff. *Fusarium,* unlike *Aspergillus,* can sporulate in deep tissue by a process called adventitious sporulation. Identification of these spores along with hyphae can suggest the diagnosis. Conidia (spores) in tissue do not resemble the banana-shaped, septate conidia in culture but are ellipsoidal, spherical, or cylindrical and not septated.[104] They may be seen free or attached to the end of a hypha. Gram stain of a blood culture may also show these spores. The spore-bearing structure, called a phialide, may appear as a flat, truncated hyphal end if the spore has become detached.[104] In culture, the characteristic feature of *Fusarium* is the production of sickle (banana)-shaped multiseptate macroconidia.[105] Identification beyond the genus level is best done by molecular methods, with multilocus sequence typing currently favored.[106] MALDI-TOF MS has the promise of rapid identification of *Fusarium* isolates, although not necessarily to the species level.[106] Patients with fusariosis may have positive beta-D-glucan tests and *Aspergillus* galactomannan assays in serum.[107]

The optimal treatment for disseminated fusariosis has not been established. Overall mortality with this infection has been reported to range from 50% to 80%.[87,94] Survival is almost always associated with the recovery from neutropenia, although corticosteroid use also impairs response to therapy.[91,93,108] In an analysis of responders versus nonresponders in one study of 43 patients, investigators noted associations with malignancy in remission (100% vs. 10%), adequate neutrophil counts (100% vs. 0%), and lack of significant (grade II or greater) graft-versus-host disease (0% vs. 66%).[91] Removal of indwelling venous catheters has been associated with improvement and thus should be considered in all cases of fungemia.[96] Surgical débridement of soft tissue infections resulting from trauma is helpful. Currently, voriconazole or a lipid formulation of amphotericin B is favored for deep infections.[31] Natamycin 5% ophthalmic suspension is used for keratitis (see Chapter 113). Echinocandins are not recommended for deep infections, although terbinafine, posaconazole, and combination regimens have been reported as useful.[109–111,112,113,114,115] Animal models have shown liposomal amphotericin B,[116] posaconazole,[117] and voriconazole[118] to be potentially useful. On the basis of successful therapy in 9 of 21 patients (43%) who received voriconazole, the FDA approved this agent for second-line use in fusariosis. Successful therapy in 10 of 21 patients (48%) with posaconazole has also been reported.[119] Unfortunately, cases of breakthrough infection in patients receiving voriconazole or posaconazole have been reported.[101–103] The addition of colony-stimulating factors (G-CSF or granulocyte-macrophage colony-stimulating factor) or granulocyte transfusions to specific antifungal therapy have also been reported,[86,91,93,120] but the benefit of these adjunctive therapies is also not proven.

OTHER OPPORTUNISTIC MOLDS

In immunocompromised hosts, virtually any of the normally nonpathogenic fungi may cause disease. In addition to more common infections caused by *Aspergillus* and *Fusarium* spp., other rare light-colored (hyaline) molds, including species of *Paecilomyces,*[121] *Acremonium,*[122] and *Trichoderma,*[123] have been described as causing clinical disease more frequently than do other rare fungi. Some authorities have grouped diseases caused by molds with light-colored cell walls into a group, termed the hyalohyphomycoses. As with the dark-walled fungi, description of these infections by the causative organisms is preferable to minimize confusion. *Paecilomyces* has been reported to cause keratitis, endophthalmitis, and cutaneous and subcutaneous infections, as well as catheter-related fungemia, sinusitis, and disseminated infection. Like *Fusarium,* both *Paecilomyces* and *Acremonium* organisms have been reported to form reproductive structures in vivo in a process called adventitious sporulation.[104] This is believed to account for the much higher frequency of recovery in blood culture observed in infections involving these three genera. Also like *Fusarium,* both are typically associated with poor response to amphotericin B and the older azoles, although resistance varies among species. *Paecilomyces varioti* is susceptible to amphotericin B, and infections have been treated successfully with this agent. *Paecilomyces lilacinus,* now named *Purpureocillium lilacinum,* responds poorly to amphotericin B and, in vitro, is resistant to this agent, caspofungin, and the older azoles.[124] In vitro testing has shown multiple strains of these fungi to be more susceptible to voriconazole and posaconazole, but clinical treatment results are limited to case reports and small case series.[26,121,125–127] *Scopulariopsis* spp. are common in the soil, air, and decaying organic debris. Isolates identified as forming ascospores are placed in the genus *Microascus,* the teleomorph of *Scopulariopsis.* Onychomycosis, keratitis, and deeply invasive or disseminated infections have been reported.[128] Hyphae are septated and branch at acute angles. Bulbous swelling of hyphal segments may be seen, a feature usually encountered in agents of phaeohyphomycosis.

