New Developments in Blastomycosis

Jeannina A. Smith, MD1 Greg Gauthier, MD1

1 Division of Infectious Diseases, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

Semin Respir Crit Care Med 2015;36:715–728.

Abstract

Blastomyces dermatitidis, the etiologic agent of blastomycosis, is a thermally dimorphic fungus that grows as a filamentous mold in the environment and as budding yeast in human tissue. This pathogen is endemic to North America, particularly in the states bordering the Mississippi and Ohio rivers, the Great Lakes, and the St. Lawrence Seaway. Infection with B. dermatitidis causes a broad array of clinical manifestations ranging from asymptomatic infection to fulminant sepsis with acute respiratory distress syndrome and death. B. dermatitidis can infect almost any organ in the body, but has a predilection for lungs and skin. There have been recent advances in the understanding of the pathogenesis, diagnosis, and treatment of this fungus. The Infectious Diseases Society of America published updated guidelines in 2008 to guide clinicians in the treatment of this important pathogen.

Keywords

► blastomycosis
► Blastomyces dermatitidis
► amphotericin
► itraconazole

Historical Perspective

Blastomycosis was first described at a dermatology conference in 1894 by Thomas C. Gilchrist and in 1898, the pathologic agent was named Blastomyces dermatitidis.1,2 In 1907, Walter W. Hamburger discovered that in response to changes in temperature, B. dermatitidis switched between mold and yeast forms.3 In 1951, Drs. Schwarz and Baum reported that the lower respiratory tract, not the skin, was the primary portal of entry for B. dermatitidis.4 In the mid-1980s, B. dermatitidis was successfully isolated from the soil in association with a large outbreak in northern Wisconsin.5 Since the 1990s, several important advances at the molecular level have enhanced the understanding of how B. dermatitidis causes clinical disease. This includes the identification of an essential virulence factor, Blastomyces adhesion-1 (BAD-1; formerly WI-1), and a dimorphism-regulating kinase-1 (DRK1) critical for the morphologic switch from mold to yeast. In 2009, the complete genome of B. dermatitidis was sequenced. In 2013, genome-wide in vivo transcriptional profiling of B. dermatitidis yeast during experimental murine infection was performed to identify genes and gene pathways important for pathogenesis.6

Mycology

B. dermatitidis is a thermally dimorphic fungus that grows as yeast at 37°C and as hyphae at 22 to 25°C (→ Fig. 1). The yeast form, which is found in human tissue, is 8 to 20 μM in diameter and is characterized by broad-based budding and a doubly refractile cell wall.7 Uncommonly, giant yeast forms (28–40 μM) can be observed in clinical specimens and confused with Coccidioides spp.8,9 At room temperature, B. dermatitidis grows as septate hyphae (1–2 μM diameter) that produce asexual spores (4–5 μM diameter) called conidia.10 In contrast to distinctive appearance of yeast, hyphal morphology is not specific for B. dermatitidis and requires molecular confirmation for definitive identification.

Multilocus microsatellite typing of 112 B. dermatitidis isolates obtained from humans, animals, and soil demonstrated genetic heterogeneity and that Blastomyces can be divided into two distinct groups.11 A subsequent analysis of 227 human B. dermatitidis isolates suggested group 1 isolates were more likely to be associated with disseminated disease and cause outbreaks, whereas group 2 isolates tended to cause pneumonia.12 Moreover, recent phylogenetic analysis using genealogical concordance phylogenetic species recognition (GCPSR) suggested that the Blastomyces genus might...
have two distinct species, *B. dermatitidis* and *B. gilchristii* sp. nov.\textsuperscript{13}

**Geographic Distribution and Epidemiology**

The geographic range of *B. dermatitidis* in North America is primarily limited to the Midwest, south-central, and southeastern regions of the United States and four Canadian provinces (\textsuperscript{\textit{\small Fig. 2}}). This includes the Ohio-Mississippi and Saint Lawrence River valleys as well as the Great Lakes region. Within the endemic region, *B. dermatitidis* is not uniformly distributed, rather it is found in an ecological niche characterized by forested, sandy soils with an acidic pH and decaying vegetation or rotting wood that are located by water sources (e.g., rivers, lakes).\textsuperscript{5} Similar to other fungi such as *Histoplasma capsulatum* and *Cryptococcus* spp., *B. dermatitidis* can grow in bird guano. Human activities that disrupt soil (e.g., home and road construction, exploration of beaver dams or underground forts, use of a community compost pile, hunting, clearing brush, or cutting trees) or involve water (e.g., canoeing, boating, fishing) have been associated with the acquisition of *B. dermatitidis* as well as outbreaks of disease (\textsuperscript{\textit{\small Table 1}}).\textsuperscript{5,14-28}

Epidemiologic knowledge about *B. dermatitidis* is limited because the data are based on passive surveillance and retrospective studies. Blastomycosis is reportable in six states (WI, MN, MO, AK, LA, and MS) and two provinces (Ontario, Manitoba). Serological testing has insufficient sensitivity and no skin test exists to survey large populations. Moreover, approximately 50% infected persons develop subclinical or asymptomatic illness that remains undiagnosed.\textsuperscript{5} Thus, epidemiologic data are limited to clinically apparent infections that are diagnosed and reported. Although an underestimate, the reported annual incidence ranges from 0.2 to 1.94 cases per 100,000 persons.\textsuperscript{29-36} Certain geographic locations are considered

