TABLE 257.1 Characteristics of *Aspergillus* Species Associated with Invasive Infection

ASPERGILLUS SPECIES	FREQUENCY OF SPECIES COMPLEX ISOLATED IN CLINICAL INFECTION (%)	COLONY CHARACTERISTICS	MICROSCOPIC FEATURES	CLINICAL SIGNIFICANCE
A. fumigatus (see Fig. 257.1A and B)	50–67	Smoky gray green; may have pale yellow or lavender reverse; grows at 50°C	Columnar; uniseriate; smooth to finely roughened conidia 2–3.5 µm	Most common invasive species; most pathogenic
A. flavus (see Fig. 257.2A and B)	8–14	Olive to lime green	Radiate to loosely columnar; uniseriate or biseriate; rough conidiophore; conidia 3–6 µm	Sinusitis; skin infection; produces aflatoxin
A. terreus (see Fig. 257.3A– C)	3–5	Beige to cinnamon buff	Columnar; biseriate; globose; small 2–2.5 µm conidia; globose accessory conidia along hyphae	Increasingly detected; resistant to amphotericin B; more susceptible to newer azoles
A. niger. (see Fig. 257.4A and B)	5–9	Initially white, rapidly turning black with yellow reverse	Radiate; biseriate; globose, black, very rough conidia 4–5 µm	Uncommon in invasive infections; superficial agent of otic disease; colonization

Data from Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine (Baltimore). *2000;79:250–260 [invasive infections: United States]; Balajee SA, Kano R, Baddley JW, et al. Molecular identification of Aspergillus species collected for the Transplant-Associated Infection Surveillance Network.* J Clin Microbiol. *2009;47:3138–3141 [transplant-associated infections: United States]; Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M. Current section and species complex concepts in Aspergillus: recommendations for routine daily practice.* Ann NY Acad Sci. *2012;1273:18–24 [invasive infections: Spain].*

such as sequencing of ribosomal, β-tubulin, calmodulin, or rodlet A genes, which has allowed more natural subgroupings of ascomycetous fungi.[10,11,16–18] It is important to note that with identity established by means of molecular sequencing, the result of a familiar species may be reported as an unfamiliar teleomorph, which has led to assigning one name to one fungus to clarify this potential confusion in clinical mycology.[19,20]

The genus *Aspergillus* is an anamorphic member (asexual form) of the family Trichocomaceae. The teleomorphs of *Aspergillus* spp. are classified in eight genera in the order Eurotiales in the phylum Ascomycota. Identification of the genus and of common pathogenic species is usually not difficult, but species-level identification of less common members can be laborious, and misidentification of atypical or "cryptic" members of sections, such as poorly sporulating forms, is common using solely phenotypic methods.[11,18,21]

The most common species causing invasive infection is *Aspergillus fumigatus*, the most common pathogen in the section *Fumigati*, which historically has made up a vast majority of invasive isolates: *A. flavus; Aspergillus terreus*; and, less commonly for invasive infection, *Aspergillus niger*.[3] Recent studies have shown emergence of less common species, including *A. terreus* and unusual less pathogenic species as the etiologic agents of invasive infection, including many cryptic species that are identifiable only by molecular studies.[18,21–23] With more prolonged and profound immunosuppression, along with molecular identification of cryptic species within a species complex, the list of rare species causing invasive infection continues to increase, including *A. alabamensis, A. alliaceus* (teleomorph *Petromyces alliaceus*), *A. avenaceus, A. caesiellus, A. candidus, A. carneus, A. clavatus, A. calidoustus, A. flavipes, A. glaucus, A. granulosus, A. insuetus*, *A. keveii*, *A. lentulus, A. nidulans (Emericella nidulans), A. novofumigatus, A. ochraceus, A. puniceus, A. pseudodeflectus, A. restrictus, A. sydowii, A. quadrilineatus, A. tamarii, A. tanneri*, *A. udagawae (Neosartorya udagawae), A. tubingensis*, *A. versicolor, A. viridinutans*, *A. vitus* (teleomorph *Eurotium amstelodami*), *A. wentii, A. thermomutatus* (teleomorph *Neosartorya pseudofischeri*), and many others, although the authenticity of at least some of these has been questioned and perhaps misidentified before molecular studies.[9,11,18,24–28]

Pathogenic *Aspergillus* spp. are easily cultured from pathologic samples and grow rapidly (24–72 hours) on a variety of media. Blood cultures are uncommonly positive and usually reflect contamination rather than invasive disease.[29] A distinguishing characteristic of pathogenic *Aspergillus* spp. is their ability to grow at 37°C. *A. fumigatus* is able to grow at 50°C, a feature that, in addition to morphology, can also be used to identify this species and can help distinguish *A. fumigatus* from cryptic *Aspergillus* spp. within the *A. fumigatus* complex.[21,25] Most species initially appear as small, fluffy white colonies on culture plates within 48 hours. Presumptive identification of an *Aspergillus* species complex is usually accomplished by appearance of the fungus on gross and microscopic inspection of the colony, which provides typical sporulation, although specific species-level identification requires molecular confirmation so that laboratories should report isolates identified phenotypically as a species complex.[11]

Microscopic features and colony morphology for the most common clinical isolates—*A. fumigatus, A. flavus, A. terreus*, and *A. niger*—are described in Table 257.1 and shown in Figs. 257.1 to 257.4. Species-level identification of *Aspergillus* has become increasingly important due to antifungal drug susceptibility differences.[11,30]

Aspergillus fumigatus is the most frequent species in invasive infection, constituting >90% in some series, although, recently, a prevalence of 50% to 67% has been described.[3,11,18,29] Colonies of *A. fumigatus* are typically gray-green with a wooly to cottony texture (see Fig. 257.1A). Like other species of *Aspergillus*, hyphae are hyaline (lightly pigmented), have septa, and are usually branched at acute (typically 45-degree) angles. The conidial head is columnar with conidiophores that are smooth walled and uncolored, or darkened in the upper portion near the vesicle. This species is uniseriate (a term describing phialides that are attached directly to the vesicle), with closely compacted phialides borne only on the upper portion of the vesicle (see Fig. 257.1B). Conidia are smooth to finely roughened and are 2 to 3.5 µm in diameter. The fruiting head (the conidiophore and conidia) is not commonly seen in clinical specimens, although it may be detected in sites exposed to air, such as wounds or lung cavities. Like other *Aspergillus* spp., it is widespread in nature—found in soil, on decaying vegetation, in the air, and, more recently, in water supplies.[31,32]

Other cryptic members of the section *Fumigati*, meaning identification by molecular methods, have been described that are human pathogens, including *A. lentulus, A. calidoustus, A. tubingensis, A. novofumigatus, A. thermomutatus* (teleomorph *N. pseudofischeri*), *A. viridinutans*, and others.[11,18,21,33–35] These species are frequently poorly sporulating and fail to grow at 50°C.[21,25] Of importance, some of these species, such as *A. lentulus*, *A. viridinutans*, and *A. thermomutatus*, may exhibit decreased antifungal susceptibility and can be associated with a poor clinical outcome.[11,30,36] When these species are identified only phenotypically, they should be referred to as members of the *Aspergillus fumigatus* species complex.

Aspergillus flavus is a common isolate in sinusitis, skin, and other invasive infections. This species, which produces an aflatoxin, is found in soil and decaying vegetation.[37] Colonies are olive to lime green and grow at a rapid rate (see Fig. 257.2A). Some isolates are uniseriate but are more typically biseriate. In biseriate species sterile cells known as metulae are attached to the vesicle, and these, in turn, support the

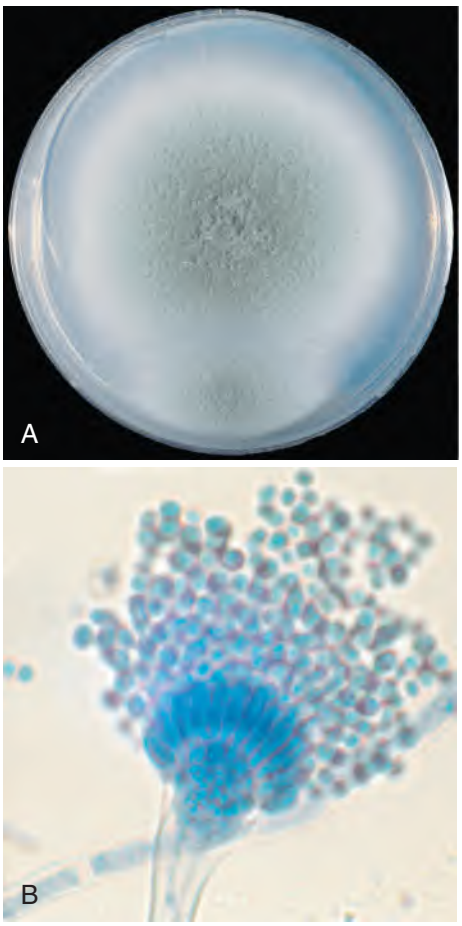

FIG. 257.1 Aspergillus fumigatus. (A) Gray-green colony morphology on potato flakes agar. (B) Uniseriate conidiophore with columnar conidia (all photomicrograph magnifications ×420). *(Courtesy Dr. Deanna Sutton.)*

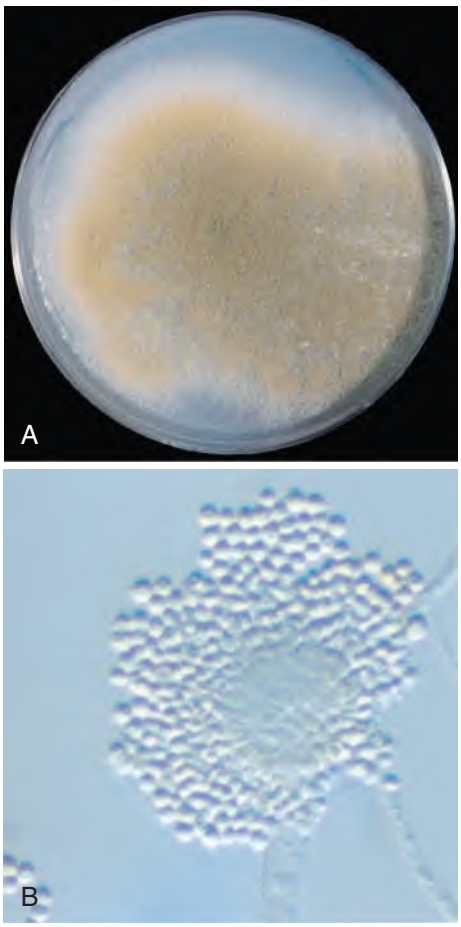

FIG. 257.2 Aspergillus flavus. (A) Olive–lime green colony on potato flakes agar. (B) Radiate, biseriate conidia. *(Courtesy Dr. Deanna Sutton.)*

phialides. *A. flavus* has noticeably rough conidiophores and smooth conidia (3–6 µm) (see Fig. 257.2B). If characterized by morphology alone, *A. flavus* and other species in the section *Flavi* should be referred to as members of the *Aspergillus flavus* species complex. Other species, such as *A. alliaceus*, have been reported to cause clinical infection and may have decreased susceptibility to polyenes.[11,38]

Aspergillus terreus is a common soil-related isolate that has been increasingly reported in invasive infection in immunocompromised hosts.[22] *A. terreus* conidia are small (2.0–2.5 µm), and the colony color and fruiting structures are characteristic for this species (see Fig. 257.3). Colonies range in color from buff to beige to cinnamon (see Fig. 257.3A).[11] Conidiophores are smooth walled and hyaline, and the conidial heads are biseriate and columnar (see Fig. 257.3B). A distinguishing feature of this species is the presence of globose accessory conidia that are produced on hyphae. These accessory conidia can be detected in histopathologic samples, which may be used to establish presumptive identification of the species.[39] Identification of this species has become increasingly important because of its resistance to antifungals, including amphotericin B, although improved susceptibility with newer azoles has been reported.[40–42] Molecular characterization has shown *A. terreus* is also a species complex with species such as *A. alabamensis* and *A. carneus* causing infection in immunocompromised hosts and, like *A. terreus*, is less susceptible to polyene antifungals.[11,28,43]

Aspergillus niger (see Fig. 257.4A and B) is found in soil, on plants, and even in food and condiments, such as pepper. Colonies are initially white but quickly become black, with the production of the pigmented fruiting structures (see Fig. 257.4A). It grows rapidly with a pale yellow reverse. Conidial heads are biseriate and cover the entire vesicle. Conidia are brown to black and are very rough (4–5 µm) (see Fig. 257.4B), although the hyphae are hyaline. The species may produce oxalate crystals in clinical specimens.[44] The role of *A. niger* in invasive infection is less well established, with its decreased pathogenicity perhaps due in part to the fact that its larger conidia do not readily reach deep into lung tissues. It is a common colonizing isolate and can cause superficial infection, such as otitis externa.[29,45] This species complex also contains several related species, including *A. tubingensis*, which is one of the more common cryptic aspergilli identified, suggesting it may have a role in invasive infection.[11,30,46]

Other species of *Aspergillus* are less common in invasive infection but are increasingly reported, perhaps reflecting increased awareness and molecular techniques confirming their identity.[3,11,18,29] *Aspergillus nidulans* (section *Nidulantes*) and *A. tanneri*, (section *Circumdati*, species complex *terreus*), for example, have been reported as causes of infection in patients with chronic granulomatous disease and are species that may be resistant to amphotericin B.[26,30,47] *Aspergillus calidoustus* is a species in the section *Ustus*, which grows at 37°C (formerly called *A. ustus*, a species that fails to grow at 37°C) and exhibits high minimum inhibitory concentrations (MICs) to several classes of antifungal agents.[48–50] Thus even previously "nonpathogenic" *Aspergillus* spp. must be considered potentially clinically significant in an appropriate clinical setting and host.[9,18]

EPIDEMIOLOGY

Aspergillus is ubiquitous worldwide—found in soil, water, food, and air—and is particularly common in decaying vegetation. The inoculum for infection is not known, but hosts with normal pulmonary host defenses rarely develop disease despite exposure to the organism with normal daily living through airborne conidia, including foodstuffs like pepper, herbs, and so forth.[51] Patients with altered host immunity, particularly those with reduced pulmonary host defenses (e.g., those

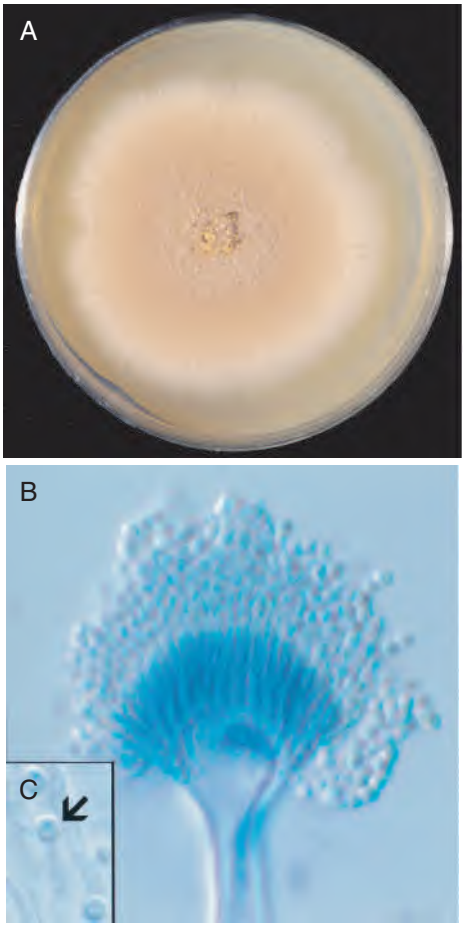

FIG. 257.3 Aspergillus terreus. (A) Buff-cinnamon colony on potato flakes agar. (B) Columnar, biseriate smooth conidia. (C) Globose, sessile accessory conidia along hyphae. *(Courtesy Dr. Deanna Sutton.)*

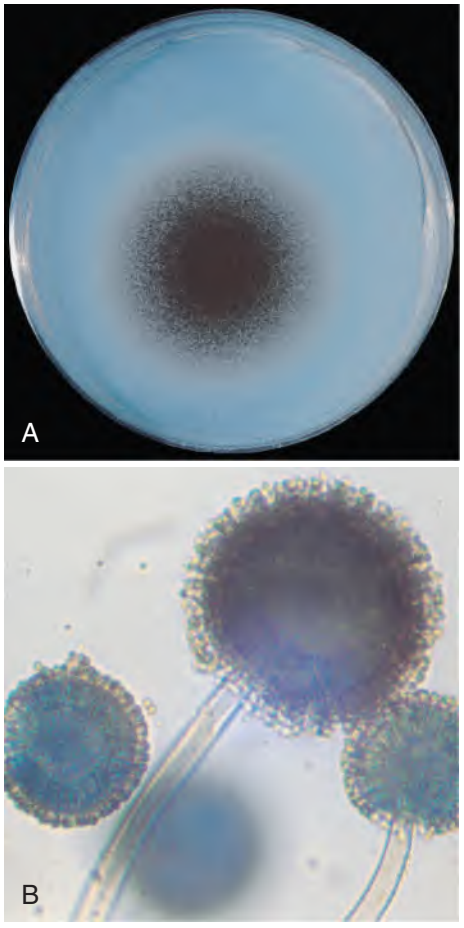

FIG. 257.4 Aspergillus niger. (A) Black colony on potato flakes agar. (B) Large, radiate, biseriate and uniseriate conidia. *(Courtesy Dr. Deanna Sutton.)*

who use corticosteroids) that inhibit the activity of pulmonary macrophages or those who are neutropenic, have increased susceptibility to these organisms.[52]