TRICHOSPORON SPP.

The genus *Trichosporon* is characterized by the production of septate hyphae, arthroconidia, yeasts, and pseudohyphae and by yeastlike growth on culture media. As a result of revisions in taxonomy, there are now more than 50 species of *Trichosporon,* 16 of which are clinically relevant.[129] Invasive human infection is most commonly due to *Trichosporon asahii,* and less commonly with *Trichosporon mucoides, Trichosporon mycotoxinivorans,* or *Trichosporon faecale.*[130–134] *Trichosporon asteroides* and *Trichosporon cutaneum* cause superficial infection. White piedra of the scalp is caused by *Trichosporon ovoides,* and similar disease of the pubic hair is caused by *Trichosporon inkin* (see Chapter 266). *Trichosporon* can be found in soil and water, on plants, and colonizing the human mouth, gastrointestinal tract, respiratory tract, vagina, skin, and urine.[129,135] More than 100 patients with deep trichosporonosis have been described; approximately 60% have been severely neutropenic, usually with acute leukemia.[133,136,137] A few have had organ transplantation, HIV infection, burns, chronic ambulatory peritoneal dialysis, or catheter-acquired fungemia.[138] Seven patients had prosthetic valve infections.[135]

Trichosporonosis is an acute, febrile, often fatal infection with dissemination to multiple deep organs and is associated with a mortality rate as high as 64%. Pneumonia is not a consistent or early feature, and thus the portal of entry is often not apparent. Renal involvement is common in disseminated disease and is associated with hematuria and funguria. Multiple red papular skin lesions may occur early on and assist diagnosis.[139,140] On biopsy, *Trichosporon* is seen as a mixture of true hyphae, pseudohyphae, budding yeasts, and tubular elements with square ends, called arthroconidia. Trichosporonosis is easily mistaken for candidiasis (which does not produce arthroconidia). *Trichosporon* grows readily on most culture media, but blood cultures tend to yield positive results late in the course. In the past, therapy with amphotericin B was recommended, but poor response and failures with this drug have occurred. MICs of the echinocandins for these fungi are high, and multiple incidences of breakthrough infections in patients receiving echinocandins have been reported.[141] These fungi are typically susceptible in vitro to voriconazole, as well as fluconazole, isavuconazole, itraconazole, and posaconazole[142,143]; thus therapy should include use of one of these azole antifungals.

MALASSEZIA FURFUR

Malassezia furfur, a lipophilic yeast, commonly colonizes normal human skin and is the cause of a superficial mycosis, pityriasis (tinea) versicolor (see Chapter 266). The fungus can also cause catheter-related sepsis, almost always in patients who are receiving parenteral lipids through a central venous catheter.[144] Most reported patients have been neonates with extended stays in intensive care units, although a few have been adults with malignancy or immunosuppression.[145] Fever has been the most common finding, but bradycardia, apnea, thrombocytopenia, and catheter blockage have been observed in some infants. In the autopsy study of one case, the yeast was observed in lipid-containing areas of pulmonary vascular endothelium. *M. furfur* is rarely detected by conventional culture techniques because the yeast requires fatty acids for growth. Organisms are better recovered with culture of blood drawn back through the catheter through the use of the lysis-centrifugation technique and lipid-enriched agar.[144] Results of cultures or smears of peripheral blood are occasionally positive.[146] The yeast is identified on smear by its size and shape and the distinctive collarette between mother and daughter cells. Lipid requirement for growth also aids identification. It is likely that some cases attributed to *M. furfur* were caused by other lipid-requiring species in the *M. furfur* complex, which includes *Malassezia sympodialis, Malassezia globosa, Malassezia obtusa, Malassezia restricta,* and *Malassezia slooffiae.*[147] *Malassezia pachydermatis* has the