**Fig. 1** *Blastomyces dermatitidis* mold and yeast. (A) Hyphae with conidia at 22°C. (B) Broad-based budding yeast at 37°C. Scale bar is 10 μm. (Reprinted with permission from Gauthier.\textsuperscript{60})

**Fig. 2** Geographic distribution of *B. dermatitidis* in the United States and Canada. (Reprinted with permission from Gauthier.\textsuperscript{60})
hyperendemic for blastomycosis including Kenora, Ontario (117.2 human cases/100,000 population); Vilas County, WI (40.4/100,000); Eagle River, WI (101.3/100,000); Washington Parish, LA (6.8/100,000); and central/south-central Mississippi (> 5/100,000). From 2007 to 2011, a total of 4,688 patients in 46 states were hospitalized for blastomycosis. The majority of these patients were hospitalized in the state in which they resided; however, 8% of patients were admitted to hospitals outside of the endemic region. Unlike most human pathogenic fungi, B. dermatitidis can infect persons with intact or impaired immune systems. Immune-compromised patients at risk for blastomycosis include those who have undergone solid-organ transplantation (SOT), received tumor necrosis factor-α (TNF-α) inhibitor therapy, have underlying malignancy, or develop HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome). Blastomycosis occurs in less than 0.2% of SOT recipients, has an onset between 0.4 and 250 months after transplantation, and can be associated with opportunistic pathogens, especially cytomegalovirus. When compared with histoplasmosis or coccidioidomycosis, the frequency of blastomycosis in persons with HIV/AIDS or immunosuppressed by TNF-α inhibitor therapy is lower.

Autochthonous cases of culture-proven blastomycosis have been reported from Africa and India. Approximately 100 patients with blastomycosis have been described from 18 African countries, whereas less than 10 confirmed autochthonous cases have been reported from India. The limited number of human and animal cases has precluded accurate discernment of the geographic distribution or ecological niche within Africa and India.

### Pathogenesis

#### Overview

*B. dermatitidis* is a primary fungal pathogen because it can infect persons with normal or impaired immune defenses. Features that facilitate growth of dimorphic fungi in human tissue include the production of small-sized conidia that can penetrate deep into the respiratory tree, ability to grow at 37°C (i.e., thermotolerance), conversion to yeast morphology, expression of yeast-phase-specific virulence factors, and evasion of host immune cells.

#### The Phase Transition

The conversion between hyphal and yeast morphologies, known as the phase transition, is essential for the pathogenesis for all thermally dimorphic fungi including *B. dermatitidis*. In the soil (22–25°C), *B. dermatitidis* grows as filamentous hyphae that produce conidia (asexual spores). Following soil disruption from human activities (e.g., construction), conidia and hyphal fragments are aerosolized and when inhaled into the lungs of a human host (37°C) convert into budding yeast that cause pneumonia. The predominant stimulus that induces the morphologic switch between hyphal and yeast forms is temperature. Recent investigation has demonstrated that conversion to yeast is accelerated following phagocytosis of *B. dermatitidis* spores by alveolar macrophages. In addition to intracellular spore germination, yeasts are able to survive and replicate within macrophages during the early stages of infection. Thus, *B. dermatitidis* exhibits an intracellular lifestyle, which is similar to other dimorphic pathogens including *H. capsulatum*, *Coccidioides* spp., and *Paracoccidioides brasiliensis*.

### Table 1 Outbreaks of human blastomycosis

<table>
<thead>
<tr>
<th>State</th>
<th>Years(s)</th>
<th>Persons infected</th>
<th>Outbreak source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Carolina</td>
<td>1975</td>
<td>5</td>
<td>Harvest at peanut farm</td>
<td>CDC (1976)</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1988</td>
<td>32</td>
<td>Hotel construction</td>
<td>Baumgardner and Burdick (1991)</td>
</tr>
<tr>
<td>Tennessee</td>
<td>1989</td>
<td>3</td>
<td>Construction at a rayon factory</td>
<td>Frye and Seifer (1991)</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>2006</td>
<td>21</td>
<td>Community yard waste site</td>
<td>Pfister et al (2011)</td>
</tr>
</tbody>
</table>
Previously, *B. dermatitidis* was thought to be an extracellular pathogen. Other stimuli such as estradiol, which affects the phase transition in *P. brasiliensis* or growth of *Coccidioides* spp., has minimal effect on *B. dermatitidis* morphology. The development of molecular tools to manipulate gene transcription in *B. dermatitidis* has facilitated the discovery of genes important for the phase transition and virulence including *DRK1* (dimorphism-regulating kinase), *SREB* (siderophore biosynthesis repressor in Blastomyces), *NGT1* and *NGT2* (*N*-acetylglucosamine transporters), and *BAD1* (*Blastomyces* adhesin-1; formerly WI-1).

*DRK1* encodes a histidine kinase that is essential for the conversion from hyphae to yeast following an increase in temperature from 22 to 37°C. Deletion of *DRK1* in *B. dermatitidis* and *H. capsulatum* results in fungal cells that grow as hyphae (instead of yeast) at 37°C and fail to express yeast-phase–specific virulence factors (*BAD1* for *B. dermatitidis*; *CBP1* for *H. capsulatum*). Moreover, these cells fail to produce conidia at 22°C and have altered distribution of cell wall carbohydrates including α-(1,3)-glucan and chitin. In a murine model of pulmonary infection, *B. dermatitidis* and *H. capsulatum* strains with reduced *DRK1* transcription were avirulent. This provided genetic proof that the morphologic switch is essential for virulence. *DRK1* is conserved in the dimorphic fungi with homologs identified in *Sporothrix schenckii* and *Penicillium marneffei*. *DRK1* transcript is 24-fold more abundant in *S. schenckii* yeast than hyphae. In *P. marneffei*, the *DRK1* homolog, *DRKA*, is important for germinating conidia to develop into the pathogenic yeast within macrophages at 37°C.