Patients with prolonged and profound neutropenia are at high risk for IA, but changing treatment patterns in chemotherapy and transplantation, along with the use of growth factors, have limited the numbers of persistently neutropenic patients.[53,54] It is important to recognize that, even among high-risk patients with hematologic malignancy, there is substantial heterogeneity of risk.[55,56] In patients with acute myelogenous leukemia, the incidence of IA has been reported to range from as low as 1% to 2% to rates as high as 25% to 28% or more, with mean incidence in most series at 4% to 7%.[54,57–59] Most cases of IA occur in the period of neutropenia after primary induction chemotherapy or in patients with refractory or recurrence of malignancy, with the risk of aspergillosis during consolidation chemotherapy very low.[55,56]

In patients undergoing HSCT or bone marrow transplantation, a recent increase in the incidence of IA has been reported, and the epidemiology of infection has changed (Table 257.2).[54,60] In patients undergoing HSCT the major periods of risk are bimodal, with peak incidence occurring fewer than 40 days but more than 100 days after transplantation, with mean time to diagnosis more than 180 days in some series.[53,54,61–63] In the Transplant-Associated Infection Surveillance Network (TRANSNET) study the median time to onset of IA was 99 days, with 22% and 53% of 335 IA cases occurring 1 and 4 months after transplantation, respectively.[54] One of the factors in the changing epidemiology in this patient population is the use of nonmyeloablative transplant procedures, which has shifted the major risk factor in these patients from neutropenia to that of the use of high doses of corticosteroids or other immunosuppressive therapy for the treatment of acute or chronic graft-versus-host disease (GVHD).[53] Only 31% of the HSCT recipients with IA reported by Wald and colleagues[53] were neutropenic. Acute or chronic GVHD may occur late after HSCT and increases the risk for *Aspergillus* infection.[53,54,61]

TABLE 257.2 Incidence and Mortality of Invasive Aspergillosis in Transplantation

TYPE OF TRANSPLANT	INCIDENCE (%)		12-WEEK MORTALITY (%)
	RANGE	MEAN	
Allogeneic stem cell	2.3–11	7	40–75
Autologous stem cell	0.5–2	1	50
Lung	2.4–9	6	5–21
Liver	1–8	4	45–59
Heart	0.3–6	2	15–21
Kidney	0.1–2	0.5	25–48

Data from references 54, 62, 63, 65–67, and 134 .

Other immunosuppressed patients are also at risk for IA, including patients undergoing organ transplantation, particularly lung transplantation (see Table 257.2).[64,65] The increased risk in lung transplantation is because the transplanted organ is constantly exposed to the environment, ciliary clearance is reduced, and many of these patients are colonized with *Aspergillus* in either the native or transplanted lung.[66] In most organ transplant recipients the diagnosis of IA is a late occurrence; the mean time to diagnosis is typically more than 180 days and may occur a year or more after the organ transplant, although the median onset in liver transplants appears shorter (~100 days).[65,67] Other patients at

higher risk for IA include patients receiving corticosteroids or biologic agents, including the tumor necrosis factor-α (TNF-α) antagonists, such as infliximab, etanercept, adalimumab, and others, including ibrutinib.[68] Newer biologic agents have variable risks for host acquisition of invasive fungal infections, and risks remain incompletely defined. IA occurs in other immunosuppressed patients, including patients with pulmonary diseases, acquired immunodeficiency syndrome (AIDS), chronic granulomatous disease, and other hereditary immunodeficiency syndromes.[3,52] IA can occur in critically ill patients in intensive care units (ICUs) independent of traditional risk factors, suggesting additional cumulative risk factors for disease in this setting, although the overall impact of IA in that population is unclear and varies in different series.[69,70]

Outbreaks of IA have occurred in patients exposed to aerosols of *Aspergillus* conidia in association with construction and other environmental risks.[71–74] In severely immunosuppressed patients aspergillosis may occur from other exposures as well, including aerosols of contaminated water.[32,75] Aspergillosis may also occur as endogenous reactivation from prior infection or colonization. Therefore it may not be possible to prevent all cases by reducing environmental exposures within the hospital setting.[76] It should also be noted that, with the prolonged period of risk, these infections become very difficult to prevent with protective environments because much of their health care will occur in a non–hospital-based setting.[76]

PATHOGENICITY AND HOST DEFENSES

The usual route of infection for IA is through inhalation of *Aspergillus* conidia into the lungs. Although less common, invasive infection may also follow local tissue invasion, such as through surgical wounds or contaminated intravenous (IV) catheters or armboards, leading to cutaneous infection.[77]

In the absence of effective host defenses after pulmonary exposure, the inhaled small resting conidia enlarge and germinate, resulting in transformation into hyphae with subsequent vascular invasion and eventual disseminated infection. The incubation period for conidial germination in pulmonary tissue is variable, estimated to range from 2 days to months and may even vary by species and strain.[39] The growth rate at 37°C may be one determinant of the rate of disease progression and possible pathogenicity of the organism. Hydrocortisone significantly increases the growth rates of *Aspergillus*, further enhancing the role of corticosteroids as a risk factor for invasive disease.[78] The process of hyphal growth and tissue invasion results in a hallmark feature of IA—vascular invasion (Fig. 257.5) and pulmonary infarction (Fig. 257.6), which are classic features of invasive pulmonary aspergillosis due to the angioinvasive nature of the organism.

Although infection in apparently normal hosts can occur, IA is uncommon in immunocompetent hosts.[3] Normal pulmonary defense mechanisms are usually able to contain the organism in a host with intact pulmonary defenses. The first line of defense against *Aspergillus* is ciliary clearance of the organism from the airways and limited access to the alveoli due to conidial size. This feature is one reason for the increased pathogenicity of *A. fumigatus* compared with other species of *Aspergillus*.[79] After conidia reach the alveoli, the major line of defense becomes the pulmonary macrophages, which are capable of ingesting and killing *Aspergillus* conidia.[80] After the cells germinate, polymorphonuclear leukocytes act to extracellularly kill both swollen conidia and hyphae.[81] Efficacy of host defenses against the organism may be enhanced by opsonization of conidia with complement or other molecules, such as mannose-binding protein and surfactant proteins.[82] *A. fumigatus* possesses a hydrophobic external component, which inhibits fungal recognition[83]; produces melanin, which inhibits host phagocytosis[84]; and a complement inhibitor, which may increase its pathogenicity.[85] Antibodies against *Aspergillus* are common because of the ubiquitous nature of the organism, although they are not protective, nor are they useful in the diagnosis of infection in high-risk patients owing to the lack of consistent seroconversion after exposure or infection.[86]

Many *Aspergillus* spp. produce toxins, including aflatoxins, ochratoxin A, fumagillin, and gliotoxin, the last of which may reduce macrophage and neutrophil function, although the role of these toxins as major virulence factors is not established.[87] *Aspergillus* spp. possess other potential virulence factors, including production of proteases, phospholipases, and metabolites, although strains deficient in those genes are still capable of producing experimental infection.[88] These metabolites are additionally under investigation for diagnostic utility.[89]

T-helper (Th) cytokines have vital roles in innate and adaptive defense against *Aspergillus*. Studies in experimental aspergillosis have shown that a Th1 response is associated with a favorable response.[90] Pathogen recognition receptors, including Toll-like receptors (TLRs) and dectin-1, also mediate defense against *A. fumigatus*.[91,92] Recognition of *Aspergillus* motifs by TLR2 and dectin-1 results in activation of intracellular

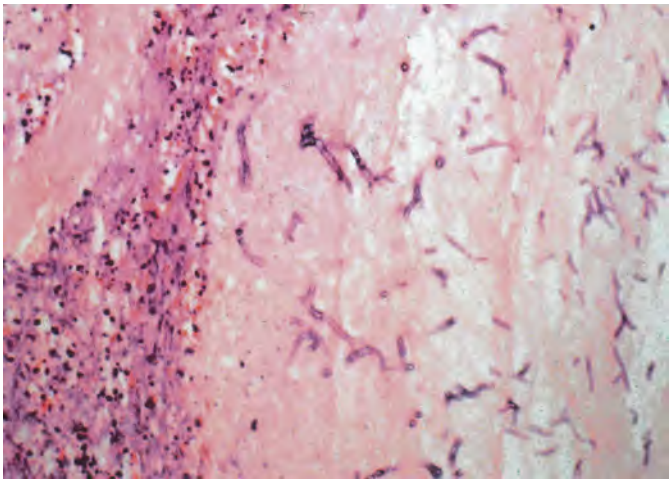

FIG. 257.5 Lung tissue section showing narrow, acutely branching septated hyphae on hematoxylin and eosin stain showing vascular invasion (original magnification ×420).

FIG. 257.6 Infarcted lung tissue due to *Aspergillus* angioinvasion on gross lung specimen.

pathways, leading to proinflammatory cytokine production.[93,94] These events are needed for an effective initial antifungal defense and bridge the gap between the innate and acquired immunity.[95] Polymorphisms in host genes mediating innate immune defenses influence susceptibility to IA. TLR4 haplotypes and polymorphisms in plasminogen gene alleles have both been shown to be associated with increased risk of IA in HSCT patients.[96,97] Additional pathways have been recently identified in large-scale genomic studies, and these polymorphisms may have implications on transplant donor screening and the risk stratification of recipients.[98]

In contrast to the deficient host responses that lead to invasive *Aspergillus* infections, the pathogenesis of ABPA and allergic fungal sinusitis relates to aberrant inflammatory host responses to the organism.[99] The immune response to *Aspergillus* antigens in ABPA, as in both asthmatic patients and those with cystic fibrosis, is a Th2 response.[79] ABPA begins with an allergic inflammatory response that follows after *Aspergillus* conidia are inhaled into the bronchi, where they germinate and form hyphae.[99] These mycelial cells release allergens that are processed by antigen-presenting cells bearing human leukocyte antigen (HLA)-DR2 or HLA-DR5 and presented to T cells within the bronchoalveolar lymphoid tissue. The T-cell response to these allergens favors a Th2 response, with release of cytokines interleukin (IL)-4, IL-5, and IL-13.[79] The inflammatory response in the bronchial submucosa leads to excessive mucin production, extravasation of eosinophils into the bronchial mucin, intermittent bronchial obstruction with atelectasis, and, over time, to bronchiectasis in some patients. Allergic fungal sinusitis is also characterized by submucosal inflammation and eosinophil-rich mucin in the sinus cavity.

Aspergilloma, called *fungus ball of the lung*, is a mass of hyphae in a preexisting cavity. *Aspergillus* causes a brisk immunoglobulin G (IgG) antibody response to the organism even though invasion of the cavity wall is rarely observed. Increased risk for chronic forms of pulmonary aspergillosis and noninvasive forms of pulmonary aspergillosis have been linked to subtle immune defects, including polymorphisms in mannose-binding protein and TLR polymorphisms.[100,101]

CLINICAL PRESENTATION

The spectrum of clinical syndromes associated with aspergillosis is diverse, ranging from allergic responses, asymptomatic colonization, superficial infection, and acute or subacute invasive disease. The clinical presentation reflects the underlying immune defects and risk factors associated with each patient group, with greater immune suppression correlating with increased risk for invasive disease.

Allergic Manifestations of Disease

Allergic Bronchopulmonary Aspergillosis

ABPA is a long-term allergic response to *Aspergillus* that is characterized by mucoid impaction of the bronchi, eosinophilic pneumonia, and transient pulmonary infiltrates due to atelectasis. Central bronchiectasis occurs in some patients after several years of disease.[99] The incidence of ABPA is estimated to range from 1% to 2% in patients with persistent asthma and is approximately 7% (range, 2%–15%) in patients with cystic fibrosis.[79,99,102] Diagnostic criteria for ABPA include: (1) asthma, (2) central bronchiectasis on chest computed tomography (CT), (3) immediate skin test reactivity to *Aspergillus* spp. (or *A. fumigatus)*, (4) total serum immunoglobulin E (IgE) concentration > 417 IU/mL (1000 ng/mL), (5) elevated serum IgE and/or IgG antibody to *A. fumigatus*, (6) fleeting infiltrates on chest radiography, (7) serum precipitating antibodies to *A. fumigatus*, and (8) peripheral blood eosinophilia.[103] Other clinical features may be present that can be used to support the diagnosis, including positive sputum cultures for *Aspergillus* or smears with hyphae consistent with *Aspergillus*, brown mucus plugs with degenerated eosinophils (Charcot-Leyden crystals) in sputa, and chest radiographic findings suggesting bronchial inflammation.[104] These latter chest radiographic findings include the "ring sign," indicating bronchial thickening without mucus plugs, and "parallel lines" or "tram tracks," suggesting bronchiectasis, which contrasts to tapering seen in the normal bronchus.[102]

The diagnosis of ABPA in cystic fibrosis may be particularly difficult because many of the diagnostic criteria overlap with common manifestations of cystic fibrosis (Table 257.3).[79] In these patients eosinophilia is not a useful diagnostic tool because the patients may have elevated peripheral blood eosinophils from other causes, or it may be suppressed from corticosteroid therapy.

ABPA typically progresses through a series of remissions and exacerbations but can eventually lead to pulmonary fibrosis and chronic pulmonary aspergillosis, which is associated with a poor long-term prognosis.[102,104,105] Management of ABPA is directed at reducing acute asthmatic symptoms and avoiding end-stage fibrosis. Corticosteroid therapy is commonly used for treating exacerbations, although few randomized trials have been conducted for their use.[106] Guidelines suggest that worsening diagnostic or clinical parameters may warrant a trial of corticosteroid therapy.[1] The role for antifungal therapy was evaluated with a randomized, double-blind, placebo-controlled trial that showed itraconazole at 200 mg/day for 16 weeks significantly reduced daily corticosteroid use, reduced levels of IgE, and improved exercise tolerance and pulmonary function.[107] In patients with severe asthma and fungal sensitization, itraconazole therapy resulted in significant clinical improvement in approximately 60%.[108] The efficacy of voriconazole, posaconazole, and isavuconazole have not been evaluated extensively in this setting but resulted in approximately 70% responses in ABPA or asthma patients who had previously failed itraconazole.[109]

TABLE 257.3 Criteria for Diagnosis and Management of Allergic Bronchopulmonary Aspergillosis in Patients with Cystic Fibrosis

Diagnostic Criteria[103]	
Predisposing Conditions (One Must Be Present)	
Asthma Cystic Fibrosis	
Obligatory Criteria (Both Must Be Present)	
Aspergillus skin test positivity or elevated IgE levels to *Aspergillus fumigatus* Elevated total IgE (typically >1000 IU/mL)	
Other Criteria (At Least Two Must Be Present)	
Precipitating serum antibodies to *A. fumigatus* or elevated serum *Aspergillus* IgG Radiographic abnormalities consistent with ABPA Total eosinophil count >500 cells/μL (may be historical)	
Recommendations for Screening for ABPA	
Maintain clinical suspicion for diagnosis	Especially in patients older than 6 years
Determine total serum IgE annually	If total IgE is greater than 500 IU/mL, consider skin test or measure IgE to *Aspergillus*
If total IgE 200–500 IU/mL, repeat if clinical suspicion is high	Consider retesting with disease exacerbation and perform other diagnostic tests
Therapy for Exacerbations of ABPA	
Corticosteroids: 0.5–2 mg/kg/day oral prednisone equivalent (maximum 60 mg/day) for 1–2 weeks, tapered over 2–3 months	Recommended for disease exacerbation in all patients except those with steroid toxicity
Antifungal therapy (itraconazole or other azole with *Aspergillus* activity—voriconazole, posaconazole)	Slow steroid response or toxicity; itraconazole 5 mg/kg/day up to 400 mg/day[108]—levels necessary (voriconazole or posaconazole may be effective when itraconazole fails but not extensively studied)[109]; monitor liver function tests; duration 3–6 months
Adjunctive therapy: inhaled corticosteroids; bronchodilators; environmental manipulation	No evidence for efficacy in ABPA but may be useful in asthma; reasonable to search for source of extensive mold exposure