same appearance and has caused similar infections, including an outbreak in a neonatal intensive care unit,[148] but does not require lipids for growth. The fungus adheres to the lumen of the catheter and has not been eradicated by discontinuing lipid infusions or administering miconazole or amphotericin B through the catheter.[149] Catheter removal and discontinuing parenteral lipids have been curative. In vitro, *M. furfur* appears to be susceptible to both amphotericin B and azole antifungals, including itraconazole and voriconazole.[150,151]

OTHER UNCOMMON YEASTS

Magnusiomyces capitatus (formerly called *Saprochaete capitata*, *Blastoschizomyces capitatus, Trichosporon capitatum,* and *Geotrichum capitatum*) has been reported as the cause of severe infection in more than 75 patients, most of whom also had acute leukemia.[152,153,154,155] Blood culture findings are usually positive, and skin lesions similar to those seen in leukemic patients with disseminated candidiasis have been observed. *M. capitatus* may colonize the skin, respiratory tract, and gastrointestinal tract. Intravenous catheters are a possible portal of entry.[156] Intravenous amphotericin B with or without flucytosine[156] and voriconazole or high-dose fluconazole with amphotericin B[153] have been advocated for treatment of these infections. High-dose fluconazole was shown in an animal model to be more efficacious than amphotericin B, flucytosine, or voriconazole monotherapy.[157] Resistance to echinocandins has been reported.[154] Catheter removal was associated with improved outcome in the largest and most recent report.[153] *Saprochaete clavata,* a closely related species, is sometimes misidentified as *Magnusiomyces capitatus (Saprochaete capitata).*[158] *S. clavata* has caused an outbreak of sepsis in neutropenic patients from an unidentified source.[159]

Other noncandidal yeasts may also rarely cause infection in humans.[143,160] These include *Wickerhamomyces anomalus* (formerly *Pichia [Hansenula] anomala*),[161–163] the black yeast *Exophiala (Wangiella) jeanselmei,*[57] *Rhodotorula* spp.,[161,164,165] and *Saccharomyces cerevisiae.*[166] Infection is usually seen in immunocompromised individuals, most commonly as catheter-associated fungemia. Localized outbreaks secondary to *E. jeanselmei* and *W. anomalus* (*P. anomala*) have been described.[57,163]

TALAROMYCES (FORMERLY *PENICILLIUM*) *MARNEFFEI*

Talaromyces marneffei is a thermally dimorphic fungus that causes life-threatening disseminated infection (penicilliosis marneffei) in a geographically distinct area of the world. *T. marneffei* is a thermally dimorphic, geographically restricted, intracellular pathogen that exudes a brilliant red pigment into the agar around the colony. Endemic to Southeast Asia, *T. marneffei* infection was extremely rare before the AIDS epidemic: fewer than 30 cases had been described since the first human infections in 1959 and 1984.[167,168] Between 1984 and 2004, more than 6000 *T. marneffei* infections were diagnosed in Thailand.[169] In the mid-1990s, this was the third most common opportunistic infection seen in HIV-infected individuals in northern Thailand.[170] Infection with *T. marneffei* has a limited geographic distribution, affecting persons residing in or those who have visited Southeast Asia or southern China. Endogenous cases have been reported from Myanmar (Burma), Hong Kong, Indonesia, Laos, Malaysia, Singapore, Taiwan, Thailand, Vietnam, and the Guangxi province of China.[171–173] Although most commonly seen in young adults infected with HIV, the disease has been reported in children[174,175] and adults, both with and without detectable immunocompromise.[171,172] *T. marneffei* has been isolated from healthy bamboo rats (*Rhizomys pruinosis, Rhizomys sinensis, Rhizomys sumatrensis,* and *Cannomys badius*)[176,177] and the soil around their burrows,[178] but the role of these rats in human infection is unknown. A case-control study of the disease in 80 persons with AIDS revealed an association with recent history of occupational or other exposure to soil, but not to rats.[179] Infection occurs more commonly during the rainy season in both northern Thailand and Vietnam.[180,181] It is likely that this infection is acquired by inhalation of conidia from an environmental source such as the soil.