Over the past decade, there has been increasing interest in understanding the yeast-to-hyphal transition following a drop in temperature from 37 to 22–25°C. Growth in the hyphal phase is postulated to promote environmental survival, transmission to new hosts through spores, and genetic diversity by sexual reproduction. In *B. dermatitidis*, the *SREB* transcription factor and *N*-acetylglucosamine transporters influence the morphologic shift to hyphal growth. *SREB* encodes a GATA transcription factor that promotes the conversion from yeast to mold at 22°C and regulates the biosynthesis of specialized iron gathering molecules (i.e., siderophores). *GATA* transcription factors bind to GATA binding sites (e.g., A/T-GATA-A/G, ATC-A/T-GATA-A/G) in the promoters of target genes to induce or repress transcription. Deletion of *SREB* results in yeast cells that are unable to complete the conversion from yeast to mold after a drop in temperature and fail to accumulate substantial biomass at 22°C. The morphologic and growth defects are independent of exogenous iron concentrations. *SREB* null mutant (SREBΔ) cells at 22°C are viable and convert to yeast when temperature is increased to 37°C. RNA interference targeting *SRE1*, a *SREB* homolog, resulted in *H. capsulatum* cells that exhibited similar defects as SREBΔ. In addition to *SREB* (and *SRE1*), the morphologic switch is also influenced by exogenous *N*-acetylglucosamine (GlcNAc), which accelerates the transition from yeast to hyphae; this is mediated by *NGT1* and *NGT2* transmembrane transporters.

### BAD1 and Immune Evasion

*B. dermatitidis* upregulates the expression of BAD1 (formerly WI-1), which is an essential yeast-phase–specific virulence factor important for adhesion and immune evasion. BAD1 is secreted extracellularly and can either bind to the yeast cell wall via interaction with chitin or remain soluble. An individual yeast cell is estimated to have 4.7 × 10⁶ BAD1 molecules on its surface; BAD1 is not found on the cell surface of hyphae or conidia. The 120-kDa BAD1 protein is characterized by a core region with 41 tandem repeats (10 degenerate, 31 highly conserved) of a 24 amino acid sequence, an N-terminus with Cardin-Weintraub motif, and a C-terminus with an epidermal growth factor (EGF)-like domain. Each tandem repeat contains a tryptophan-rich motif that binds heparan sulfate, but not laminin or collagen. Thus, BAD1 functions as a cellular adhesin to bind yeast cells to host tissue. Moreover, both bound and soluble forms of BAD1 bind macrophage receptors (CD14, CR3) to depress TNF-α production by innate immune cells (macrophages, neutrophils) in a transforming growth factor-β (TGF-β)-dependent and -independent fashion, respectively. TNF-α is an important cytokine that contributes to host defense against *B. dermatitidis* infection, and neutralization of TNF-α in mice results in progressive lung infection. In addition to its effects on innate immune cells, BAD1 inhibits CD4+ T lymphocyte activation, which results in reduced interleukin-17 (IL-17) and interferon-gamma (INF-γ). Deletion of *BAD1* renders *B. dermatitidis* avirulent in a murine model of pulmonary infection. The lungs of mice infected with *BAD1* null (*BAD1Δ*) strains appear grossly normal and contain few granulomas. In addition to facilitating adhesion and immune evasion, BAD1 can bind calcium via its tandem repeats and block deposition of complement (C3) on the yeast cell wall. The biological impact of BAD1 calcium binding is unclear, but it appears to facilitate attachment of BAD1 to the yeast cell surface.

In addition to BAD1, changes in cell wall composition associated with the morphologic switch may contribute to immune evasion. During the transition from hyphae to yeast, cell wall α-(1,3)-glucan increases and β-(1,3)-glucan decreases. β-(1,3)-glucan represents 40 to 50% of the cell wall glucan in hyphae, but less than 5% in yeast. Moreover, exposed β-(1,3)-glucan molecules bind mannose-binding lectins (MBL-A, MBL-C). The reduction of β-(1,3)-glucan and its binding to MBLs may impede recognition of β-(1,3)-glucan by dectin-1 receptors on innate immune cells. Clinically, the reduced amount of β-glucan in the yeast cell wall renders the (1,3)-β-glucan diagnostic test unreliable and echinocandins ineffective for persons infected with *B. dermatitidis*.

### Host Response

Innate and adaptive immune responses are important for combating *B. dermatitidis* infection. In contrast, antibody-mediated immunity is dispensable. In the lung, alveolar macrophages and neutrophils can kill conidia; however, under experimental conditions, not all conidia are killed. Conidia that survive the innate immune defenses can germinate as yeast. Yeasts are more difficult to kill by host cells...
Clinical Presentation of Human Disease