ABPA, Allergic bronchopulmonary aspergillosis; *IgE,* immunoglobulin E; *IgG,* immunoglobulin G.
Data also from Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. Clin Infect Dis. *2003;37(suppl 3):S225–S264.*

Other Allergic Manifestations

Aspergillus is an occasional cause of allergic fungal sinusitis, although most cases in the United States are due to melanized molds.[110] This entity occurs in patients with a history of chronic allergic rhinitis, often with hyperplastic nasal mucosa forming nasal polyps. A mass of inspissated mucus forms in a sinus cavity with *Aspergillus* hyphae and Charcot-Leyden crystals. The sinus mucosa is hyperplastic but not invaded.[111] Management is directed at aerating the sinus and ensuring that tissue invasion is not present. The benefit of treating with either intranasal steroids or systemic antifungal agents has not been shown.[1]

Saprophytic Colonization and Superficial Aspergillosis

Fungus Balls Due to *Aspergillus*

A pulmonary fungus ball due to *Aspergillus*, or aspergilloma, is a solid mass of hyphae growing in a previously existing pulmonary cavity. Typically, *Aspergillus* fungus balls of the lung develop in preexisting cavities in the pulmonary apex of patients with chronic lung disease, such as bullous emphysema, sarcoidosis, tuberculosis, histoplasmosis, congenital cyst, bacterial lung abscess, or, very rarely, in a pulmonary bleb from *Pneumocystis* pneumonia in patients with AIDS.[112] On chest radiograph a pulmonary aspergilloma appears as a solid round mass in a cavity. The detection of *Aspergillus* in sputum cultures or detection of high titers of *Aspergillus* antibodies are further evidence that the radiographic findings are consistent with a diagnosis of fungus ball due to *Aspergillus* so that a biopsy is not usually necessary except to diagnose the underlying lung disease.[113]

In many patients the fungus ball due to *Aspergillus* remains asymptomatic, but in a significant number of patients hemoptysis occurs and can be fatal.[113] Surgical resection is considered the definitive therapy, but the dense pleural adhesions adjacent to the fungus ball and the poor pulmonary reserve of most patients make surgery hazardous. Contamination of the pleural space with *Aspergillus* and the common complication of bronchopleural fistula in the postoperative period can lead to chronic *Aspergillus* empyema. Dense adhesions make pleural drainage difficult, often requiring pleural stripping, further compromising lung function.[113]

Aspergillus can also be associated with fungus balls of the sinuses without tissue invasion.[110] The maxillary sinus is the most common site for a sinus aspergilloma to occur.[114] Clinical presentation is similar to that for any chronic sinusitis. CT of the sinus can be used to confirm the fungus ball, along with cultures of *Aspergillus*, usually *A. flavus* or *A. fumigatus.* Management is usually directed at surgical removal and a generous maxillary antrostomy for sinus drainage, along with confirmation that invasive disease has not occurred.

Denning and colleagues[115] have described a spectrum of chronic pulmonary aspergillosis ranging from chronic cavitary pulmonary aspergillosis, characterized by the formation and expansion of multiple cavities and the presence of fungal balls, to chronic fibrosing or necrotizing aspergillosis, in which slowly progressive infection occurs, usually in a single thin-walled cavity with demonstration of hyphae invading tissue. To differentiate chronic cavitary pulmonary aspergillosis from a simple *Aspergillus* fungus ball, patient symptoms, radiographic evidence of inflammation, and radiographic stability are useful parameters. The global burden of chronic pulmonary aspergillosis after tuberculous is thought to be substantial, which is important because of the potential response to antifungal agents in some patients.[116] In all of these conditions the diagnosis is suggested by radiologic and clinical features, and the role of therapy remains speculative, although it appears that long-term antifungal therapy may be beneficial in a subset of patients.[115,117]

Other Superficial or Colonizing Syndromes of Aspergillosis

Otomycosis is a condition of superficial colonization typically due to *A. niger*.[117] The clinical features include findings similar to other causes of external otitis, with the external auditory canal potentially revealing mold growing on cerumen and desquamated epithelial debris. *A. fumigatus* looks greenish, and *A. niger* forms a black tuft. Treatment is to focus on the underlying chronic otitis externa and débridement rather than the fungus. In immunocompromised patients, invasive otitis externa resembles that due to *Pseudomonas aeruginosa* clinically and may respond to antifungal therapy.[45,118]

Onychomycosis due to *Aspergillus* is another superficial condition that, although rare, can become chronic and responds poorly to antifungal agents.[119] Antifungal agents in the setting of nail infection may have a spectrum of activity limited to yeasts; thus a nail culture can be useful in patients with nonresponsive disease to establish a specific fungal etiology so that appropriate therapy can be initiated.

Aspergillus is an occasional etiology of keratitis, particularly after trauma or corneal surgery (see Chapter 113).[120,121] The diagnosis can be established with smears demonstrating hyphae, which may be indistinguishable from other molds, such as *Fusarium,* that also cause keratitis, but culture results are usually positive and confirm the diagnosis. Therapy consists of topical antifungal agents, usually topical natamycin 5% or topical voriconazole, although studies demonstrating efficacy of antifungal therapy are limited.[117,120–123] Surgical intervention may be required for deep lesions or those nonresponsive to medical therapy.[117] Amphotericin B has been injected intracamerally when corneal penetration has occurred.[124]

Invasive Syndromes Caused by *Aspergillus*

IA most frequently begins in the lung after inhalation of *Aspergillus* conidia. Invasion of hyphae into pulmonary vasculature is common, occurring in as many as a third of patients with invasive pulmonary aspergillosis. Disseminated disease occurs either by hematogenous spread to distant sites or by contiguous extension from the lung. Hematogenous dissemination to the CNS or other organs, including the thyroid, liver, spleen, kidney, bone, heart, and skin, is common in patients with severe immunosuppression, such as those undergoing HSCT, and heralds a poor prognosis.[3]

Invasive Pulmonary Aspergillosis

The most common manifestation of IA is invasive pulmonary aspergillosis (IPA). IPA rarely manifests before 10 to 12 days of profound neutropenia, which is a major risk factor for infection.[125] The incubation period for developing IPA after inhalation of conidia is not known but in patients with neutropenia has been estimated at approximately 15 days.[126] A significant number of patients have manifestations of IPA on admission or within the first 2 weeks of hospital admission, suggesting that community-acquired exposure is common.[76,127]

Symptoms of IPA include progressive cough, dyspnea, pleuritic chest pain, fever despite coverage with broad-spectrum antibiotics, and pulmonary infiltrates. These symptoms may be reduced in patients who are unable to mount an inflammatory response owing to profound neutropenia. In addition, although fever is common, it may be absent in those receiving high doses of corticosteroids. Other clinical features of IPA include hemoptysis, pleural effusion, and pneumothorax. However, all the physical findings are nonspecific and may lag significantly behind the disease process. Clinical characteristics may resemble a pulmonary embolism with pleuritic chest pain, hemoptysis, and dyspnea, which reflect the angioinvasive nature of the organism. Laboratory studies are also nonspecific but may include elevation in bilirubin, lactate dehydrogenase, and C-reactive protein, as well as coagulation abnormalities. Life-threatening hypoxia may occur in patients with extensive or progressive infection.

In extensive infection multiple diffuse nodular pulmonary infiltrates are readily seen on chest radiographs or chest CT (Fig. 257.7). Although these are not diagnostic, dense nodular lesions are associated with a poorer prognosis.[128,129] Other pulmonary radiographic findings of IPA include the classic pleural-based, wedge-shaped densities or cavitary lesions, although the former are not commonly detected, and the latter are present late in the course of infection.[128,130] Pleural effusions are more common than previously considered, but whether they are a specific manifestation of IPA is not established.[128] The presence of a "halo" of low attenuation surrounding a nodular lesion on CT is an early finding in IA (Fig. 257.8A).[128,130] Later in the course of infection, these nodular lesions may cavitate, usually in temporal association with recovery of neutrophils, forming an "air-crescent" sign (see Fig. 257.8B). These radiographic features are characteristic of IPA, but similar findings can

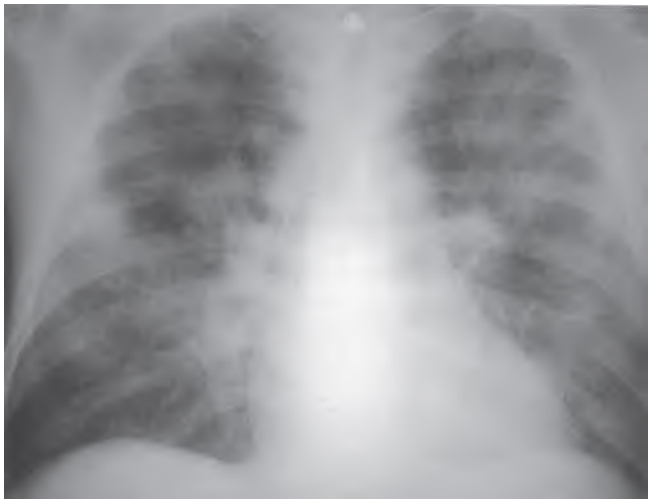

FIG. 257.7 Chest radiograph showing diffuse pulmonary infiltrates of invasive pulmonary aspergillosis.

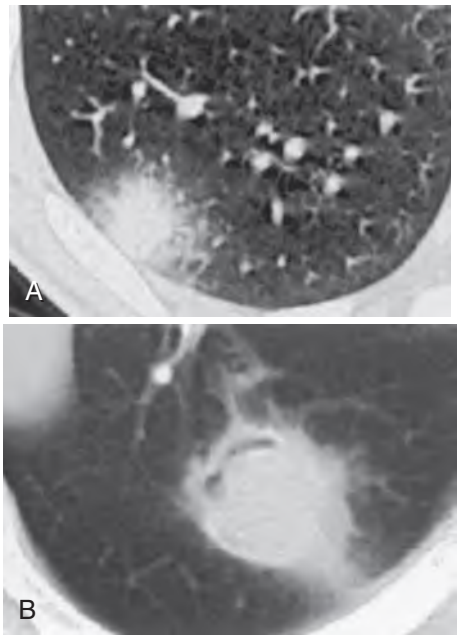

FIG. 257.8 Computed tomography of chest. (A) "Halo" sign of low attenuation surrounding a nodular lung lesion detected in early pulmonary aspergillosis. (B) "Air-crescent" sign in a nodular lung lesion found in late disease. *(Radiographs courtesy Dr. Reginald Greene.)*

also occur with other angioinvasive organisms, including *Mucorales, Fusarium*, and *Scedosporium*, as well as bacterial pathogens.

Tracheobronchitis

Aspergillus in the airways can range in significance from colonization, which is common in lung transplantation, to ulcerative tracheobronchitis.[66] The syndrome of *Aspergillus* tracheobronchitis typically occurs in patients undergoing lung transplantation and in patients with AIDS and is characterized by extensive pseudomembranous or ulcerative lesions due to *Aspergillus*. In patients undergoing lung transplantation the infection often occurs at the suture line of the lung transplant and can lead to dehiscence of the anastomotic site. Symptoms of tracheobronchitis are nonspecific and include dyspnea with associated pulmonary function abnormalities, cough, chest pain, fever, or hemoptysis. Symptoms may be mild and can be confused with other causes, including rejection. Results of plain radiographs may be normal so that clinical suspicion is needed to establish the diagnosis, which is accomplished by bronchoscopy with biopsy to document tissue invasion. A prolonged course of a systemic antifungal agent therapy is usually required for treatment, although aerosols of liposomal formulations of amphotericin B have been used for localized disease.[117]

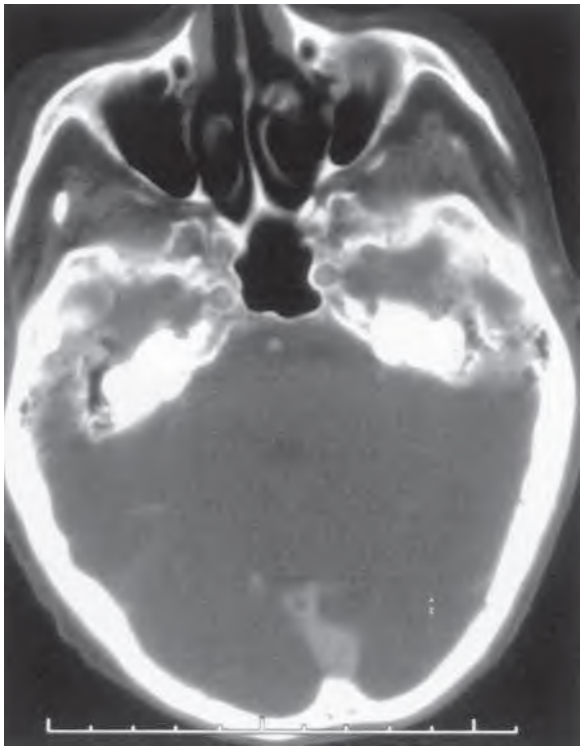

FIG. 257.9 Computed tomography of sinuses showing sinus wall thickening.

Sinusitis

Aspergillus infection of the sinuses and nasal cavities in immunocompromised patients manifests as acute invasive rhinosinusitis often in association with invasive pulmonary aspergillosis.[110,111] The clinical manifestations are not specific to *Aspergillus* and include fever, cough, epistaxis, sinus discharge, and headaches. Clinical signs are also nondiagnostic, but findings of an ulcerative nasal lesion with an eschar or nonsensitive area may be a clue to a fungal diagnosis.[131] Presence of epistaxis or unexplained fever in a high-risk patient may warrant endoscopy and biopsy of a nasal mucosal lesion. In patients with progressive infection the disease spreads to contiguous paranasal sinuses, palate, orbit, or brain. The mortality in invasive cases is high, ranging from 20% in patients with leukemia in remission to up to 100% in patients with relapsed leukemia or those undergoing bone marrow transplantation.[117] Plain radiographs are not diagnostic, may be falsely negative, and do not distinguish fungal etiologies from other causes of sinusitis. Sinus CT scans are useful for establishing extent of infection and determining local tissue invasion (Fig. 257.9). Routine surveillance cultures of the nose have been advocated, but these lack specificity and sensitivity. Cultures from sinus aspirates are useful to demonstrate the presence of *Aspergillus*, but a biopsy with tissue invasion is needed for definitive diagnosis.[132] Therapy for these infections is often difficult and requires long-term administration of antifungal medications.[117] The role of surgery is controversial. Efficacy of antifungal prophylaxis has not been demonstrated, but attempts at reduction of environmental exposures in high-risk patients may be beneficial.[133]

Disseminated Infection

Progressive invasive pulmonary aspergillosis often results in disseminated IA, a complication associated with an extremely high mortality.[2] In patients with severe and ongoing immunosuppression, disseminated infection can occur with mortality rates historically >90%.[2,3] Even with advances in antifungal therapy, mortality rates in

disseminated disease occurred in approximately 50% in the TRANSNET study.[134]

Other Invasive Syndromes

Cerebral Aspergillosis

Cerebral aspergillosis is associated with the highest mortality of IA syndromes, with mortality rates of >90% in most series.[3,134,135] The incidence of cerebral aspergillosis is difficult to determine because the diagnosis is often unsuspected, but it has been estimated to occur in 10% to 20% of all cases of IA, usually in patients with persistent immunosuppression and disseminated disease.[3,136] In one series of patients undergoing HSCT *Aspergillus* was found in 58% of biopsied cerebral mass lesions.[137] The diagnosis may occur an extended time after transplant (>100 days) and is nearly always associated with extensive immunosuppression, such as therapy for GVHD.[138] Concomitant pulmonary infection is usually, but not always, present.[137] Isolated cerebral aspergillosis can occur in immunocompetent patients or in the setting of injection drug use, in which case it may be associated with a slightly better prognosis, provided the diagnosis is made and surgical drainage or removal is performed.[136] *Aspergillus* meningitis is rare. The clinical presentation of cerebral aspergillosis is nonspecific and is characterized by focal neurologic signs, alteration in mental status, and headaches.[139] On CT of the brain the appearance is nonspecific and is similar to that of other infectious causes of brain abscess, with ring enhancement of the abscess, along with surrounding edema (Fig. 257.10) and may be hemorrhagic.[140] Magnetic resonance imaging (MRI) scans may reveal additional lesions, but the findings are still nonspecific. Confirmation of the diagnosis requires biopsy, but the diagnosis is often presumed. In patients without a clear diagnosis biopsy is recommended because the differential diagnosis is extensive, including other fungi and an extensive array of opportunistic diseases. Until recently the outcome of this infection has been almost universally fatal, although voriconazole has been associated with favorable responses in approximately 30% of patients.[135,136]

Bone Aspergillosis

Aspergillus osteomyelitis is an uncommon finding of IA. Vertebral osteomyelitis can result from local extension of an *Aspergillus* empyema. *Aspergillus* osteomyelitis can also be seen as a complication of disseminated infection or as a primary infection in certain risk groups, such as chronic granulomatous disease, in IV drug users, or after spinal procedures.[141] Vertebral osteomyelitis is the most common site for hematogenous spread to bone, usually involving the lumbar region.[142] The lesions can be seen on plain radiographs (Fig. 257.11) and on a CT or an MRI scan, which can be useful to stage the infection and to guide needle biopsy of the lesion. Favorable responses with voriconazole in *Aspergillus* osteomyelitis of the spine exceeded 60% in one review, although the need for long-term therapy and surgical intervention in medically nonresponsive patients is noted.[143] Infection of an intervertebral disk is a rare complication of hematogenous spread or surgery on the disk.