Patients typically present with a chronic illness averaging 4 weeks in duration associated with low-grade fever, weight loss, and one or more skin lesions.[170,182] Apart from the skin lesions, disease is quite similar in presentation, course, and treatment to that of acute disseminated histoplasmosis. The most common clinical characteristics are fever, malaise, anemia, leukocytosis, weight loss, and, in 60% to 70% of patients, skin lesions.[170,172,182] Fungemia, generalized lymphadenopathy, and cough are reported in more than half of patients. Subcutaneous and mucosal lesions, diarrhea, colonic lesions, hepatomegaly with or without splenomegaly, hemoptysis, osteoarticular lesions, and pericarditis have also been described.[171,183,184] Skin lesions commonly occur on the face, upper trunk, and extremities. They may occur as papules, pustules, nodules, ulcers, or abscesses. In HIV-infected individuals, lesions commonly become umbilicated and resemble those of molluscum contagiosum. Pharyngeal and palatal lesions are also more commonly seen in HIV-infected patients.[184] Lung lesions can appear as reticulonodular, nodular, or diffuse alveolar infiltrates, but on occasion they are cavitary and cause hemoptysis.[185] Autopsy studies have revealed involvement of lymph nodes, liver, spleen, lungs, kidneys, skin, bone, bone marrow, adrenal glands, tonsils, bowel, and meninges.[171,186] Mortality of higher than 80% has been reported in a series of 21 HIV-infected persons with *T. marneffei* meningitis.[187]

Consideration of the diagnosis of *T. marneffei* infection should be made in persons who have resided in or visited an endemic area. Laboratory exposure to the organism was causally linked to disseminated infection in one immunocompromised individual.[188] The duration of incubation is not currently known, and reactivation disease may be possible. In one report, a severely immunocompromised individual acquired disseminated infection more than 10 years after visiting an endemic area.[189] Diagnosis is based on identification of the organism on smear, histopathologic studies, or culture. Diagnosis has been made most frequently from smears of skin lesions and biopsy samples of lymph nodes and bone marrow.[170] The organism has also been noted on peripheral blood smear in at least one report.[190] Isolation of *T. marneffei* from culture of bone marrow, blood, lymph nodes, skin lesions, bronchoalveolar lavage, or sputum can be diagnostic. Microscopic examination of clinical materials reveals yeast forms (2×2 to 3×6.5 µm) both within phagocytes and extracellularly.[171] The intracellular forms are smaller, resembling *Histoplasma capsulatum,* whereas the extracellular forms are larger and often have a transverse septum (asexual fission or schizogony) (Fig. 268.11). The extracellular forms may also appear as "sausage forms," consisting of three cells (8–13 µm in length) divided by two transverse septa or, rarely, as short hyphae. Three types of histopathologic reactions have been noted in association with *T. marneffei* infection: granulomatous, suppurative, and necrotizing inflammation.[186] Granulomatous or suppurative changes are most commonly seen in patients with normal immunity. The necrotizing reaction is more commonly seen in immunocompromised patients and is characterized by focal necrosis with surrounding histiocytes and extracellular fungi. Culture at 30°C produces a mold with sporulating structures typical of *Penicillium.* Identification is aided by the formation of a soluble red