The clinical manifestations of infection with *B. dermatitidis* can be quite variable, from asymptomatic infection to fulminating sepsis with acute respiratory distress syndrome (ARDS). *B. dermatitidis* can infect nearly every organ of the body including the eye, endocrine glands, muscles, peritoneum, and breast.\(^8\,\text{32}^\) It is because of the great variety of clinical manifestations that *B. dermatitidis* infection has been called “the great pretender.” As noted previously, more than half of infected patients are asymptomatic.\(^5,\text{84}\) Of the patients who do have symptoms, the majority present with pulmonary symptoms and up to 25 to 40% develop disseminated disease.\(^40\) Common end-organ presentations of extrapulmonary infection include the skin, bone, genitourinary tract, and central nervous system (CNS). Extrapulmonary manifestations can occur after a significant delay from the primary pulmonary infection, so much so that the remnants of the prior pulmonary infection are no longer present. It is also not uncommon for patients to manifest more than one location of infection at the time of presentation such as simultaneous pulmonary and cutaneous infection.\(^84\)

Pulmonary Blastomycosis

Pulmonary infection is reported in more than 90% of patients with documented blastomycosis.\(^85\) Pulmonary manifestations range from subclinical pneumonia to acute fulminant presentation with ARDS. In one large series of 118 people with pulmonary blastomycosis, cough (90%) was the most common symptom, followed by fever (75%), night sweats (68%), weight loss (68%), chest pain (63%), dyspnea (54%), aches or myalgias (50%), and hemoptysis (18%).\(^86\)

Acute Pneumonia

The symptoms of acute pulmonary blastomycosis may be mild and are often mistaken for other self-limited pulmonary infection such as viral or community-acquired bacterial pneumonia. The majority of patients present with cough (productive or nonproductive) and fever. Other symptoms include headache, chills, dyspnea, chest pain, and malaise. Symptoms typically resolve in 2 to 4 weeks.\(^5,\text{85},\text{87},\text{88}\) Given the brief self-limited nature of most acute pneumonias, diagnosis is rarely made in the absence of an outbreak investigation or epidemiologic study. Despite the potential for spontaneous resolution of infection, experts agree that all diagnosed cases of blastomycosis should be treated because of the risk of progressive and disseminated infection.\(^40\)

Chronic Pneumonia

Unrecognized or untreated acute pulmonary blastomycosis can progress to chronic pneumonia. Symptoms include persistent cough with or without hemoptysis, fever, night sweats, poor appetite, weight loss, and malaise. Radiographic presentations are heterogeneous and can mimic malignancy (e.g., nodules or masses) or mycobacterial infection (e.g., cavitation). Again because this clinical picture is nonspecific, blastomycosis is frequently not clinically suspected, unless the patient has other findings such as skin lesions or has failed to respond to antibacterial therapy.\(^89,\text{90}\) Thus, symptoms are often present for several months prior to diagnosis. Chronic pulmonary blastomycosis is nearly always progressive in the absence of specific antifungal treatment.

Acute Respiratory Distress Syndrome

Patients with acute pulmonary blastomycosis can present with a rapidly progressive infection resulting in respiratory failure or ARDS. Initially, patients may be misdiagnosed with community-acquired pneumonia that becomes fulminant in 5 to 7 days, or may present in florid septic shock that is clinically indistinguishable from bacterial sepsis and is often fatal. In a recent study of patients with pulmonary blastomycosis who required hospitalization, 10% patients received some care in the intensive care unit (ICU). More than one-third of all patients treated for respiratory failure in ICU died, and death usually occurred within several days.\(^91\) This presentation is seen more commonly in patients with defective cell-mediated immunity such as those with hematological malignancy, SOT, or AIDS but also occurs in patients who were previously healthy.\(^5,\text{82}^\text{45}\) In two-thirds of patients who died of ARDS caused by blastomycosis, the diagnosis was either not suspected or considered only after the patient was moribund.\(^95\) Thus, early diagnosis of blastomycosis in patients with ARDS is critical for decreasing mortality.

Disseminated Disease

*B. dermatitidis* may disseminate to any organ in the body. Early studies of blastomycosis conducted prior to effective antifungal therapy reported high rates of dissemination, but more recent studies have reported dissemination rates of 20 to 25%.\(^33,\text{88},\text{95}\) Nearly all cases of extrapulmonary blastomycosis represent disseminated disease and should be managed as such, regardless of the number of organs involved at the time of presentation.

Cutaneous Blastomycosis

The skin is the most common extrapulmonary site of infection and skin involvement occurs in as many as 60% of those with disseminated infection.\(^96,\text{97}\) Patients typically present with ulcerated lesions on any skin surface but exposed areas of the head, neck, or extremities are most common (\textit{e.g.}, ear, nose, or eye). Ulcers are characterized by well-defined edges with heaped-up borders. Other cutaneous manifestations include violaceous nodules, plaques, abscesses, large verrucous lesions, or keloids.\(*6,\text{96}^\text{99}\) Cutaneous infection is rarely the result of direct inoculation (e.g., penetrating trauma, accidental needle stick
Osseous Blastomycosis
Blastomycosis has a known predilection for bone involvement. Osseous lesions are painful and often associated with draining sinus tracts. Long bones of the legs are commonly affected, and can be complicated by extension into the joint space resulting in septic arthritis. Vertebral infection represents the second most common osseous site of involvement and may clinically resemble Pott’s disease. Complications of vertebral osteomyelitis include compression fractures and extension into the soft tissue resulting in paravertebral and psoas abscesses. A recent case series noted that 12 of 14 patients with osseous blastomycosis were initially thought to represent malignant osseous tumors, and therefore the authors advocated for intraoperative histopathologic analysis by frozen section to reduce morbidity associated with resection of the involved areas, which could otherwise be treated with antifungal chemotherapy with better preservation of function.