Cutaneous Infection

Skin involvement by *Aspergillus* can represent either disseminated hematogenous infection or local inoculation of infection that may arise around an IV catheter insertion site or the surrounding areas covered by adhesive dressings.[77] Whereas most lesions occur in patients with neutropenia or in other immunocompromised patients, *Aspergillus* can also invade patients with burns or surgical wounds. Clinically, the lesion is an area of rapidly increasing erythema with a necrotic, often ulcerated, center (Fig. 257.12). The lesions may resemble pyoderma gangrenosum. Pathologically, invasion of blood vessels and cutaneous ulceration occurs. Cutaneous disease can also be a manifestation of widespread disseminated disease, and in that setting a skin biopsy can be a relatively easy method to obtain tissue to establish the diagnosis of IA.

Other Sites

IA has also been reported in anecdotal cases to cause infection in virtually all body sites, including the heart, kidney, esophagus, intestine, and

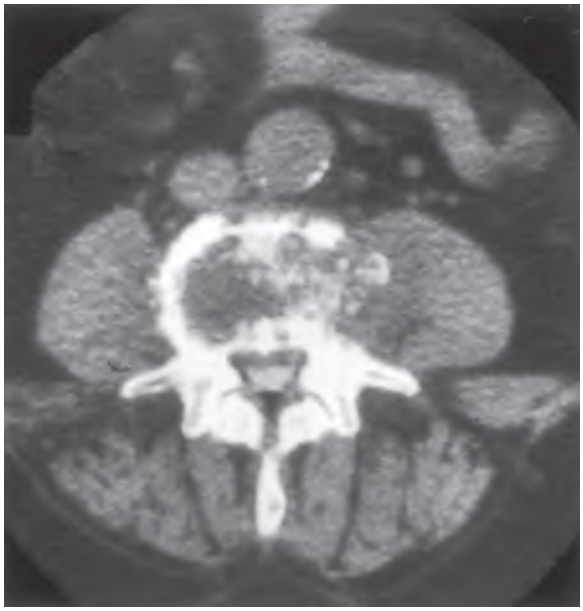

FIG. 257.11 Radiograph of spine showing bone destruction associated with spinal aspergillosis.

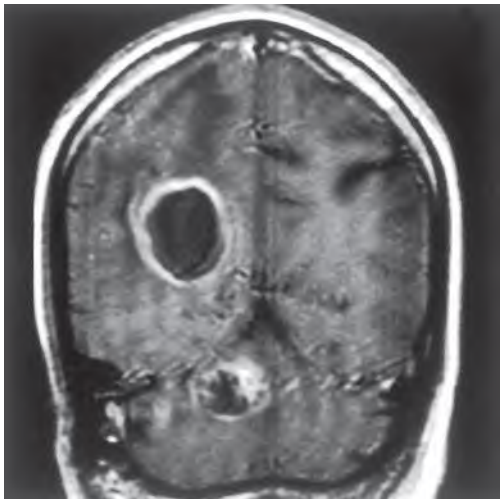

FIG. 257.10 Brain abscess of invasive aspergillosis with ring enhancement and extensive edema.

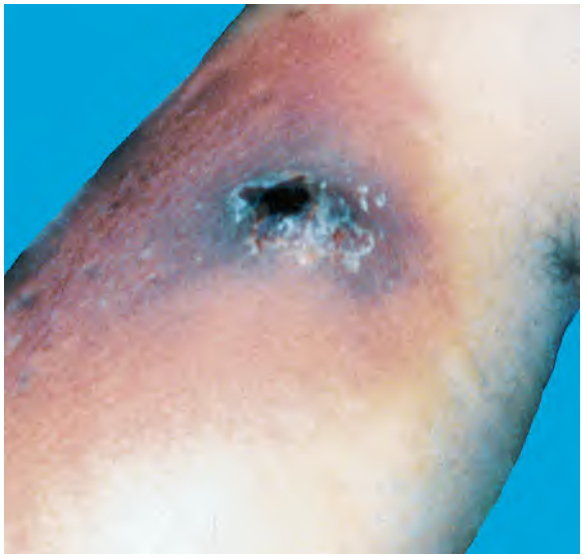

FIG. 257.12 Necrotic skin lesion of cutaneous aspergillosis.

others.[117] *Aspergillus* endocarditis can occur in either native or prosthetic heart valves.[144] Diagnosis is difficult because blood cultures usually remain negative even with extensive disease.[145] Even with surgical intervention, long-term survival is limited, although favorable outcomes with newer agents, particularly voriconazole, have been reported.[146,147] *Aspergillus* pericarditis is also associated with disseminated infection but can occur because of local extension of invasive pulmonary aspergillosis and can be complicated with cardiac tamponade. Some of these uncommon syndromes appear more common in certain epidemiologic settings. Renal infection occurs in patients with AIDS or with a history of injection drug use.[117]

DIAGNOSIS AND ANTIFUNGAL RESISTANCE

A proven diagnosis of IA requires a tissue biopsy showing invasion with hyphae and a positive culture for *Aspergillus*.[132] The diagnosis can also be established with positive cultures from a normally sterile site, such as a needle biopsy or cerebrospinal fluid (CSF), although blood cultures are rarely positive.[148] Tissue biopsies may not be possible because of potential risks, although *Aspergillus* hyphae are easily seen with common fungal stains, such as Gomori methenamine silver or periodic acid–Schiff. *Aspergillus* hyphae are hyaline, septate, acute-angle branched, even diameter, and 3 to 6 μm in width. Although these features usually distinguish *Aspergillus* from agents of mucormycosis, they are not distinguishable from a number of other opportunistic molds, including *Fusarium, Lomentospora* (formerly *Scedosporium*), *Paecilomyces*, and others so that a positive culture is needed to confirm the diagnosis.[149]

Cultures for *Aspergillus* in respiratory samples in high-risk patients, particularly if obtained via bronchoalveolar lavage (BAL), can support the diagnosis of probable IA.[132] *Aspergillus* can also be cultured from patients in whom no clinical illness is apparent, and positive cultures in patients with a low risk for IA should be interpreted with caution.[29]

Radiographic findings can also be used in the diagnosis and management of invasive pulmonary aspergillosis. Plain chest radiographs are of limited diagnostic utility because they are insensitive and findings are nonspecific.[128] A "halo" of low attenuation surrounding a nodular lesion on CT imaging is an early finding in invasive pulmonary aspergillosis and has been used as a marker for initiating early antifungal therapy.[4,128,150] The volume of lesions may increase over the first 7 days of infection, even when therapy is successful, so that early radiologic progression should be interpreted cautiously.[130] The CT findings of IPA have been validated in high-risk, neutropenic, and bone marrow transplant recipients, but in other patients, including solid-organ transplant recipients and those with nontraditional risk factors in the ICU, the CT findings are not as well defined.

Non–culture-based methods have been used to establish a rapid diagnosis of IA.[151] Antibody detection is of limited utility because immunosuppressed hosts fail to mount an antibody response even with invasive infection.[86] Detection of galactomannan (GM) by enzyme immunoassay has contributed substantially to the diagnosis of probable IA.[152] This assay has been validated in a variety of patient groups, particularly with serial measurements. Studies have suggested a sensitivity as high as 89% and a specificity of 92% in high-risk HSCT patients not receiving antimold prophylaxis.[153] Other studies have found the assay to be less sensitive (40%–50%), reflecting the impact of prior antifungal therapy in reducing the level of circulating GM, a limited number of samples per patient, extent of infection, and other variables.[152–154] This has resulted in recommendations for a lower cutoff for a positive test result, especially in high-risk patients with greater probability of infection.[153,155] A meta-analysis using an optical density index cutoff of ≥0.5 showed a sensitivity of 78% and a specificity of 81%.[156] False-positive results have been reported in some children, including in some neonates, which may be due to dietary intake or the presence of cross-reacting antigens with bacteria such as *Bifidobacterium*, and in patients receiving therapy with piperacillin-tazobactam—which now appears to be uncommon—and other antibiotics.[152,157] GM detection has also has been used for other body fluids, such as CSF, and in BAL fluid.[152,158] GM results from BAL fluid testing demonstrate sensitivity that is greater than that seen in serum testing and may be less affected by antifungal therapy.[159–161] A negative test result may be useful in ruling out a diagnosis of IPA.[160] Some colonized patients, such as those with chronic obstructive pulmonary disease or those undergoing lung transplantation, may have a positive GM result and not have evidence of invasive infection.[162] Serial assessments of GM may offer prognostic value in outcomes of infection. Other potential markers also include the nonspecific fungal marker (1→3)-β-D-glucan.[163,164]

Several reports demonstrate the potential for using polymerase chain reaction (PCR) as an early diagnostic marker for IA, which may be more sensitive that other methods.[165] These assays have not been traditionally standardized, and it was thus problematic to determine their diagnostic utility.[166,167] More recently, PCR has become commercially available, allowing standardization and quality control schemes to be implemented. The specificity of BAL PCR appears to be greater than GM testing, providing better utility for a diagnostic test.[168]

Susceptibility testing for *Aspergillus* has been standardized, but correlation with clinical responses has not been well established.[169] Antifungal resistance to itraconazole has been reported developing after exposure to antifungal therapy and is associated with point mutations in the *cyp51A* gene target of triazoles antifungals.[170] That resistance mechanism, which correlated with lack of efficacy in an animal model, has not been a common finding. However, resistant isolates have also been detected in azole-naïve patients.[171,172] In the initial report all cases were observed since 1999, and the prevalence of resistant *A. fumigatus* ranged from 1.7% to 6%. The authors speculated that resistance in *A. fumigatus* could be related to the widespread use of agricultural triazole fungicides.[172] These isolates have a particular genetic alteration consisting of a 34–base pair tandem repeat in the promoter coupled with a point mutation in the *cyp51A* target gene: an amino-acid substitution at codon 98 (TR34/L98H), causing multi–azole resistance. In contrast to the point mutations that occur in resistant isolates after azole exposure, no isogenic strains demonstrating this mechanism of resistance have been shown, suggesting a potential environmental source of infection. This mechanism has been most prevalent in the Netherlands, where agricultural azole fungicides are common, and it has also been demonstrated in several other European countries, India, and Asia.[173–175] More recently, other similar but distinct mechanisms of resistance (TR46/Y121F/T289A) have been reported in clinical isolates.[176] It is not clear how frequently these mutations occur clinically, but the lack of azole responsiveness in patients with these isolates suggests it may become prudent to consider that possibility. Different mutations induce differential triazole susceptibility, with some mutations causing resistance to voriconazole and isavuconazole, whereas others cause resistance to posaconazole and itraconazole, and others cause panazole resistance.[177] Other species, such as *Aspergillus terreus* and cryptic *Aspergillus* spp., may be resistant to amphotericin B or other antifungals, so testing of those species may be warranted.[11,30,178]

THERAPY (Table 257.4)

Historical efficacy of antifungal therapy in IA has been extremely poor, with favorable responses in less than 40% of patients and overall mortality rates of 60% or more.[2,3] Although mortality rates in high-risk patients continue to be unacceptably high, mortality rates have decreased.[63,134] Response to antifungal therapy depends on several factors, including the immune status of the host and the extent of infection at time of diagnosis.[3] In severely immunosuppressed patients, such as those undergoing allogeneic HSCT, and in patients with CNS or widely disseminated infection, extremely poor outcomes are seen, with mortality rates >75% in those groups.[a]

Primary Antifungal Therapy

Voriconazole

Voriconazole is a potent, broad-spectrum triazole that has become the recommended primary therapy for most patients with IA.[1] Voriconazole can be given either orally or intravenously (see Chapter 40B). The recommendation for voriconazole for primary therapy is based on a randomized trial that compared voriconazole with amphotericin B deoxycholate,

[a]References 3, 65, 117, 134, 136, 179, 180.

TABLE 257.4 Antifungal Agents for Invasive Aspergillosis

AGENT	CLASS	ROUTE OF ADMINISTRATION	DOSE	COMMENTS
Primary Therapy				
Voriconazole	Azole	IV/PO	6 mg/kg (IV) q12h × 2 doses, followed by 4 mg/kg (IV) q12h or 200 mg (PO) q12h. Some experts advise oral dosing as 4 mg/kg (PO) q12h.	Recommended for primary therapy in most patients due to randomized trial demonstrating improved survival compared with amphotericin B deoxycholate[4,117,181]; caution for use in patients with potential liver toxicity and for drug interactions; measurement of serum levels for efficacy and avoidance of toxicity[187]
Alternative Primary Therapy				
Isavuconazole	Azole	IV/PO	372 mg q8h × 6 doses, IV or PO followed by 372 mg daily (372 mg isavuconazonium sulfate = 200 mg isavuconazole)	Well tolerated, efficacy in treatment of pulmonary aspergillosis, and fewer adverse events than voriconazole-treated patients[194]
Liposomal amphotericin B	Polyene	IV	3–5 mg/kg/day	Well tolerated; minimal infusion reactions or nephrotoxicity; initial doses of 10 mg/kg/day more toxic and not more effective[199]
Other Agents				
Amphotericin B	Polyene	IV	1–1.5 mg/kg/day	Previous gold standard; significant toxicity in higher doses; limited efficacy in high-risk patients[3,200,201]
Amphotericin B lipid complex	Polyene	IV	5 mg/kg/day	Indicated for patients intolerant or refractory to standard therapy; case-controlled data suggest better efficacy than amphotericin B deoxycholate[203]
Amphotericin B colloidal dispersion	Polyene	IV	3–6 mg/kg/day	More infusion-related toxicity than other lipid formulations; efficacy similar to amphotericin B in primary treatment[204]
Posaconazole	Azole	IV/PO	Investigational in United States for *Aspergillus* treatment: tablet or IV, 300 mg q12h × 2 doses, followed by 300 mg daily	Oral and IV formulation; efficacy in salvage therapy and prophylaxis[58,207,208]
Itraconazole	Azole	PO	200 mg twice daily (PO) or 65 mg twice daily of SUBA-itraconazole	Erratic bioavailability improved with oral solution and new 65-mg capsule, serious cardiac adverse events with higher doses, drug interactions common; IV formulation not currently available[3]
Caspofungin	Echinocandin	IV	70 mg first day then 50 mg/day	Approved for refractory infection and intolerance to standard therapy; well tolerated[213]; limited efficacy as monotherapy for primary infection[180]; preclinical and anecdotal data showing improved efficacy in combination with azoles[210,217]
Micafungin	Echinocandin	IV	Investigational for *Aspergillus* treatment (United States) (50–100 mg/day)	Efficacy for prevention and salvage treatment of aspergillosis[214,245]
Anidulafungin	Echinocandin	IV	Investigational for *Aspergillus* (200 mg first day then 100 mg/day)	Combination trial data showing improved outcomes in patients with galactomannan diagnosis of invasive pulmonary aspergillosis

IV, Intravenous; *PO,* orally.

with each agent followed by other licensed antifungal therapy if needed for intolerance or progression of disease.[4] In this trial voriconazole was successful in 52% of patients versus 31% in those receiving amphotericin B deoxycholate. Superiority of voriconazole was demonstrated in patients at high risk for mortality, including those undergoing HSCT and in those with disseminated and CNS infection.[136,181] A survival benefit of voriconazole was also shown.[4] Voriconazole has also demonstrated efficacy in very difficult-to-treat clinical conditions, with responses of approximately 34% in CNS infection and in 52% of patients with osteomyelitis.[136,143]

Although voriconazole is generally adequately tolerated and exhibits a favorable pharmacokinetic profile, there are a number of issues to consider, including drug intolerance and drug interactions, especially those with immunosuppressive agents, such as cyclosporine, tacrolimus, and sirolimus, the latter of which is contraindicated for use with voriconazole. Other drug-drug interactions are common with medications metabolized through cytochrome P-450 (CYP)3A4, CYP2C9, or CYP2C19 enzymes. The most common adverse event has been a transient and reversible visual disturbance that has been reported in approximately 30% of patients receiving the drug.[4] This effect is dose related and described as an altered or increased light perception that is temporary and not associated with pathologic sequelae. Other adverse events have been less common, including dose-limiting liver abnormalities in 10% to 15%, skin rash in 6%, nausea and vomiting in 2%, and anorexia in 1%. Other serious adverse events have also been associated with long-term voriconazole use, including alopecia (which appears reversible with discontinuation of the drug), periostitis (perhaps related to the fluoride molecules in the compound), skin malignancies, and potentially severe peripheral neuropathies.[182–185] Therapeutic drug monitoring is recommended for voriconazole to improve efficacy[186] and limit adverse events such as liver toxicity and hallucinations, which are perhaps more common, with levels >6 μg/mL, and decreased efficacy with trough levels <1 μg/mL.[187–189] Weight-based dosing may increase the likelihood of achieving target drug levels with voriconazole, but considerable interpatient and intrapatient variability occurs.[190–192]

Alternative Primary Therapy

Isavuconazole

Isavuconazole is also a broad-spectrum triazole and an alternative first-line agent in the treatment of pulmonary aspergillosis.[1] Isavuconazole can be given orally or intravenously (see Chapter 40B). After administration, the prodrug, isavuconazonium, is cleaved by plasma esterases to the active agent isavuconazole.[193] Isavuconazole is an alternative primary therapeutic agent based on a single, comparative phase 3, randomized

controlled trial. This study found isavuconazole noninferior to voriconazole in the primary treatment of invasive mold infection. In patients with proven or probable aspergillosis there was no difference in all-cause mortality at day 42 or 84.[194] Patients with disseminated infection were not included in this trial. Adverse events from isavuconazole were less frequently observed compared with the voriconazole group (hepatobiliary disorders 9% vs. 16%, eye disorders 15% vs. 27%, and skin disorders 33% vs. 42%, respectively).