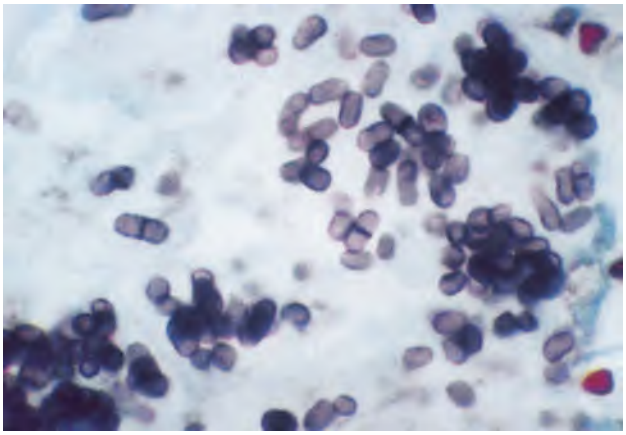

FIG. 268.11 ***Talaromyces* (formerly *Penicillium*) *marneffei* in a splenic abscess.** Note the transverse septa. (Gomori methenamine silver stain.) *(From McGinnis MR, Chandler FW. Penicilliosis marneffei. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

pigment that diffuses into the agar. The mold form can be converted to a yeast form by incubation at 37°C.[191] Because disease normally appears to result from inhalation of conidia, it seems reasonable to use Biosafety Level 2 precautions when working with the mold form. Diagnosis by specific immunologic techniques, including serum antibody and antigen tests, is still in the experimental stage.[192,193] The currently available galactomannan test for *Aspergillus* may assist in the diagnosis; two-thirds of patients with *T. marneffei* infection had positive test results in one study.[194]

Excellent response (97.3%) was achieved with a regimen of intravenous amphotericin B for 2 weeks (0.6 mg/kg/day) followed by oral itraconazole for 10 weeks (200 mg twice daily) in 74 HIV-infected patients.[195] This regimen allowed shortened hospital stays while producing a clearing of fungemia that was more rapid than that produced by itraconazole alone. An open-label, randomized trial comparing initial therapy with IV amphotericin B versus oral itraconazole in 440 HIV-infected patients found a much higher incidence of death at 24 weeks in the itraconazole arm.[196] Successful treatment of disseminated infection has been reported with amphotericin B with or without the addition of flucytosine,[171] and with oral itraconazole or voriconazole monotherapy.[197] Failure rates in a study of 86 HIV-infected patients included 8 of 35 patients (22.9%) receiving amphotericin B, 3 of 12 (25%) taking itraconazole, and 7 of 11 (63.6%) taking fluconazole.[198] Itraconazole has been shown to prevent relapse of this disease in patients with HIV infection,[199] and secondary prophylaxis of HIV-infected patients with itraconazole (200 mg once daily) is suggested to prevent relapse.[171] As with secondary prophylaxis in other fungal diseases, it appears safe to stop this therapy after response to antiretroviral therapy ($CD4^+$ cell count of ≥100 cells/μL for at least 6 months).[200] Immune reconstitution inflammatory syndrome has been reported with the initiation of antiretroviral therapy in HIV-infected persons.[201]

LACAZIA LOBOI

Lobomycosis (Lobo disease, keloidal blastomycosis) is a chronic skin infection most commonly afflicting the indigenous people of the Amazon regions of Colombia and Brazil and dolphins. The etiologic agent of lobomycosis has never been isolated in culture, but it has been shown to be closely related to *Paracoccidioides brasiliensis* by 18S ribosomal DNA sequencing.[202] This fungus has been known by many genus names; *Lacazia loboi* has been proposed to replace *Loboa loboi* and other previous designations.[203] More than 100 cases have been reported from countries in Central and South America. Lobomycosis found outside the endemic area, including one case from the United States,[204] typically occurs in persons with history of travel to an endemic country. Recently, two cases, one from South Africa and one from Greece, have been reported in people who had no history of travel to the Western Hemisphere.[205,206] Although it is difficult to confirm these cases with our inability to culture the organism (molecular techniques were also not employed), they raise the possibility that the range of lobomycosis is larger than previously believed. The fungus remains confined to the skin, progressing slowly over decades. Probably because of this slow progression, rare cases outside of the endemic area have been reported, often many years after potential exposure.[207] Lesions are typically nodules or keloidal plaques that are red, hard, and shiny, in association with fibrosis and a granulomatous reaction on histologic examination (Figs. 268.12 and 268.13). The diagnosis is made by finding the typical globose to lemon-shaped cells (about 9 μm in diameter), either singly or in short chains (Fig. 268.14). Surgical excision is the only useful therapy. One patient has been reported to respond to posaconazole.[208]

AGENTS OF ADIASPIROMYCOSIS (*EMMONSIA* SPP.)