Genitourinary
Dissemination of B. dermatitidis to the genitourinary system is uncommon. In men, the most common sites of involvement include the prostate and epididymis. In several large case series, the prostate involvement was reported in less than 10% of patients. Patients with prostatitis have urinary obstruction, dysuria, perineal or suprapubic discomfort, and may have a tender prostatic mass on examination. Symptoms of epididymitis include testicular enlargement and pain. In women, dissemination to the genitourinary system can result in tubo-ovarian abscess and pyogranulomatous inflammation of the endometrium. Tubo-ovarian abscesses can be complicated by extension of B. dermatitidis into the abdominopelvic cavity resulting peritoneal and omental nodules along with new onset ascites.

Central Nervous System
CNS blastomycosis is estimated to occur in less than 5 to 10% of immune competent patients with disseminated blastomycosis. Dissemination to the CNS results from hematogenous spread or direct invasion from untreated osteomyelitis involving the skull. B. dermatitidis may infect any part of the CNS, but most commonly causes meningitis or brain abscess. Symptoms include headache, focal neurologic defects, confusion, altered vision, and seizures. In patients with meningitis, cerebrospinal fluid (CSF) analysis demonstrates neutrophilic or lymphocytic pleocytosis along with elevated protein and hypoglycorrhachia. Less than half of patients have positive CSF cultures; however, the B. dermatitidis antigen test on CSF may facilitate diagnosis.

Blastomycosis in Immunocompromised Hosts
HIV/AIDS
Patients with AIDS have been shown to have an increased risk of developing symptomatic disease after exposure to B. dermatitidis. Up to 25% of AIDS-related cases of blastomycosis can be caused by reactivation of a previously dormant infection. Because AIDS is associated with defective cell-mediated immunity, the infection is more likely to elude initial host attempts to contain the infection. Patients with AIDS therefore manifest disease differently than do most immunocompetent hosts. For example, there is an increase in disseminated, miliary, and fatal disease. Additionally, CNS involvement is common in this population and is estimated to occur in up to 40% of cases. ARDS and miliary disease are seen in nearly 20% of patients with AIDS at the time of diagnosis.

Solid-Organ Transplant
Transplant-associated blastomycosis typically presents as pneumonia and it is frequently complicated by severe disease including respiratory failure and ARDS. The development of ARDS increases mortality from 33–38% to 67%. Extrapulmonary disease occurs in 33 to 50% and most commonly affects the skin. In contrast to patients with AIDS, dissemination of B. dermatitidis to the CNS is uncommon in solid-organ transplant recipients.

Patients Receiving TNF Inhibitors
There is a paucity of clinical data on patients who developed blastomycosis while receiving TNF inhibitor therapy; however, blastomycosis was listed in the warning issued by the Food Drug Administration on September 4, 2008, regarding increased risk of fulminant infections with the endemic mycosis in patients receiving TNF inhibitor therapy.

Blastomycosis in Pregnancy
Blastomycosis in pregnancy is rare and clinical information is limited to case reports. Infection is most commonly diagnosed in the second or third trimester and disseminated disease occurs in 62% of patients. Although B. dermatitidis...
yeasts have been identified in the placenta of an infected patient, the frequency of placental dissemination is unknown.\textsuperscript{116,117} There is potential for the transmission of blastomycosis to the neonate during the peripartum period; however, the underlying pathogenesis is unclear and may involve either transplacental transmission or aspiration of infected vaginal secretions during delivery.\textsuperscript{116–119}

**Diagnosis**

Owing to the nonspecific clinical and radiographic manifestations, a delay in the diagnosis as well as initiation of therapy for blastomycosis is common. Even in hyperendemic areas, as few as 5% of patients are correctly diagnosed at initial presentation and diagnostic delays longer than 1 month can occur in more than 50% of patients.\textsuperscript{88,89,95} Coexisting pulmonary and cutaneous involvement is a clinical clue that can facilitate the recognition of blastomycosis. In addition, blastomycosis in a family pet such as a dog suggests a common source of exposure.\textsuperscript{18} A detailed history regarding potential exposures such as place of residence, travel, hobbies, recent home remodeling, nearby road construction, use of a wood burning stove, or community compost pile should be obtained in patients with pneumonia. This may facilitate the diagnosis of blastomycosis, which requires a high index of clinical suspicion.

The most expeditious method to diagnose blastomycosis remains examination of stained clinical specimens. Sputum can be stained with 10% potassium hydroxide or calcofluor white, whereas tissue samples can be stained with Gomori methenamine silver (GMS) or periodic acid–Schiff (PAS).\textsuperscript{120} *B. dermatitidis* yeasts are difficult to visualize with Gram or hematoxylin and eosin (H&E) stains. The characteristic broad-based budding pattern of yeast can lead to presumptive diagnosis before culture and nonculture diagnostic test results are available. This strategy correlated well with culture results in a recent review which demonstrated positive histopathology in nearly 80% of culture-confirmed cases.\textsuperscript{121} Despite the utility of fungal-specific stains for diagnostic testing, this technique is often underutilized.\textsuperscript{122} In tissue specimens, the presence of neutrophils with granulomatous inflammation should prompt a detailed examination for broad-based budding yeast.