Isavuconazole is generally well tolerated and has been found a useful agent in patients intolerant of other mold-active triazoles.[195] Drug-drug interactions occur with this agent as well, and caution with coadministration of agents metabolized by CYP3A4 is needed. Adverse reactions included hepatotoxicity (16%), infusion-related reactions, and hypokalemia (15%).[194,196] In contrast to other triazoles isavuconazole has been associated with shortening of the QT interval, although the significance of this remains unclear.[197] An association of serum drug levels with efficacy or toxicity has not been found,[198] and there are currently no recommendations for monitoring serum concentrations of isavuconazole,[1]

Lipid Amphotericin Formulations

In patients in whom voriconazole is contraindicated based on drug interactions, liver dysfunction, intolerance, or other contraindication, liposomal amphotericin B is recommended as an alternative primary therapy.[1] Liposomal amphotericin B offers the advantage of less toxicity than amphotericin B deoxycholate and allows administration of higher doses of therapy.[199] This recommendation is based on a clinical trial that compared high initial doses of liposomal amphotericin B (10 mg/kg/day) versus 3 mg/kg/day for 2 weeks, followed by standard antifungal regimens. In this trial similar efficacy of 50% and 46% was seen in the two groups, respectively.[199] These results suggest that standard doses of liposomal amphotericin are adequate as primary therapy in some patients.[117]

Other Antifungal Agents

Amphotericin B Deoxycholate

For more than 4 decades, amphotericin B deoxycholate was the gold standard therapy for patients with IA.[117] Several studies documented the limited efficacy and substantial toxicity of amphotericin B deoxycholate in high-risk patients.[4,200,201] The overall response rates of amphotericin B deoxycholate are <25%, with substantial drug-related toxicities.[3,4,201] Similar findings were documented by Bates and colleagues,[200] who found that renal toxicity occurred in approximately 30% of patients receiving amphotericin B deoxycholate and that this toxicity was associated with a sixfold increase in mortality and a dramatic increase in hospital costs. Thus, with unacceptably high mortality rates and significant toxicity, amphotericin B deoxycholate is not recommended for IA.[117]

Other Polyenes

Amphotericin B lipid complex is a lipid formulation of amphotericin B approved for IA salvage therapy.[202,203] It is generally used in doses of 5 mg/kg/day and is better tolerated than amphotericin B deoxycholate. Another study evaluated amphotericin B colloidal dispersion for primary therapy for IA, which showed similar efficacy, with reduced renal toxicity of the lipid formulation but with serious pulmonary toxicities so that this preparation offers little advantage for its use.[204] Meta-analysis of studies using lipid formulations of amphotericin B show reduced nephrotoxicity and favorable results as salvage therapy with these agents for IA.[205]

Other Triazoles

Itraconazole is approved for use as salvage therapy of aspergillosis, but its utility in invasive infection has been limited because of an unfavorable pharmacokinetic and tolerability profile. Although an IV formulation obviated bioavailability concerns, that formulation is no longer clinically available. Itraconazole is more frequently used in less immunosuppressed patients who are able to take oral therapy and for use as sequential oral therapy and for those patients with saprophytic or allergic conditions.[3,108] A new formulation of itraconazole (SUBA-itraconazole) has been developed and may offer pharmacokinetic advantages over conventional itraconazole.[206]

Other extended-spectrum triazoles, including posaconazole and ravuconazole, have activity in vitro against *Aspergillus*. Posaconazole is approved for prophylaxis of invasive fungal infection, including aspergillosis, and has demonstrated efficacy in salvage therapy.[58,207,208] As with voriconazole, drug level monitoring of posaconazole is recommended, particularly in patients with GVHD.[209]

Echinocandins

The echinocandins are an additional class of antifungals with *Aspergillus* activity.[180,210,211] These agents, which are administered intravenously, target glucan synthase, which is needed for production of β-1,3-glucan in fungal cells walls.[212] These effects are not fungicidal but significantly alter the growing fungal cell wall. Included in these agents are caspofungin, micafungin, and anidulafungin. Caspofungin is approved for treating patients refractory to, or intolerant of, standard therapies for IA but is not recommended for primary therapy.[180,213] In one prophylaxis study micafungin reduced the number of *Aspergillus* infections compared with standard prophylaxis with fluconazole and has activity in salvage therapy of the disease.[214] Anidulafungin is another echinocandin with *Aspergillus* activity and exhibits a favorable toxicity profile similar to those of the other echinocandins.

Combination Antifungal Therapy

The rationale for combination therapy is to maximize treatment by targeting multiple targets or metabolic pathways, or different points in the same pathway, to improve efficacy by an additive or synergistic effect. Antifungal drug combinations have been evaluated in vitro and in animal models in a number of prior studies with variable results.[215–219] Antagonism has been demonstrated in some but not all studies, particularly between polyenes and triazoles; however; the combination of a triazole and an echinocandin exhibits synergistic to additive interactions.[219–222] A randomized clinical trial has been completed comparing voriconazole with the combination of voriconazole plus anidulafungin. This trial in HSCT patients failed to demonstrate superiority of the combination regimen for the primary study end point of fungal-free survival. However, in the subgroup of patients diagnosed by GM detection (presumably those with early disease), mortality was significantly reduced. Guidelines do not currently recommend routine use of combination primary therapy, but these data suggest that combination therapy may be beneficial in some patients.[1]

Adjuvant Therapy

Adjuvant therapies including surgery, granulocyte transfusions, and growth factors have been used to augment antifungal therapy, although their utility has not been established. Surgical resection of isolated pulmonary nodules has been shown to improve outcome of infection,[129,223] although favorable outcomes with triazoles suggest that in some patients surgical resection may not be necessary. Surgical resection should also be considered in patients with severe hemoptysis or lesions near the hilar vessels or pericardium.

Other adjuvant therapies, including granulocyte and granulocyte-macrophage colony-stimulating factors, interferon-γ, and granulocyte transfusions,[224] have all been shown in anecdotal reports to improve outcomes but are not generally recommended for routine use, given little demonstrated efficacy.[1,225]

Approach to Therapy

Guidelines for treating IA have been published by the Infectious Diseases Society of America and other groups internationally, with similar recommended approaches to therapy.[226–228] There are few randomized trials to support evidence-based approaches to therapy and heterogeneity of underlying host factors, and diagnostic criteria further impact clinical outcomes in this disease. A prompt diagnosis and effective initial therapy are both critical in improving the outcome of this infection.[229] Radiography and use of biomarkers, such as GM, may facilitate an early detection of aspergillosis in high-risk patients.[230,231] Most patients should receive primary therapy with voriconazole, which has been shown to be superior to amphotericin B deoxycholate and has been used in a variety of clinical

syndromes since its availability for use in this disease.[4] In patients who are intolerant of voriconazole, have a contraindication to the drug, or have progressive infection, alternative agents include isavuconazole or lipid formulations of amphotericin B, the echinocandins, or another triazole.[199,207,213,214] Primary use of combination therapy in all patients is not recommended at the present time because of the lack of encouraging clinical trial data, but combination therapy in some groups of patients, such as those with an early diagnosis of infection, may prove beneficial. In a salvage setting the addition of another agent or a switch to another antifungal class may be considered due to the poor outcomes of a single agent in progressive infection.[117] Although the optimal duration of antifungal therapy is not known, improvement in underlying host defenses is crucial to successful therapy.

PREVENTION AND PROPHYLAXIS

Prevention of IA in high-risk patients is difficult. Nosocomial outbreaks of aspergillosis have been linked to construction, contaminated ventilation systems, and possibly to contaminated water.[75,232,233] For high-risk patients, such as those undergoing HSCT, the use of high-efficiency particulate air filters, frequent air exchanges, and positive pressure ventilation has been recommended to limit exposures in the hospital setting.[71–74] Infection control measures, such as construction barriers, will limit exposure to aerosols.[127] Attention to routine maintenance and cleaning of showers and water systems may further reduce risk.[72,234] Some patients will still develop infection with these precautions, and an increasing number of immunosuppressed patients receive care outside of the hospital setting so that community-acquired infection is common.[76]

Efficacy of antifungal prophylaxis has been limited because of the toxicity of amphotericin B and the limited activity of other oral agents against *Aspergillus.* Agents evaluated in this setting include low-dose amphotericin B, low doses of lipid formulations of amphotericin B, and nasal and aerosolized forms of amphotericin B, none of which has demonstrated conclusively to be beneficial in a large randomized clinical trial. Studies of aerosolized formulations of lipid amphotericin B have demonstrated its safety and potential efficacy in lung transplant recipients and in patients with prolonged neutropenia who are at high risk for IA.[235,236] Itraconazole has been suggested to have benefit against prevention of molds, but its poor tolerance in high-risk patients has also limited its use,[237,238] although a newer formulation may be useful in this setting.[206] In a long-term study of fungal prophylaxis in patients with chronic granulomatous disease, itraconazole reduced the incidence of serious fungal infections, including those due to *Aspergillus*.[239]

Two randomized trials have established the safety and efficacy of posaconazole in high-risk patients. Posaconazole was compared with fluconazole in a randomized, double-blind trial in patients undergoing HSCT with GVHD. In that trial posaconazole significantly reduced the number of breakthrough fungal infections, including IA.[208] In the companion trial posaconazole was compared with either fluconazole or itraconazole for patients with acute myelogenous leukemia or myelodysplastic syndrome.[199] In that trial prophylaxis with posaconazole reduced breakthrough fungal infections and significantly prolonged survival. More serious adverse drug events occurred with posaconazole therapy so that a risk-benefit analysis should be considered when recommending posaconazole prophylaxis.[57] Guidelines recommend the use of posaconazole prophylaxis in patients with acute myelogenous leukemia or with GVHD, who are at high risk for IA.[b] Voriconazole prophylaxis has been evaluated in two randomized trials, the first being a strategy of comparing fluconazole with voriconazole, with GM monitoring for fungal infection.[242] The primary end point of improved fungal free survival was not met, although there were fewer *Aspergillus* infections in the voriconazole arm and improved fungal-free survival in patients with acute myeloid leukemia. The second trial compared voriconazole with itraconazole in allogeneic HSCT and demonstrated better efficacy of voriconazole due to improved tolerability compared with itraconazole.[243] A meta-analysis of antimold active prophylaxis showed fewer episodes of IA but more adverse events and no impact on overall survival.[244] In high-risk patients the consideration for antimold active prophylaxis should be considered, but in patients at lower risk careful monitoring and preemptive therapy may be an appropriate course of action.

[b]References 1, 117, 179, 226, 240, 241.

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The complete reference list is available online at Expert Consult.

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258 Agents of Mucormycosis and Entomophthoramycosis

Dimitrios P. Kontoyiannis and Russell E. Lewis

SHORT VIEW SUMMARY

Definition

- Mucormycosis is an aggressive, angioinvasive fungal infection, caused by filamentous fungi in the subphylum Mucoromycotina, order Mucorales, that afflicts immunocompromised patients with severe metabolic conditions, such as uncontrolled diabetes mellitus. Skin and soft tissue infections in immunocompetent patien hosts may be encountered in patients with severe soft tissue trauma (e.g., from tornadoes, combat injuries, or burns).
- Entomophthoramycosis is a rare infection of the paranasal sinuses, subcutaneous tissues, or gastrointestinal (GI) tract caused by filamentous fungi in the subphylum Entomophthoramycotina that are principally encountered in the tropics.

Epidemiology

- Mucormycosis is acquired primarily via inhalation of environmental sporangiospores in immunocompromised hosts or through direct inoculation during trauma. The infection may break through antifungal prophylaxis regimens used to reduce the risk of aspergillosis.
- Entomophthoramycosis typically is an indolent subcutaneous infection localized to the sinuses, head and face (conidiobolomycosis), or trunk and arms (basidiobolomycosis) and acquired by inhalation or minor trauma. GI basidiobolomycosis has occurred in Arizona and the Near East and is perhaps acquired by ingestion.

Microbiology

- Most common culture-confirmed cases reported in the literature are *Rhizopus* spp. (47%), *Mucor* sp. (18%), *Cunninghamella bertholletiae* (7%), *Apophysomyces elegans* (5%), *Lichtheimia (Absidia)* spp. (5%), *Saksenaea* spp. (5%), and *Rhizomucor pusillus* (4%), with other species (8%) accounting for the remaining cases.
- *Conidiobolus coronatus* and *Conidiobolus incongruous* cause conidiobolomycosis. *Basidiobolus ranarum* causes basidiobolomycosis.

Diagnosis

- A high index of suspicion in immunocompromised patients is essential because most signs, symptoms, and radiographic signs of mucormycosis are nonspecific. Cultures have poor sensitivity. Diagnosis is typically established by histopathologic documentation of "ribbon-like" angioinvasive hyphae in tissue, although this is prone to error.

Treatment

- Lipid formulations of amphotericin B are the drugs of choice. Oral posaconazole, or perhaps isavuconazole, is used for treatment once infection is clinically stable and if absorption is adequate.

Prevention

- Given the rarity of mucormycosis and entomophthoramycosis, primary prophylaxis is not recommended. Secondary or potentially indefinite prophylaxis should be considered for immunocompromised patients with a previous episode of mucormycosis, depending on the status of underlying immunosuppression.