Adiaspiromycosis is a rare disease of humans characterized by the formation of nonreplicating adiaspores, typically in the lungs. Disease severity correlates with the host's immunologic response and likely the inoculum size. Disease most commonly affects the lungs (approximately two-thirds of cases) and is often associated with occupational dust exposure. Men, average age 40, are most commonly afflicted. Disease can range from asymptomatic to severe and sometimes fatal cases.

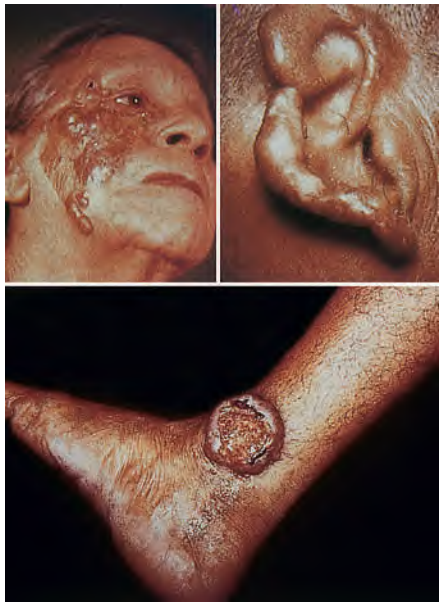

FIG. 268.12 Lobomycosis. *(From Nikolaidis G, Rosen T. Lobomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

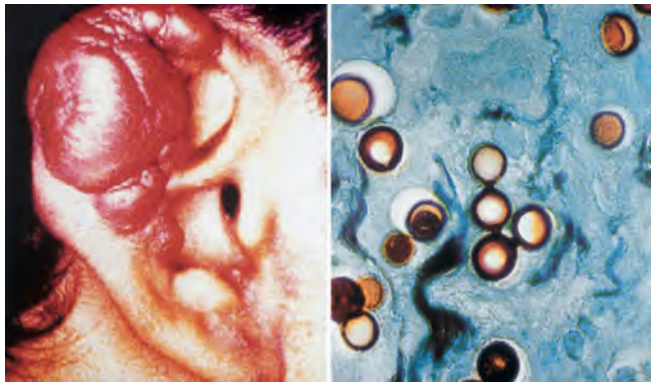

FIG. 268.13 Lobomycosis of the ear *(left)* with associated histologic findings *(right).* (Gomori methenamine silver stain.) *(From Herr RA, Herr E, Tarcha PR, et al. Phylogenetic analysis of* Lacazia loboi *places this previously uncharacterized pathogen with the dimorphic Onygenales.* J Clin Microbiol. *2001;39:309–314.)*

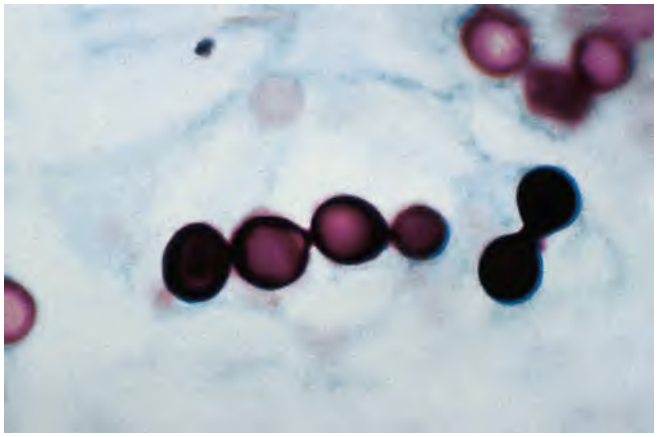

FIG. 268.14 Histologic appearance of *Lacazia loboi,* the agent of lobomycosis. (Gomori methenamine silver stain.) *(From Nikolaidis G, Rosen T. Lobomycosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

Adiaspiromycosis may be discovered when asymptomatic lung lesions are resected to rule out malignancy. Presentation may include fever, cough, dyspnea, hemoptysis, weight loss, fatigue, or respiratory failure. In addition to involvement of the lungs, adiaspiromycosis may present with ocular lesions and even with involvement of the gastrointestinal tract.[209] An outbreak in Brazil of ocular disease in children associated with fresh water sponges has been described.[210] Two cases of appendiceal involvement have also been reported.