**Culture** of *B. dermatitidis* provides a definitive diagnosis. The yield on culture from bronchoscopy is excellent with cultures of bronchial secretions being positive in 100% and bronchoalveolar lavage (BAL) fluid in 67% of patients.\textsuperscript{122} Even when the specimens were obtained by noninvasive methods, sputum cultures grew *B. dermatitidis* in 86% of patients.\textsuperscript{122} Culture requires the use of specialized media such as Sabouraud dextrose agar, potato dextrose agar, and brain–heart infusion media.\textsuperscript{120} Most clinical laboratories incubate fungal cultures at 25 to 30°C, which results in growth of *B. dermatitidis* as a mold. Although highly specific, growth of *B. dermatitidis* in culture is slow and takes on average 5 to 14 days before fungal colonies can be visualized; however, if there is a low burden of infection, growth can take longer than 4 weeks.\textsuperscript{120}

Molecular diagnostic systems have been developed to facilitate the rapid identification of *B. dermatitidis* from clinical specimens including culture. Currently, a chemiluminescent DNA probe assay is the most frequently used molecular test to confirm the growth of *B. dermatitidis* in culture; however, this test cross-reacts with *P. brasiliensis*.\textsuperscript{120} In addition, real-time polymerase chain reaction (PCR) assays targeting BAD1 and DRK1 for amplification have been developed; however, they are not commercially available.\textsuperscript{123,124}

**Serological Testing**

Antibody testing by complement fixation or immunodiffusion is not clinically useful because of poor sensitivity and specificity.\textsuperscript{125} Recently, a newer antibody-based assay that uses microplates coated with the *B. dermatitidis* BAD1 protein has enhanced sensitivity and specificity of 87% and 94–99% in patients with active blastomycosis.\textsuperscript{126} In contrast, the sensitivity of the immunodiffusion assay is 15%.\textsuperscript{125,126} Moreover, this diagnostic assay can distinguish patients with blastomycosis from histoplasmosis. The combination of the BAD1 antibody test with the Blastomyces antigen assay can increase diagnostic sensitivity to 97%.\textsuperscript{126}

**Antigen Testing**

In 2004, an antigen assay against a galactomannan component of the *B. dermatitidis* cell wall was developed and can be used to test urine, serum, BAL fluid, and CSF. The sensitivity of the urine antigen in patients with proven disease is 85.1 to 92.9% with a specificity of 79.3%.\textsuperscript{127,128} Sensitivity of the urine antigen test can be enhanced by concentration of the urine, whereas the serum antigen test has improved sensitivity when blood samples are treated with EDTA to dissociate immune complexes.\textsuperscript{127} False-positive tests can occur in patients with other fungal infections such as histoplasmosis, paracoccidioidomycosis, and penicilliosis. However, the clinical impact of a false-positive antigen test is mitigated by the fact that initial management with these organisms is similar. Therefore, a positive *Blastomyces* antigen test would still facilitate early initiation of appropriate antifungal therapy.\textsuperscript{129,130} Antigen testing has been reported to enhance the diagnosis of blastomycosis when testing is done on BAL fluid or CSF.\textsuperscript{108,131,132} Two patients have recently been reported to have “false-positive” BAL Platelia Aspergillus enzyme immunoassay tests. This phenomenon has been previously noted with histoplasmosis and this test may suggest the diagnosis as well as prompt initiation of antifungal therapy.\textsuperscript{133,134}

**Radiographic Presentation**

No pathognomonic radiographic imaging patterns have been reported for pulmonary blastomycosis. The radiographic appearance is not specific and may mimic bacterial pneumonia, tuberculosis, or lung cancer. Classic radiographic findings for pulmonary blastomycosis include diffuse or focal airspace disease (i.e., consolidation), nodular masses, interstitial disease, cavitation, and miliary disease (\textsuperscript{Fig. 4}). Air-space disease is the most common radiographic pattern and can be present on chest radiographs in patients without
pulmonary symptoms. The next most frequent radiographic presentation is either single or multiple mass lesions, which can mimic malignancy. Cavitary disease is associated with chronic blastomycosis and can be mistaken for tuberculosis. In contrast to histoplasmosis, hilar and mediastinal adenopathy as well as calcified lung lesions are uncommon in patients with blastomycosis. Pleural involvement and effusion are also rare. For patients with CNS blastomycosis, MRI imaging is the preferred diagnostic technique and nearly all patients with CNS involvement have abnormal imaging findings.

**Treatment**

In 2008, Infectious Diseases Society of America published guidelines for the diagnosis and treatment of blastomycosis. Treatment recommendations are based on the site and severity of infection, immune status of the host, and pregnancy. Prior to starting amphotericin B (AmB) or azole–base therapy, baseline laboratory testing of hepatic and renal function along with a thorough evaluation for potential drug–drug interactions should be performed. All women of childbearing age must be tested for pregnancy because prolonged use of high-dose azole antifungals can induce fetal deformities or cause spontaneous abortion.

**Itraconazole**

Itraconazole has demonstrated excellent efficacy and tolerability and is the first-line agent for the treatment of mild to moderate, non-CNS blastomycosis, and for step-down therapy after treatment with AmB. Oral administration of itraconazole results in variable serum concentrations and the use of therapeutic drug monitoring is necessary to ensure appropriate dosing. Serum concentrations are approximately 30% higher with use of the solution formulation than with the capsule formulation, but wide interpatient variability is evident with any of the formulations. Itraconazole capsules should be taken with food and an acidic beverage (e.g., orange juice, soda). Gastric acidity is required for optimal absorption of the capsule formulation. In contrast, itraconazole solution can be taken without food and does not require gastric acidity for absorption. Thus, in patients who are taking H₂-blockers or proton-pump inhibitors, itraconazole solution is the preferred formulation. Itraconazole concentrations in serum should be determined after 2 weeks of therapy, which is when a steady state concentration is reached. Because of the long half-life of itraconazole (~24 hours), serum levels can be obtained independent of the time of drug administration. A total serum level of >1.0 μg/mL is recommended (both itraconazole and hydroxy-itraconazole levels are added together). Serum levels >10.0 μg/mL are unnecessary and increase the risk for drug toxicity. In addition to therapeutic drug monitoring, liver function tests should be obtained at baseline, 2 and 4 weeks into therapy, and then every 3 months.