The disappearance of the class Zygomycetes from current taxonomy has made the term *zygomycosis* obsolete. The term *zygomycosis* encompassed both mucormycosis and entomophthoramycosis. These two infections are so different that no new name has been proposed to include both infections. Most of this chapter discusses mucormycosis, a group of filamentous fungi in the subphylum Mucoromycotina that belong to the order Mucorales. Mucorales can cause life-threatening infections in humans, especially in immunocompromised hosts. The first documented report of human mucormycosis is credited to Paltauf,[1] who in 1885 reported a disseminated infection in a patient with rhinocerebral involvement caused by angioinvasive, ribbon-like hyphae that he termed *Mycosis mucorina*. Subsequent descriptions of the infection in the following decades relied on tissue morphology and, as often is the case today, were infrequently confirmed by culture. Hence the findings of coenocytic (aseptate or pauciseptate) fungal hyphae in tissue invading blood vessels was assumed to be due to *Mucor* spp. and have become synonymous with the clinical term *mucormycosis* or, simply, *Mucor* infection. This terminology is further justified by the fact that all but the rare *Mortierella* spp. are within the order Mucorales.[2] However, the number of species causing human mucormycosis has expanded considerably in the past 2 decades with improvements in culture-based morphologic identification and the application of more precise molecular diagnostics and sequencing for fungal identification (Table 258.1). In fact, members of the genus *Rhizopus,* not *Mucor,* are reported as the most predominant cause of human infections, although in Europe *Mucor* and *Lichtheimia* account for approximately one-quarter of reported cases.[3–5]

ETIOLOGY

Agents of mucormycosis are ubiquitous fungi in the environment that are commonly found in decaying organic substrates, including bread, fruits, vegetable matter, soil, compost piles, and animal excreta.[6] These fungi characteristically produce large, ribbon-like hyphae that are irregular in diameter with only occasional septae, hence the characterization of these organisms as aseptate fungi. Identification can be confirmed by observing the characteristic, saclike fruiting structures (sporangia), which produce internally spherical yellow or brown spores (sporangiospores) (Fig. 258.1).[6] Spores ranging from 3 to 11 μm in diameter are easily aerosolized and dispersed and cause infections in humans when inhaled or introduced through a cutaneous or percutaneous route.[6]

Although several species of the order Mucorales have been reported to predominate as causes of human mucormycosis, culture recovery of these fungi from infected tissue is suboptimal and may skew the current understanding of the etiologic spectrum of mucormycosis. The widening application of molecular diagnostic techniques (i.e., polymerase chain reaction [PCR]) in culture-negative cases may expand the current understanding of the prevalence and etiology of this infection. In a global review of more than 900 reported cases of mucormycosis, *Rhizopus* spp. (47%) were the most frequently reported causes of culture-confirmed mucormycosis, followed by *Mucor* spp. (18%), *Cunninghamella bertholletiae* (7%), *Apophysomyces elegans* (5%), *Lichtheimia (Absidia)* spp. (5%), *Saksenaea* spp. (5%), and *Rhizomucor pusillus* (4%), with a variety of other uncommon species representing the remaining 8% of culture-confirmed cases.[3]

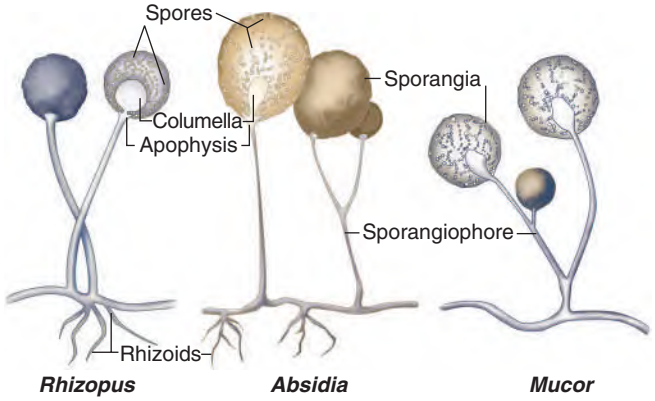

FIG. 258.1 Mucormycosis agents. Illustration of the major differentiating morphologic features of three of the most common agents of mucormycosis isolated from patients. Note the presence and location of the rhizoids and columella, as well as the shape of the sporangia. The infectious spores reside within the sporangia. *(Illustration by Lori Messenger.)*

TABLE 258.1 Taxonomic Organization of the Most Common Agents of Mucormycosis and Entomophthoramycosis

Mucormycosis
Rhizopus rhizopodoformis
Rhizopus arrhizus (Rhizopus oryzae)
Rhizopus microsporus
Rhizomucor pusillus
Rhizopus stolonifer
Cunninghamella bertholletiae
Apophysomyces elegans
Saksenaea vasiformis
Lichtheimia (Absidia) corymbifera
Mucor circinelloides
Mucor velutinosus
Syncephalastrum racemosum
Actinomucor elegans
Cokeromyces recurvatus
Mortierella wolfii
Entomophthoramycosis
Conidiobolus coronatus
Conidiobolus incongruus
Basidiobolus ranarum

Seasonal variations may affect the incidence of mucormycosis, with most infections occurring during periods of higher temperatures and decreased precipitation.[7,8] Likewise, major weather events have also been associated with infections with less frequently isolated species, such as *Syncephalastrum racemosum* from respiratory samples after Hurricane Katrina, *Apophysomyces trapeziformis* soft tissue infections in patients with traumatic injuries associated with the Joplin, Missouri tornado,[9] or posttsunami wound infections due to *Apophysomyces elegans*.[10] Combat wound infections are also influenced by environmental factors, as in Afghanistan, where wounds sustained in the southern regions, with lower elevation, warmer temperatures, and greater isothermality, had greater mold contamination.[11]

Insights From Genomic Sequencing

The first published genomic sequence of an agent of mucormycosis was *Rhizopus arrhizus* strain 99-880 (subsequently reclassified as *Rhizopus delemar*), which was isolated from a diabetic patient with fatal cerebral mucormycosis.[12] Sequencing revealed the presence of abundant transposable elements accounting for 20% of the genome and evidence of a whole-genome duplication event during the evolution of the fungus.[12] The whole-genome duplication event resulted in duplication of nearly all subunits of the protein complexes involved in respiratory electron transport chains and ubiquitin-proteasome systems, as well as multiple gene families related to cell growth and signal transduction and known fungal virulence factors, including secreted aspartic and subtilase protein families.[13] Sequencing also revealed duplication of the ergosterol biosynthetic pathway (e.g., lanosterol 14α-demethylase), the major target of azoles including voriconazole, isavuconazole, and posaconazole.[14] These genetic features may explain why Mucorales have a uniquely aggressive capacity for rapid growth in patients, even in the face of exposure to antifungal agents and the host immune response.[15] More recent sequencing studies of representative isolates from Mucorales and noninvasive Entomophthorales have identified differences in gene copy numbers that encode spore coat (Cot[H]) proteins, which facilitate invasion of blood vessel endothelial cells.[16] The most commonly isolated Mucorales (*Rhizopus, Mucor,* and *Lichtheimia*) contain three to seven copies of the *cotH* gene, whereas those that occasionally cause disease in humans (*Apophysomyces, Cunninghamella, Saksenaea,* and *Syncephalastrum*) contain only one to two copies. Notably, the *cotH* gene was lacking in Entomophthorales, which are taxonomically similar to Mucorales but do not cause blood vessel invasion.[16]

Acquisition of Infection

The primary mode of acquisition of mucormycosis is inhalation of spores from environmental sources.[17] Trauma, penetrating wounds, burns, and direct injection of sporangiospores can cause infection through a cutaneous or percutaneous route. Gastrointestinal (GI) mucormycosis, although less common, has been reported in both immunocompetent and immunocompromised patients with repeated ingestion of spores during periods of severe malnutrition, ingestion of nonnutritional substances (pica), ingestion of contaminated pharmaceutical products, prepackaged foods, fermented porridges and alcoholic drinks prepared from corn, or eating with contaminated chopsticks.[18,19] More recently, an outbreak of food poisoning was linked to intake of Greek yogurt contaminated with *Mucor circinelloides*.[20] It is unclear whether the fungus itself, which could survive transit through the GI tract and retain virulence, or a secondary metabolite or toxin was the cause of clinical symptoms experienced by the consumers.

Patient Populations at Risk

Agents of mucormycosis are unique among filamentous fungi in their ability to infect a broader, more heterogeneous population of human hosts compared with other opportunistic molds. The epidemiology of mucormycosis is similar in adult and pediatric patients.[21] Although most cases of mucormycosis are community acquired, nosocomial acquisition or pseudooutbreaks[22] have been linked to contaminated bandages and bandage tape,[23–25] needles,[26,27] and tongue depressors used to construct splints for intravenous (IV) and arterial cannulation sites in preterm infants.[28,29] An outbreak of invasive mucormycosis in a pediatric hospital was linked to contaminated bed linens resulting from improper storage, packaging, and transport from an offsite laundry service.[23,30]

The populations most commonly at risk for mucormycosis include patients with poorly controlled diabetes mellitus, prolonged neutropenia, high-dose corticosteroid treatment,[31,32] or immunosuppressive therapy associated with transplantation, and/or elevated levels of free iron, which enhances fungal growth.[33] The importance of iron overload in the pathogenesis of mucormycosis was first noted in patients with end-stage renal disease in the preerythropoietin era, where regular transfusions were required for persistent anemia, resulting in iron overload.[34] Treatment of these patients with deferoxamine to lower their iron levels was linked to the development of disseminated, rapidly fatal mucormycosis.[35,36] It was subsequently discovered that although deferoxamine is an iron chelator for the human host, it also acts as a siderophore in Mucorales, directly delivering iron to the fungus.[34] Less commonly, mucormycosis is a cause of infection in the setting of renal failure, diarrhea, and malnutrition in low-birth-weight infants and rarely in human immunodeficiency patients.[3]

Patients who develop mucormycosis in the absence of underlying disease or immunosuppression at the time of infection frequently have histories of penetrating trauma, burns, surgery, or illicit IV drug use before the infection. For example, a case series of mucormycosis at a nononcology, tertiary care center found that traumatic wounds or surgical sites were the most common infection sites (31%), followed by rhinocerebral (25%) and disseminated (12.5%) infections.[37] Severe

soft tissue infections from blast injuries in combat have also caused mucormycosis.[37,38]

Mucormycosis is infrequently identified as a complication associated with primary immunodeficiency. This complication has occurred in a case of severe congenital neutropenia associated with HAX-1 (HCLS1 [hematopoietic cell-specific Lyn substrate 1]–associated protein X-1) deficiency, or with a newly identified genetic abnormality, such as in a patient with a STAT-1 (signal transducer and activator of transcription-1) gain-of-function mutation who presented with disseminated *Apophysomyces trapeziformis* infection.[39,40]

Incidence of Mucormycosis

Mucormycosis is not a reportable disease. The incidence of mucormycosis is probably underestimated in many epidemiologic series given the inherent challenges associated with antemortem diagnosis and the declining rate of autopsies in high-risk populations. A recent estimate of the disease burden of mucormycosis in US hospitals from 2005–15 reported a prevalence of 0.12 to 0.16 cases per 10,000 discharges, depending on the International Classification of Diseases–9 code definitions used to define the disease.[41] Cases of mucormycosis were associated with an average hospital length of stay of 17 days, with 23% dead at discharge and more than one-third of patients requiring readmission to the hospital. The average cost per hospital stay was $112,419 (2014 US dollars).[41] Surveys from Europe have reported incidence rates of mucormycosis ranging from 0.43 to 1.2 cases per million people.[42,43] In autopsy series of high-risk hematology populations, mucormycosis has accounted for 8% to 13% of all reported invasive fungal infections.[44–46]

Mucormycosis is an important cause of fungal infections in patients with hematologic malignancy, recipients of allogeneic hematopoietic stem cell transplant (HSCT), and, less commonly, patients undergoing solid-organ transplantation (SOT) (Fig. 258.2).[47] Data from the Centers for Disease Control and Prevention (CDC) Transplant-Associated Infection Surveillance Network (TRANSNET) acquired from prospective surveys of 25 US transplantation centers from 2001–06 reported 1-year cumulative incidence rates for mucormycosis of 0.29% in allo-HSCT and 0.07% in SOT,[45,48] accounting for 8% and 2%, respectively, of fungal infections diagnosed in these populations. Of concern, some mucormycosis cases have presented as breakthrough infection on antifungal prophylaxis or treatment effective against *Aspergillus* but not Mucorales (i.e., voriconazole, echinocandins)[49] or because of potentially insufficient absorption of posaconazole.[50,51] Collectively, these data suggest that mucormycosis should be considered in any high-risk hematologic malignancy patient whenever fungal sinusitis develops on *Aspergillus*-active antifungal prophylaxis, especially in patients with prolonged immunosuppression and underlying hyperglycemia associated with prolonged corticosteroid use.

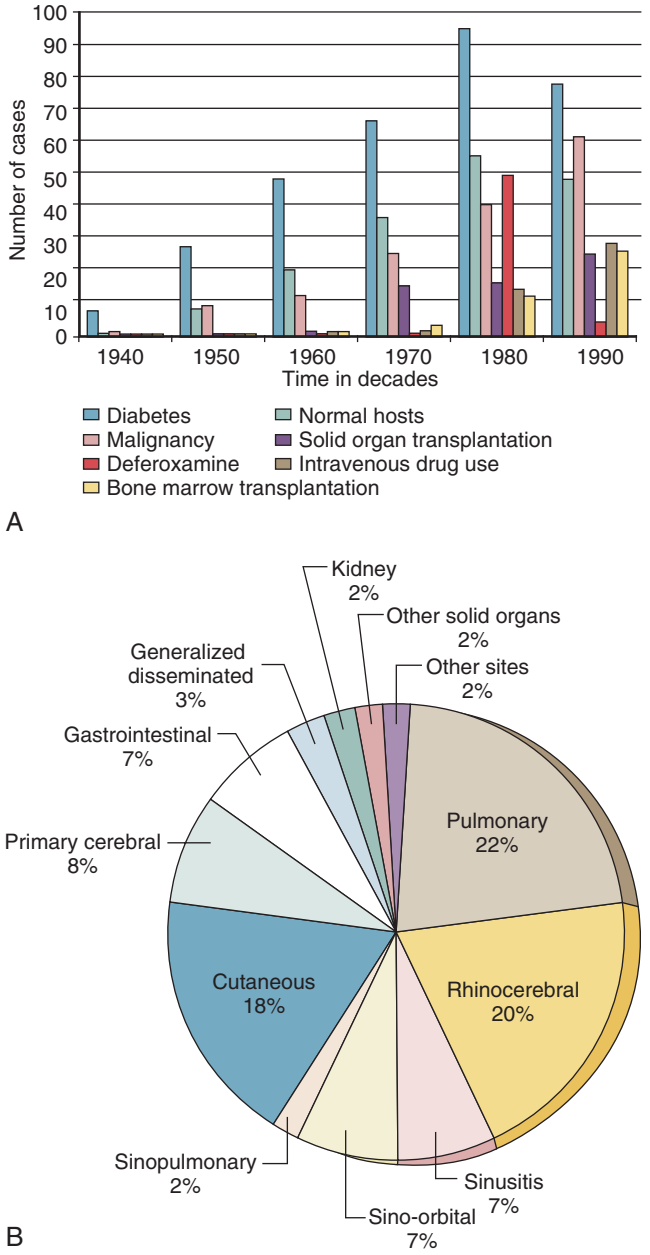

FIG. 258.2 Mucormycosis incidence. Incidence of mucormycosis over 6 decades by host population (A) and site of infection (B). *(Modified from Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases.* Clin Infect Dis. *2005;41: 634–653.)*

PATHOGENESIS (Fig. 258.3)

Most Mucorales sporangiospores are sufficiently small to evade host upper airway defenses and reach the distal alveolar spaces after inhalation.[6] Larger spores (>10 µm) may lodge in the nasal turbinates, predisposing patients to sinusitis.[6] Inhalation of a high spore inoculum, which can occur with excavation, construction, or work in contaminated air ducts, can lead to a slowly progressing pulmonary mucormycosis even in immunocompetent hosts.[52,53] In the case of primary cutaneous mucormycosis, subcutaneous inoculation of spores is the most common event leading to infection in immunocompetent hosts. Cutaneous mucormycosis in immunocompetent hosts usually follows massive soft tissue injury but has been described with even minor trauma, including insect bites[54] and tattoos.[47]

Relatively little is known about initial steps of how Mucorales sporangiospores attach to the respiratory or GI epithelium.[55] *Rhizopus* sporangiospores can adhere to extracellular matrix proteins, such as laminin and type IV collagen,[56] which may be exposed after epithelial damage caused by cytotoxic chemotherapy, infection, diabetes, or trauma. Toxins elicited by Mucorales during germination may contribute to epithelial cell damage.[57] A recent outbreak of food poisoning linked to intake of Greek yogurt contaminated with *M. circinelloides* identified secondary metabolites produced by the contaminating strain that were toxic to the GI mucosa.[20]

Mucorales appear to possess a unique mechanism for adhering to and invading endothelial cells by specific recognition of host receptor–glucose regulator protein 78 (GRP78).[58] Expression of GRP78 on the endothelial cell surface increases as a stress response after exposure to elevated concentrations of β-hydroxy butyrate (BHB), glucose, and iron, similar to those found in patients with diabetic ketoacidosis.[59] Mucorales germlings, but not sporangiospores, bind to GRP78, initiating endothelial cell–mediated fungal endocytosis, resulting in host cellular death.[58] Suppression of GRP78 expression or blocking its function by antibodies suppresses fungal invasion of host endothelial cells by Mucorales, but not *Aspergillus fumigatus* or *Candida albicans*.[58] Endothelial cell uptake is also enhanced through activation of host platelet-derived growth factor pathways (PDGF)[16] because treatment with small-molecule inhibitors of PDGF reduces endothelial cell damage caused by *Rhizopus*.