Adiaspiromycosis is chiefly caused by *Emmonsia crescens* (*Ajellomyces crescens*), a fungus closely related to *Blastomyces dermatitidis,* and *Emmonsia parva.*[211] Conidia of *E. crescens* will enlarge into 25- to 400-µm adiaspores in vitro when incubated at 37°C on brain heart infusion agar. Inhaled, these 2- to 4-µm conidia can enlarge to diameters of up to 500 µm in the human lung.

Because *Emmonsia* has not been recovered from culture in human adiaspiromycosis, diagnosis is based on its unique histologic appearance, that of a single thick-walled adiaspore within a 0.5- to 3-mm granuloma composed of epithelioid and giant cells. A report of the use of polymerase chain reaction and DNA sequencing to diagnose adiaspiromycosis has been published.[212]

Corticosteroids have appeared to be beneficial in several cases of disease. It is unclear if antifungal therapy has any role in adiaspiromycosis, but low MICs have been reported with amphotericin B, itraconazole, voriconazole, and caspofungin.

EMERGOMYCES AFRICANUS AND OTHER EMERGOMYCES SPECIES

Disseminated infection in immunocompromised individuals with the thermally dimorphic fungus *Emergomyces africanus* has recently been described.[213–215] Initially, 13 cases of disseminated infection with an *Emmonsia* spp. related to *Emmonsia pasteuriana* were reported from South Africa.[214,215] Ultimately, this pathogen was named *E. africanus,* with both it and *E. pasteuriana* included in the new genus *Emergomyces.*[216] Infection with *E. africanus* was associated with skin lesions, fungemia, and high mortality, most commonly in severely immunocompromised HIV-infected (AIDS) patients.[213–215] *E. africanus* displays temperature-dependent dimorphic growth (i.e., mold at room temperature and yeast at 37°C) and can commonly be recovered from bone marrow or blood. *E. africanus* has been identified (but not cultured) from the soil in South Africa.[217] Like other dimorphic members of the family Ajellomycetaceae (including *Blastomyces* and *Histoplasma*), MICs of amphotericin B, itraconazole, posaconazole, and voriconazole for *E. africanus* are low.[218] This supports using a therapeutic approach similar to that employed with disseminated histoplasmosis (i.e., initial IV amphotericin B, followed by oral azole therapy and long-term suppression [secondary prophylaxis]). Similar cases of disseminated infection have been reported outside of Africa, caused by *E. pasteuriana, Emmonsia helica* (renamed *Blastomyces helicus*), *Emmonsia europaeus, Emergomyces canadensis,* and *Emmonsia orientalis.*[216,219–221]

PROTOTHECA SPP.

Prototheca are unicellular algae that lack chlorophyll and reproduce by endosporulation. Although not fungi, these organisms are described in this chapter because they are often preliminarily misidentified in tissue and culture as yeasts. *Prototheca* are found in a wide range of environmental sites, including tree slime, sewage, fresh and marine water, soil, pigeon droppings,[222] and foodstuffs. They may colonize human skin, the respiratory tract, and the gastrointestinal tract.[223] More than 100 cases of human infection have been reported,[223,224] almost all in adults and from widely scattered geographic areas. *Prototheca wickerhamii* is the most common cause of infection in humans; infection secondary to *Prototheca zopfii* and a single case due to *Prototheca miyajii* have also been reported.[225]

The most common manifestation of protothecosis is a single lesion of the skin or subcutaneous tissue. The typical manifestation is a painless, slowly progressive, well-circumscribed plaque or papulonodular lesion that may become eczematoid or ulcerated. Soft tissue lesions favor the olecranon bursa, sites of minor trauma or corticosteroid injection,[226] and surgical wounds exposed to soil or water, such as that of a hand tendon repair. Lesions typically enlarge gradually over weeks to months.