**Amphotericin B Formulations**

AmB products are recommended for all serious infections with *B. dermatitidis*, which includes patients with underlying immunocompromise, severe pulmonary or disseminated infection, and CNS involvement. In addition, AmB formulations are the drug of choice for neonates and pregnant women. Large case series have reported excellent clinical response to AmB products with clinical cure rates between 77 and 91%. Despite the well-demonstrated efficacy, the use of AmB is associated with significant cumulative toxicity. Nephrotoxicity is the most treatment-limiting toxicity and occurs in more than 30% of treated patients. Other adverse effects include nausea, vomiting, rigors, fever, hypertension or hypotension, electrolyte abnormalities, hepatotoxicity, and hypoxia. Renal toxicity is mitigated by saline loading patients, avoiding diuretics and other nephrotoxic agents. Careful attention must be paid to electrolyte management because of the frequent occurrence of hypokalemia and hypomagnesemia.

Lipid formulations of AmB (e.g., liposomal amphotericin, AmB lipid complex, and AmB colloidal dispersion) have largely replaced AmB deoxycholate in the treatment of blastomycosis, mainly because of a lower incidence of toxicity. Although lipid formulations have not been directly compared with AmB deoxycholate in patients with blastomycosis, a study in AIDS patients with disseminated histoplasmosis demonstrated improved outcomes for those treated with liposomal AmB versus AmB deoxycholate. At least one case report has described the successful use of continuous infusion AmB deoxycholate in a patient with blastomycosis who was failing therapy with liposomal AmB. The authors postulated that although lipid formulations produce much higher drug concentrations than conventional AmB, they might be physiologically less active while bound to plasma proteins or contained within liposomes. The role of continuous infusion AmB deoxycholate represents an intriguing area of research in blastomycosis treatment.

**New Triazoles**

The new triazoles, voriconazole and posaconazole, have been demonstrated to have excellent in vivo and in vitro activity against *B. dermatitidis*. There have been several reports of successful use of voriconazole for the treatment of blastomycosis. Owing to its excellent CNS penetration, it has been used most frequently to treat CNS disease. Use of posaconazole for the treatment of
Blastomycosis has also recently been reported. Voriconazole should be taken without food to optimize absorption. In contrast, the liquid formulation of posaconazole should be administered with a high-fat meal. The absorption of the new tablet formulation of posaconazole is not affected by food or administration of gastric acid inhibitors (e.g., H2-blockers, proton-pump inhibitors). Similar to itraconazole, therapeutic drug monitoring along with periodic assessment of liver

Table 2 Summary of clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America

<table>
<thead>
<tr>
<th>Site of infection</th>
<th>Disease severity</th>
<th>Initial therapy</th>
<th>Step-down therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary blastomycosis</td>
<td>Mild to moderate</td>
<td>Oral itraconazole 200 mg 3 times per day for 3 d and then once or twice per day for 6–12 mo*</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Moderately severe to severe</td>
<td>Lipid formulation of AmB 3–5 mg/kg per day or AmB deoxycholate 0.7–1 mg/kg per day for 1–2 wk or until improvement is noted.</td>
<td>Oral itraconazole 200 mg 3 times per day for 3 d and then 200 mg twice per day for 6–12 mo*.</td>
</tr>
<tr>
<td>Disseminated extrapulmonary blastomycosis</td>
<td>Mild to moderate</td>
<td>Oral itraconazole 200 mg 3 times per day for 3 d and then once or twice per day for 6–12 mo*.</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Moderately severe to severe</td>
<td>Lipid formulation AmB 3–5 mg/kg per day, or AmB deoxycholate 0.7–1 mg/kg per day for 1–2 wk or until improvement is noted.</td>
<td>Oral itraconazole 200 mg 3 times per day for 3 d and then 200 mg twice per day for a total of at least 12 mo*.</td>
</tr>
<tr>
<td>CNS disease</td>
<td></td>
<td>Lipid formulation AmB 5 mg/kg per day for 4–6 wk</td>
<td>Possible options include Fluconazole (800 mg per day) Itraconazole (200 mg 2 or 3 times per day Voriconazole (200–400 mg twice per day) for at least 12 months and until resolution of CSF abnormalities</td>
</tr>
<tr>
<td>Immunocompromised patients</td>
<td></td>
<td>Lipid formulation AmB 3–5 mg/kg per day or AmB deoxycholate 0.7–1 mg/kg per day for 1–2 wk or until improvement is noted.</td>
<td>Oral itraconazole, 200 mg 3 times daily for 3 d and then 200 mg twice daily for a total of 12 mo*.c</td>
</tr>
<tr>
<td>Pregnant women</td>
<td></td>
<td>Lipid formulation AmB 3–5 mg/kg per day</td>
<td>Azoles should be avoided because of risks for teratogenicity and spontaneous abortion</td>
</tr>
<tr>
<td>Newborn</td>
<td></td>
<td>AmB deoxycholate 1.0 mg/kg per day</td>
<td>N/A</td>
</tr>
<tr>
<td>Children</td>
<td>Mild to moderate</td>
<td>Oral itraconazole 10 mg/kg per day (maximum of 400 mg/d) for 6–12 mo*</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>AmB deoxycholate 0.7–1.0 mg/kg per day or lipid formulation AmB, at a dosage of 3–5 mg/kg per day until improvement</td>
<td>Oral itraconazole 10 mg/kg per day (up to 400 mg/d) as step-down therapy for a total of 12 mo*</td>
</tr>
</tbody>
</table>