Proteins from the CotH spore protein coat family are the key ligands that bind GRP78 expressed on the host endothelium.[60] Blocking CotH

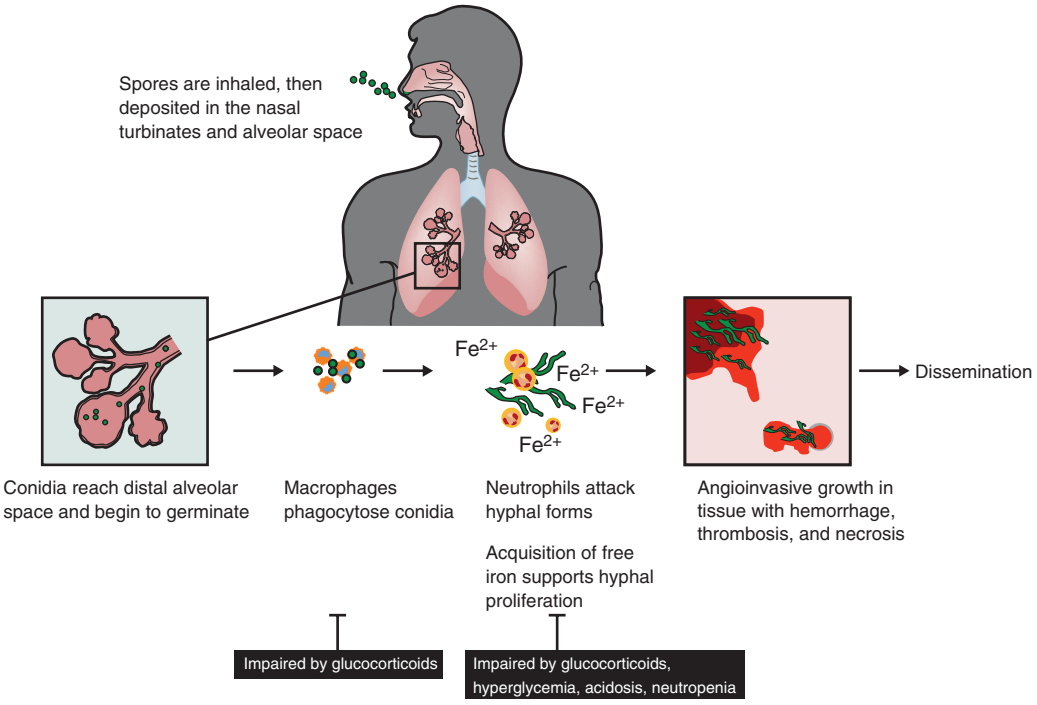

FIG. 258.3 **Pathogenesis of invasive mucormycosis.**

protein function with antibodies reduces the ability of Mucorales to invade and injure endothelial cells in vitro and improves survival in mice.[60] However, it is still unknown whether some hosts may be more susceptible to developing mucormycosis due to single nucleotide polymorphisms in GRP78, which could result in enhanced binding affinity for certain sequences of CotH proteins expressed in specific Mucorales.[61]

Role of Iron Uptake in Mucormycosis Pathogenesis

Anecdotal case reports and experimental data from the 1980s suggested that hemochromatosis is a predisposing risk factor for mucormycosis.[33,62] Fungi can acquire iron from the host by using low-molecular-weight iron chelators (siderophores) or high-affinity iron permeases, such as ferrirhizoferrin.[63] Of the two mechanisms, it is believed that high-affinity iron permeases play the more critical role for adaptive survival of the fungus in the human host. Iron overload in organs such as the liver has also been reported to enhance fungal virulence.[64]

Patients with diabetic ketoacidosis are particularly susceptible to developing rhinocerebral forms of mucormycosis, perhaps because of diminished capacity of transferrin to bind and sequester free iron at a pH less than 7.4.[33,65] The growth of *R. arrhizus* is markedly different in sera collected from patients with diabetic acidosis compared with healthy control subjects. Artis and colleagues[33,65] reported that normal human serum is not capable of supporting the growth of *R. arrhizus* even with the addition of free iron. However, under acidic conditions (pH < 7.4), the addition of exogenous iron markedly enhances the growth of *R. arrhizus* hyphae. Similarly, sera collected from patients with ketoacidosis supports exuberant growth of *R. arrhizus* without exogenous iron, provided the pH was maintained at less than 7.4, suggesting that acidosis disrupts the ability of transferrin to bind and sequester free iron.[33,65]

Historically, patients with severe hemochromatosis or aluminum toxicity received treatment with the metal chelator deferoxamine, which, paradoxically, is associated with increased susceptibility for developing disseminated mucormycosis.[34,66] Subsequent experimental models demonstrated that *R. arrhizus* can use deferoxamine as a xenosiderophore to form the ferrioxamine complex, which will make iron available for use previously unavailable to the fungus.[34,67] Specifically, *Rhizopus* can bind deferoxamine complexes and strip away free iron through a reductive process that allows intracellular uptake of free iron through the enzyme iron permease.[62] Of interest, uptake of radiolabeled iron in the presence of deferoxamine is 8- to 40-fold lower in *Candida* and *Aspergillus* compared with *R. arrhizus,* suggesting this mechanism is a relatively unique pathogenic trait of this fungus. This observation has been confirmed in animal models where administration of deferoxamine worsens survival of guinea pigs infected with *R. arrhizus* but not *Candida albicans.*[67]

Unlike deferoxamine, newer iron chelator agents, such as deferiprone and deferasirox, have not been associated with increased risk for mucormycosis because of their limited capacity to act as xenosiderophores for *Rhizopus* spp. Indeed, both deferiprone and deferasirox have shown protective effects in murine[68] and guinea pig[66] models of mucormycosis, with several case reports suggesting a possible benefit of adjunctive deferasirox therapy in human mucormycosis.[69] However, a small nonrandomized study found no survival benefit of adding deferasirox in combination with liposomal amphotericin B (L-AMB) in patients with hematologic malignancies.[70] Deferasirox may be more effective adjunct therapy for mucormycosis in the setting of diabetic ketoacidosis versus patients with neutropenia. Nevertheless, adjunctive iron chelation therapy cannot be recommended in patients with mucormycosis until more convincing clinical evidence of its benefit is available.[71]

Host Immune Response Against Mucormycosis

Both mononuclear and polymorphonuclear phagocytes, including natural killer cells, prevent germination of Mucorales spores and damage hyphal forms of the fungus.[72–74] After encountering *R. arrhizus* hyphae, human polymorphonuclear neutrophils activate robust proinflammatory gene expression through activation of Toll-like receptor 2 and nuclear factor kappa B pathways.[75] Hyphal damage is elicited by oxidative mechanisms after monocyte or neutrophil attachment to hyphal fragments.[72,76] Platelets may also play a role in host antifungal defenses against Mucorales by adhering to hyphae forms and secreting microbiocidal proteins.[77]

Defects in phagocytic activity associated with decreased cell numbers (i.e., neutropenia) or function (i.e., associated with glucocorticoids, hyperglycemia, or acidosis) allow unimpeded growth of the hyphal form. Glucocorticoids are known to impair the migration, attachment, ingestion, and phagolysosome fusion of bronchoalveolar macrophages essential for clearing spores from the alveoli.[78] Neutrophils collected from patients with severe hyperglycemia and diabetic ketoacidosis, burn-stress pseudodiabetes, or glucocorticoid-treated graft-versus-host disease (GVHD) often have impaired chemotaxis and diminished

oxidative and nonoxidative fungicidal mechanisms against Mucorales spores and hyphae.[79] In the setting of these impaired host immune defenses, rapidly growing Mucorales can outcompete the impaired immune response and rapidly spread through tissues and blood vessels.[55]

Mucorales-specific $CD4^+$ or $CD8^+$ T cells that produce interferon (IFN)-γ, interleukin (IL)-10, and, to a lesser extent, IL-17 can be detected early in infection in patients with hematologic malignancies and decrease with resolution of the infection.[80] As such, detection of such cells may support the diagnosis of mucormycosis. Mucorales have also been reported to activate human dendritic cells through Dectin-1, resulting in the production of IL-23 and induction of proinflammatory Th17 responses.[81] Elevated concentrations of glucose, iron, and BHB encountered in patients with diabetic ketoacidosis impair T-cell proliferation, IFN-γ production, and phagocyte-mediated killing.[59,82–85]

Evidence from animal models suggests that Mucorales can persist in granulomatous clusters, which reactivate during periods of intensive immunosuppression or metabolic dysfunction, such as diabetic ketoacidosis.[86] The possibility of a preexisting latent infection stage raises questions concerning the possibility of screening for latent mucormycosis in patients before undergoing intensive immunosuppressive therapy.

Histopathology

Mucorales have an exceptional capacity to invade blood vessels, resulting in necrosis of vessel walls and mycotic thrombi.[87] Thrombosis of vessels leads to infarction and hematogenous dissemination. Infected tissue typically reveals extensive necrosis with diffuse infiltration of polymorphonuclear leukocytes. However, in areas with ischemic necrosis, inflammation is sometimes minimal despite the presence of numerous hyphae.[88,89] In the otherwise healthy host a pyogenic or pyogranulomatous response without angioinvasion is more common. Reactivation from granulomas, that is, granulomatous inflammation, is possible during periods of subsequent immunosuppression or metabolic dysfunction (e.g., hyperglycemia, ketoacidosis).

CLINICAL MANIFESTATIONS OF MUCORMYCOSIS

The clinical presentation of mucormycosis is broad, depending on the underlying immune status and comorbidities of the host (Table 258.2). The signs and symptoms of mucormycosis are nonspecific, emphasizing the importance of a high index of suspicion in susceptible patient populations. In the immunocompromised host, mucormycosis can present as a fulminant angioinvasive infection that frequently disseminates with fatal consequence. The clinical manifestations of mucormycosis can be generally grouped into one of six syndromes with some overlap: (1) rhinocerebral infections and (2) pulmonary, (3) cutaneous, (4) GI, (5) disseminated, and (6) unusual presentations of mucormycosis.

TABLE 258.2 Patterns of Mucormycosis by Host Population

PREDISPOSING CONDITION	PREDOMINANT SITES OF INFECTION
Diabetes mellitus	Rhinocerebral, sino-orbital, cutaneous
Malignancy (typically hematologic malignancy)	Pulmonary, sinus, cutaneous, sino-orbital, disseminated
Hematopoietic stem cell transplantation	Pulmonary, sinus, rhinocerebral, disseminated
Solid-organ transplantation	Sinus, cutaneous, pulmonary, rhinocerebral, disseminated
Intravenous drug use	Cerebral, endocarditis, cutaneous, disseminated
Malnutrition	Gastrointestinal, disseminated
Deferoxamine therapy	Disseminated, pulmonary, rhinocerebral, cerebral, cutaneous, gastrointestinal
Trauma	Cutaneous, ocular

Rhinocerebral Infections

Rhinosinusitis, rhino-orbital, and rhinocerebral infections are classic manifestations of human mucormycosis. Infection is initially localized to the nasal turbinates and paranasal sinuses after inhalation of spores but can rapidly progress to the orbit (sino-orbital) or brain (rhinocerebral), particularly in patients with diabetic ketoacidosis or profound neutropenia.[42,90] Patterns of progression for the infection demonstrate some host predilection (see Table 258.2). The rhino-orbital form occurs more frequently in patients with poorly controlled diabetes, whereas patients with underlying leukemia or lymphoma are more likely to present with pulmonary infections (Fig. 258.4). Indeed, rhino-orbital mucormycosis is sometimes the first manifestation of undiagnosed diabetes mellitus, especially in patients from developing countries.

Clinical Presentation

Initial symptoms of sinus invasion by mucormycosis are indistinguishable from other more common causes of sinusitis. Sinus pain, congestion, headache, mouth or facial pain, otologic symptoms, and hyposmia or anosmia are common. A concomitant nonproductive cough often reflects lung involvement. Involved tissues become red, then violaceous, and finally black with thrombosis and tissue necrosis. Necrotic eschars of the nasal cavity and turbinates, facial lesions around the nose, and exophytic or necrotic lesions of the hard palate extending from the maxillary sinus are signs of rapidly progressing infection.[91] Careful endoscopic inspection of the nasal cavity and biopsy of suspicious lesions is helpful for early diagnosis. However, the absence of lesions or necrotic eschars does not rule out the possibility of rhinocerebral infection because necrotic nasal or palate lesions may be seen in only 50% of patients within 3 days of the onset of infection.[91]

Extension of sinus disease is primarily into contiguous structures. Maxillary sinus infection extends into the hard palate, nasal cavity, and ethmoid sinus. Sphenoid disease invades the cavernous sinus, contiguous temporal lobe, and internal carotid artery in the siphon. Septic emboli from the carotid artery into the frontal and parietal lobes can occur. Ethmoid sinus disease may invade the face or frontal lobe but easily crosses the lamina papyracea into the orbit.[92] The frontal sinus is an uncommon primary site. Invasion of the orbit is typically unilateral (see Fig. 258.4). Periorbital edema, ptosis, proptosis, chemosis, and preseptal and orbital edema are early signs of orbital extension. Pain and blurring or loss of vision often indicate invasion of the globe or optic nerve. Infraorbital facial numbness follows invasion of the infraorbital nerve within the orbit.[92] Patients with extensive rhino-orbital or rhinocerebral disease may present with trigeminal and ocular motor nerve palsy after cavernous sinus invasion (cranial nerves III, IV, and VI, and the superior two branches of the fifth nerve).[93] A bloody nasal discharge may be an early sign of nasal mucosal invasion.[94,95] Intracranial complications include epidural and subdural abscesses, cavernous, and, less commonly, sagittal sinus thrombosis.[96–98] Frank meningitis in patients with mucormycosis is rare.[17]

Radiology

Radiographic imaging is often suggestive of severe sinusitis but lacks the specificity to diagnose rhinocerebral mucormycosis. Patients with fungal or surgical disruption of the dura mater may present with superimposed bacterial meningitis, or bacterial sinusitis may complicate postoperative management.[99] Computed tomography (CT) of the sinuses typically reveals mucosal thickening, air-fluid levels, and bony erosion (Fig. 258.5).[100] Highly immunosuppressed patients often present with pansinusitis that is highly suggestive of an aggressive fungal infection.[50] Orbital thickening may also be detected on CT scans but can be detected earlier by magnetic resonance imaging (MRI).[101] CT and MRI scans of the orbits may be unremarkable during the initial stages of the infection, highlighting the importance of serial radiographic imaging for monitoring disease progression.[101] The frequency of radiographic imaging is patient dependent but may be required every 2 to 3 days in patients with suspected progression. Therefore rhinoscopy or nasal endoscopy is critical for confirming tissue ischemia and the extent of disease.[99] Extraorbital muscle thickening is often the first sign on CT or MRI of orbital involvement and should prompt empirical antifungal therapy until surgical exploration or biopsy of the sinus and orbits can be

FIG. 258.4 Sino-orbital involvement of mucormycosis. (A) Orbital involvement in cancer patient. Note the periorbital ecchymosis and sanguineous discharge from the eye. (B) Rapid progression of orbital involvement with necrosis of nasal bridge in less than 24 hours. (C) Necrotic eschar on the hard palate of a cancer patient with rhinocerebral mucormycosis. *(Courtesy Drs. Gerald Bodey, George Viola, and Mona Shiekh Sroujieh, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

performed, which should be done as soon as possible.[100] Every effort should be made to establish an early definitive diagnosis of mucormycosis by biopsy and culture of necrotic lesions and rapid histologic assessment of frozen sections.[102] Impression smears from the biopsy or surgical margin may also reveal hyphae consistent with mucormycosis.