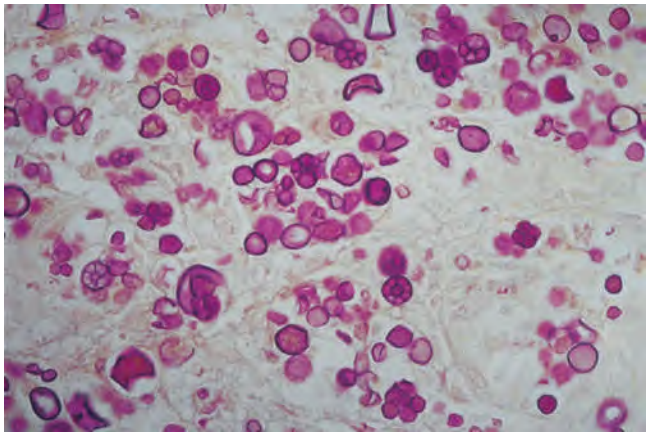

FIG. 268.15 *Prototheca wickerhamii* in an olecranon bursa biopsy sample. (Gridley stain.) *(From Ramsay E, Chandler FW, Connor DH. Prototheocosis. In: Connor DH, Chandler FW, Schwartz DA, et al, eds.* Pathology of Infectious Diseases. *Norwalk, CT: Appleton & Lange; 1997.)*

Skin lesions in HIV-infected patients have not differed from those seen in other patients.[227,228] Disseminated and deep-seated infections, such as peritonitis, meningitis, and endocarditis, have also been reported in rare cases.[224,229,230]

Protothecosis is best diagnosed through biopsy for histologic study and culture. The inflammatory response consists of both microabscesses and granulomas with multinucleate giant cells. *Prototheca* cells usually range from 3 to 30 µm in diameter, are well visualized with Gomori methenamine silver or periodic acid–Schiff stain, and often contain two to eight tightly packed endospores in each cell or sporangium (Fig. 268.15). White opaque colonies appear in a few days on standard mycologic media. Identification is made by gross and microscopic appearance plus biochemical testing (the algae is identified by most commercially available yeast identification systems).

Protothecosis has little, if any, tendency toward self-healing. There have been few cases of disseminated disease in the setting of solid organ or stem cell transplantation; all resulted in mortality.[229] Both surgical excision of lesions and intravenous amphotericin B have been used successfully.[227] *Prototheca* spp. are resistant to flucytosine, but prolonged therapy with ketoconazole, itraconazole, or fluconazole has been reported to benefit some patients with skin lesions.[226,231,232] Short-course itraconazole (200 mg daily for 2 months) has also been used successfully.[233] Voriconazole has proven effective in a case of cutaneous infection initially unresponsive to itraconazole.[234]

PYTHIUM SPP.

Pythium spp. are protists formerly classified as fungi (Oomycetes, or water molds), which rarely cause human infection. Disease is found worldwide, but the largest number of cases have been reported from Thailand.[235] Pythiosis is associated with environmental trauma and with underlying thalassemia or other hemoglobinopathies. The most common patient is male, age 20 to 60 years, with an agricultural occupation. In the largest reported series, vascular (typically arterial) infection was most common (59%), followed by ocular (33%), skin and subcutaneous (5%), and disseminated infection (3%).[236] Swimming in Thailand while wearing contact lenses has led to *Pythium insidiosum* keratitis.[237] Although ocular disease was usually associated with trauma, 85% of patients with vascular, skin and subcutaneous, and disseminated disease had a thalassemia-hemoglobinopathy syndrome. Disease has also been reported in association with burns and combat trauma.[238–241]

Pythiosis has been associated with poor prognosis,[236,242] due in part to difficulties in diagnosing the infection and lack of antimicrobial agents effective against this disease. In the aforementioned study, 79% of those with ocular disease required removal of the affected eye to control infection; 78% of vascular cases lost a limb (60% survived—all of whom underwent amputation). All patients with disseminated disease died.