Abbreviations: CNS, central nervous system; N/A, not applicable.
*aTherapeutic drug monitoring of serum itraconazole levels is required.
bPatients with osteoarticular blastomycosis should receive a total of at least 12 months of antifungal therapy.
cLife-long suppressive therapy with oral itraconazole, 200 mg/day may need to be considered in select patients including those with immunosuppression that cannot be reversed and in those who experience relapse despite appropriate therapy.
function tests is needed for patients treated with voriconazole and posaconazole. The goal serum trough concentration for voriconazole is >1 and <5.5 μg/mL. The optimal serum concentration that correlates with clinical efficacy for posaconazole is unclear, but most experts recommend serum trough levels >0.5 to 1 μg/mL. Isavuconazole and ravuconazole have potent in vitro activity against B. dermatitidis and have the potential to be added to the therapeutic armamentarium against blastomycosis.

Echinocandins
The echinocandins have poor in vitro activity against B. dermatitidis yeast and are not recommended for treatment of blastomycosis. At least one case report has described successful use of caspofungin for chronic pulmonary blastomycosis in a patient intolerant of other therapies; this should be considered only a last resort. The authors do not advocate this strategy because of the minimal amount of drug target, β-(1,3)-glucan, in the cell wall of B. dermatitidis.

Adjunctive Therapy
Despite adequate antifungal therapy, the mortality rate of blastomycosis-induced ARDS remains high. There is considerable interest in the role of adjunctive steroids for the treatment of patients with ARDS caused by B. dermatitidis. Although clinical data are limited, a few case reports suggest the potential for adjunctive steroids to improve survival. However, it is unclear if these findings can be applied to all patients with ARDS from B. dermatitidis. Moreover, the steroid doses used in these case reports were not standardized and were higher than recommended for patients with severe pulmonary histoplasmosis. Additional research is needed regarding the dose, duration, and efficacy of adjunctive steroids for the treatment of patients with severe pulmonary blastomycosis complicated by respiratory failure and ARDS. In addition to steroids, extracorporeal membrane oxygenation has been used in a single patient with blastomycosis-induced ARDS; however, this did not result in patient survival.

Mortality
The case fatality rate reported in a large group of patients from Wisconsin from 1986 to 1995 was 4.3% (29 of 670 patients) and from a large series in Manitoba the observed mortality rate was 6.3%. Mortality has been associated with shorter duration of symptoms likely suggesting more fulminant presentation as well as with a compromised immune status of the host. Blastomycosis-induced ARDS is associated with 50 to 89% mortality rate, even in patients receiving appropriate therapy. The mortality of blastomycosis in patients who have AIDS is nearly 40%, and most deaths occur within 3 weeks of diagnosis.

Conclusion
Over the past two decades, there have been significant advances in the pathogenesis, immunology, diagnosis, and treatment of B. dermatitidis. Basic science research that led to the discovery of BAD1 and DRK1 is beginning to impact clinical diagnostics with the implementation of the BAD1 antibody test and the use of DRK1 as a target for real-time PCR analysis of clinical specimens. Although the Blastomyces antigen test has revolutionized how clinicians diagnose blastomycosis, fungal smear and culture remain important. The ability to recognize the diverse clinical manifestations and consider blastomycosis as a potential diagnosis is essential. The newer azole antifungals such as voriconazole have been successfully used for treatment of blastomycosis, including patients with CNS infection. In contrast, the echinocandins, which represent the newest class of antifungals, have poor activity against B. dermatitidis yeast. AmB lipid formulations have facilitated the treatment of patients with severe blastomycosis while minimizing (but not eliminating) nephrotoxicity. Despite these advances, the treatment of patients with blastomycosis-induced ARDS remains challenging and new approaches are needed to reduce mortality in this patient population.
Blastomycosis

29 Pappas PG, Threlkeld MG, Bedsole GD, Cleveland KO, Gelfand MS, Dismukes WE. Blastomycosis in immunocompromised patients. Medicine (Baltimore) 1993;72(5):311–325


Propek PA, Scanlan KA. Blastomycosis of the breast. AJR Am J Roentgenol 1996; 166(3): 726


Blastomycosis

Smith, Gauthier 727

115 FDA Alert September 2008. Tumor necrosis factor-alpha blockers (TNF blockers), Cimzia (certolizumab pegol), Enbrel (etanercept), Humira (adalimumab), and Remicade (infliximab)
123 Babady NE, Buckwalter SP, Hall L, Le Febre KM, Binnicrjer MJ, Wengenack NL. Detection of Blastomyces dermatitidis and Histoplasma capsulatum from culture isolates and clinical specimens by use of real-time PCR. J Clin Microbiol 2011;49(9):3204–3208

Seminars in Respiratory and Critical Care Medicine Vol. 36 No. 5/2015

Downloaded by: IP-Proxy University of Wisconsin - Madison, University of Wisconsin-Madison. Copyrighted material.


Espinel-Ingroff A. Comparison of In vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. J Clin Microbiol 1998;36(10):2950–2956