Pulmonary Infections

Pulmonary mucormycosis is most commonly encountered in patients with prolonged neutropenia, recipients of hematopoietic stem cell or solid-organ transplantation, and patients receiving deferoxamine therapy.[3,17,103] The infection frequently occurs concomitantly with sinus infection.[17] However, the clinical manifestations of the infection are indistinguishable from more common opportunistic molds, such as invasive pulmonary aspergillosis (IPA). Therefore timely diagnosis is a critical factor in the outcome on the infection because first-line antifungals typically used for aspergillosis, such as voriconazole, lack activity against Mucorales. A case series of 61 leukemia and transplant patients with evidence of fungal pneumonia found that 84% of patients who were eventually documented to have pulmonary mucormycosis were receiving ineffective antifungal therapy at the time of their diagnosis.[104,105] Similarly, an analysis of 70 hematologic malignancy patients with pulmonary mucormycosis demonstrated that a delay in the administration of appropriate antifungal therapy (typically an amphotericin B formulation) of as few as 6 days was associated with a doubling of 4-week (35.1% vs. 66.6%; $P = .006$) and 12-week (48.6% vs. 82.9%; $P = .029$) crude mortality rates.[106] Data to support imaging findings that might heighten the suspicion for pulmonary mucormycosis are reported in a retrospective analysis of neutropenic leukemia patients. In the first 5 days of clinical symptoms, fever or chest pain, a reverse halo sign (focal ground-glass attenuation surrounded by a ring of consolidation) was present in 15 of 16 patients with proven pulmonary mucormycosis.[107,108] However, the reverse halo sign is not specific for mucormycosis but, rather, may be suggestive of invasive aspergillosis, mucormycosis, or organizing pneumonia.[109,109a]

Clinical Presentation

Clinical symptoms of pulmonary mucormycosis are subtle and nonspecific even at late stages in the infection, especially in patients receiving therapies that suppress immune responses (i.e., high-dose glucocorticoid therapy, anti–T-cell antibodies).[106] Patients frequently present with refractory fever on broad-spectrum antibiotics, nonproductive cough, progressive dyspnea, and pleuritic chest pain.[50,106] Pulmonary mucormycosis can traverse tissue planes in the lung, invading through the bronchi, diaphragm, chest wall, and pleura.[103] A pleural friction rub on auscultation is present in some patients. Hyphal invasion of blood vessels results in necrosis of the surrounding parenchyma, ultimately leading to cavitation or potentially fatal hemoptysis.[110–112] In patients with hematologic malignancies, clues for distinguishing pulmonary mucormycosis from IPA may include the presence of severe sinusitis; history of prophylaxis with antifungals that possess activity against aspergillosis but not mucormycosis (i.e., voriconazole or echinocandin prophylaxis); suggestive CT imaging findings, such as reverse halo sign and multiple nodular infiltrates with or without pleural effusion; and possibly the repeated absence of detectable *Aspergillus* galactomannan antigen in the serum or in bronchial alveolar lavage (BAL) fluid.[50,113–115] Unfortunately, it is common in debilitated patients to have a concomitant polymicrobial pneumonia, which can further confound early diagnosis of pulmonary mucormycosis.[103,110]

Radiology

The radiographic presentation of pulmonary mucormycosis is broad, including focal consolidation with nonspecific infiltrates, cavitary lesions, or even diffuse opacities that evolve quickly, depending on the underlying

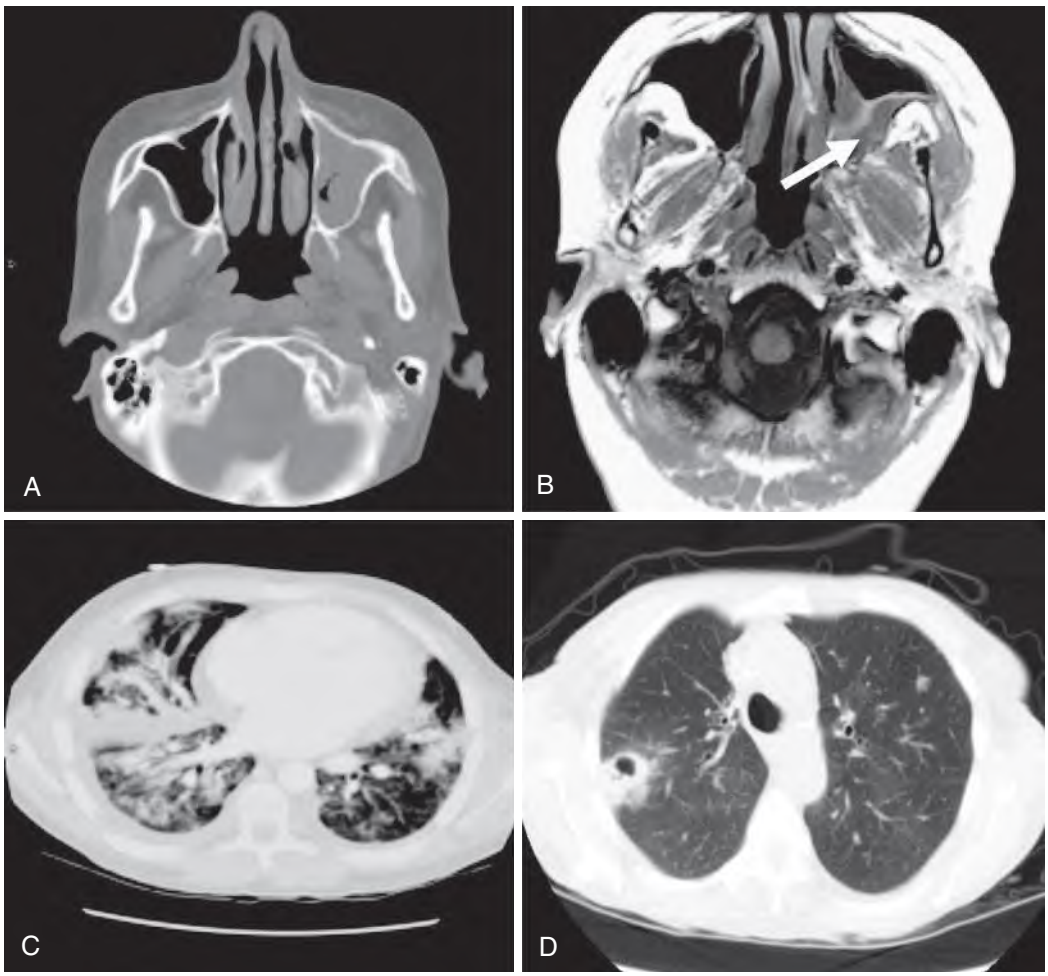

FIG. 258.5 Radiographic findings in sinopulmonary mucormycosis. (A) Left maxillary sinus air-fluid level is evident in this computed tomography (CT) scan that is indistinguishable from bacterial sinusitis. (B) Magnetic resonance image reveals T2 signal hyperintensity in the left pterygoid musculature *(arrow)* in conjunction with a left maxillary sinus air-fluid level. (C) Multiple heterogeneous nodular and consolidative lesions with a large pulmonary vessel infarct *(wedge)* and modest pleural effusions are shown in a cancer patient with pulmonary mucormycosis. (D) Contrast-enhanced CT scan demonstrates a cavity within a dense infiltrate in a patient with acute myelogenous leukemia and pulmonary mucormycosis. *(Courtesy Dr. Edith Marom, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

immune status of the patient (see Fig. 258.5).[103,116] Thrombosis of pulmonary vessels with angioinvasion often leads to large wedge-shaped infarcts.[103] Earlier studies of pulmonary mucormycosis suggested a predilection for upper lobar disease in 55% to 84% of cases.[117,118] However, any part of the lung may be involved, and bilateral disease is common.[103]

Like IPA, high-resolution chest CT is the best method of determining the extent of pulmonary mucormycosis and typically demonstrates evidence of the infection before its appearance on standard chest radiographs (see Fig. 258.5). Although nodular opacities without an air bronchogram indistinguishable from aspergillosis are the most common finding on CT scan, the presence of multiple nodules (≥10), pleural effusion, or both, may favor the diagnosis of pulmonary mucormycosis.[116] Halo and air crescent signs are encountered less frequently in leukemic patients with pulmonary mucormycosis compared with pulmonary aspergillosis.[116] However, centrally located lesions demonstrating the air-crescent sign are often associated with an increased risk for pulmonary artery erosion and massive hemoptysis.[102] Three small case series have suggested that a reverse halo sign, a focal round area of ground-glass attenuation surrounded by a ring consolidation, may be a more common early radiographic finding in patients with invasive pulmonary mucormycosis compared with aspergillosis.[107,108,119,120]

Pulmonary mucormycosis rapidly spreads to the contralateral lung and distal organs if not promptly treated. Although patients with pulmonary mucormycosis usually die from disseminated disease before respiratory failure occurs, dissemination is rarely detected antemortem.[121] The overall mortality rate of pulmonary mucormycosis ranges from 50% to 70% but can increase up to 95% in patients with extrathoracic dissemination.[121]

PULMONARY MUCORMYCOSIS IN LESS SEVERELY IMMUNOCOMPROMISED PATIENTS

In more immunocompetent hosts, pulmonary mucormycosis may present with more atypical, slowly progressing forms.[6] Mycotic pulmonary artery aneurysms and pseudoaneurysms, bronchial obstruction, and even asymptomatic solitary nodules have been described without clear underlying immune dysfunction. Patients with diabetes mellitus have a predilection for developing endobronchial lesions that present with a less fulminant course than pulmonary mucormycosis encountered in the neutropenic or transplant population.[117] On occasion, endobronchial lesions may lead to obstruction of the major airways or erosion of major pulmonary blood vessels and fatal hemoptysis.

Like *Aspergillus* spp., Mucorales in rare instances can form mycetomas in preexisting lung cavities or cause slowly necrotizing pneumonia and hypersensitivity syndromes. *Rhizopus* spp. have also been implicated in an allergic alveolitis described in farm workers and Scandinavian sawmill workers (wood-trimmer's disease).[122,123]

Skin and Soft Tissue Infections

Cutaneous mucormycosis is typically the result of direct spore inoculation or exposure of skin already compromised by burns or extensive trauma or, less commonly, after insect or spider bites.[124,125] Necrotizing soft

tissue infections with cutaneous mucormycosis have been reported in survivors with traumatic injuries after volcanic eruptions, tsunamis, and tornadoes.[9,10] Mucorales-contaminated bandages, needles, and bed linens have also been implicated in outbreaks of soft tissue infections in hospitalized patients.[23–27,30]

Cutaneous mucormycosis typically starts as erythema and induration of the skin at a puncture site and progresses to necrosis with a black eschar (Fig. 258.6). Cutaneous infections can quickly extend into the deep fascia and muscle layers. Necrotizing fasciitis has been reported in patients with progressive cutaneous mucormycosis and is associated with an extremely poor prognosis.[126–131] Neutropenic patients, in particular, are susceptible to lymphatic and blood vessel invasion, infarction, and necrosis with eventual dissemination. In patients with massive soft tissue trauma the mold invades the gray necrotic tissue at the margins of the wound and spreads to contiguous viable tissue, enlarging the wound. Unlike many other molds (*Aspergillus, Fusarium, Scedosporium,* fungi causing phaeohyphomycoses), the skin appears to be a less common site of secondary involvement in disseminated mucormycosis.[17] Atypical dermatologic manifestations have also been reported in less immunosuppressed leukemic patients and solid-organ transplant recipients, mimicking erythema nodosum or panniculitis.[132,133]

Skin biopsy is critical for diagnosis because necrotic skin lesions in neutropenic patients have a broad differential diagnosis.[134] Biopsy specimens taken from the center of the lesion down to the subcutaneous fat are most likely to reveal hyphae invading the blood vessels of the dermis and subcutis. Excision and wide débridement of cutaneous lesions, coupled with systemic antifungal therapy and, according to some, hyperbaric oxygen therapy, can further reduce mortality rates (see Chapter 50).

FIG. 258.6 Cutaneous presentation of mucormycosis. (A) Chronic, nonhealing ulcer with necrosis after traumatic inoculation. Cutaneous ecthyma gangrenosum lesions behind the ear (B) and face (C) of a neutropenic patient with disseminated mucormycosis. *(Courtesy Drs. Gerald Bodey and Saud Ahmed, University of Texas, MD Anderson Cancer Center, Houston, TX.)*

Osteomyelitis

Mucorales are an uncommon cause of bone and joint infections that are typically diagnosed late after initial symptom onset. A review of 34 individual cases from 30 publications[135] found that bone and joint infections were most frequently associated with trauma, automobile accidents with fracture, and puncture of the knee or penetrating wounds. Direct inoculation was the main mechanism of infection in 56% of cases, particularly in patients with prior trauma or surgery. Hematogenous dissemination occurred in 24% of cases in patients with hematologic malignancy or immune impairment, whereas another 21% of cases occurred from contiguous spread of the infection. Fever was uncommon, but patients frequently experience local pain and tenderness in the affected joint with occasional cellulitis with elevated inflammatory markers, such as elevated erythrocyte sedimentation rate, white blood cell counts, and possibly elevated C-reactive protein. Diagnosis was most frequently established by open surgical biopsy.[135] Mucorales osteomyelitis requires both antifungal treatment and surgical intervention that may include débridement, bone grafting/fixation procedures, full excision, or amputation if required. The overall mortality rate in a published case series was 24%.[135]

Gastrointestinal Mucormycosis

Primary GI mucormycosis is a rare infection, with protean manifestations occurring primarily in malnourished patients and premature infants, where it can present as necrotizing enterocolitis.[19] The infection often starts with an ulcer, sometimes in the stomach, but can involve any compartment of the GI tract.[136] Patients may present with peritonitis after the fungus has invaded through the gastric mucosa and bowel wall.[137] Liver abscesses have also been described after ingestion of herbal products contaminated with *Mucor indicus*.[138] In neutropenic patients seeding of the GI tract is probably more common than previously thought because only a few of the infections are identified antemortem. Patients may present with subtle findings of fever, enterocolitis, or hematochezia that can progress to colonic ischemia with transmural necrosis and perforation of the gut. Masslike appendiceal or ileal lesions have also been described.[137] Unfortunately, GI mucormycosis is often diagnosed late because of the nonspecific presentation and a high degree of suspicion is required for early diagnosis by endoscopic biopsy.[137]

Disseminated Mucormycosis

Disseminated mucormycosis is rarely apparent before death. The symptoms vary depending on the site of dissemination, degree of vascular invasion, and affected organs. The patient groups classically at risk for this infection have received treatment with deferoxamine for iron overload, are persistently neutropenic patients with active leukemia, or are allogeneic stem cell transplant recipients with GVHD receiving high-dose glucocorticoid therapy or anti–T-cell antibodies.[6,17] Pneumonia is common in patients with disseminated mucormycosis and is assumed

to be the primary source in most patients even when not detectable radiologically.[121] Because of the poor sensitivity of blood and respiratory cultures for diagnosing disseminated mucormycosis, biopsy of the suspected sites is critical for diagnosis of the infection. Patients with hematologic malignancies or diabetic patients with disseminated mucormycosis have presented with acute myocardial infarction or bowel ischemia after arterial occlusion by fungi.[139–141]

Less Common Presentations of Mucormycosis

Although rare, peritonitis has been described in patients undergoing continuous ambulatory peritoneal dialysis.[142–144] The infection tends to have a slowly progressive course, although the attributable mortality rate in patients who received delayed or inappropriate therapy can exceed 60%.[142] In peritoneal dialysis, catheter-related mucormycosis, prompt removal of the catheter, and several weeks of systemic antifungal therapy are essential.

Isolated reports of mucormycosis of the trachea,[145] mediastinum,[146] bone,[147] heart,[148,149] thyroid, and kidney[150] have been described. Other manifestations, such as otitis externa,[151] corneal infection, and superior vena cava syndrome,[152] have also been reported. IV drug abusers are particularly vulnerable to central nervous system manifestations of mucormycosis, often presenting as a brain abscess involving the basal ganglia in conjunction with infective endocarditis.[153–157]

DIAGNOSIS

Because of the ubiquitous nature of the fungus in the environment, positive cultures may occasionally reflect culture contamination rather than true infection. However, discovery of hyphae in a specimen from an immunocompromised host is an important diagnostic clue that should be confirmed whenever possible with histopathologic documentation of fungal invasion.[158] Not surprisingly, the site of infection has a major impact on the likelihood of histopathologic confirmation.[6] The ease of accessibility of the skin or sinuses allows more definite diagnosis of infections at these sites. Tissue swabs and cultures of sputum, sinus secretions, nasal mucosa, and BAL fluid are usually nondiagnostic but may be an important indication of disease in immunocompromised patients.[159] Blood cultures rarely grow Mucorales despite the angioinvasive nature of these pathogens.

In tissue, Mucorales hyphae can often be distinguished from other more common opportunistic molds, such as *Aspergillus* and *Fusarium,* by their broad (uneven diameter, 3–25 μm), empty, thin-walled, mostly aseptate hyphae.[87] Frequently, these hyphae have focal bulbous dilation and nondichotomous irregular branching at occasional right angles. Tissue sections may show a variety of mixed hyphal forms that include folded, twisted, or compressed hyphae that may be mistaken for septae or, when transected, large empty spherules of *Coccidioides immitis.* Reproductive hyphal structures containing spores (sporangia) are rarely observed in deep tissue, even in well-aerated sites of infection. Mistaken histologic identification is relatively common and can lead to inappropriate therapy.

A variety of stains, including hematoxylin and eosin, Grocott-Gomori methenamine silver, and periodic acid–Schiff stains, will reveal characteristic hyphal elements in tissue. Failure of hyphae to stain with Grocott-Gomori methenamine silver is observed in some patients. Perineural invasion is found in 90% of tissues that contain nerves.[93] The inflammatory process can range from neutrophilic, granulomatous, or pyogranulomatous to minimal inflammation with hemorrhage, depending on the degree, chronicity, and type of underlying immune deficit.[87] Fungal hyphae can also be examined directly with a potassium hydroxide preparation of the tissue specimen or BAL fluid. Staining with fluorescent dyes, such as calcofluor white, Blankofluor, or Uvitex, may enhance detection of hyphal elements during ultraviolet microscopic examination and improve the discrimination between septate and aseptate molds in biopsy specimens.[104,105,160]

Species Identification

Identification of Mucorales to the genus and species level requires cultivation of the fungus in culture to examine reproductive fruiting structures of the fungus. Most species grow rapidly on fungal media, such as Sabouraud dextrose agar incubated at 25° to 30°C. The level of development of the rhizoids, the shape of the sporangium, and the location of the sporangiospores are the morphologic features used to identify different genera of Mucorales (see Fig. 258.1). Nucleic acid sequencing of PCR is being used increasingly to identify cultures without typical morphologic features.[161] Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry may also aid identification and is becoming more available in reference laboratories, although reference databases used to aid interpretation are still limited.[162,163] Recent applications of PCR-based identification have suggested that up to 20% of species may be misidentified at the species level by morphology alone. Unfortunately, culture recovery of the agents of mucormycosis from tissue is inherently poor owing to the friability of the nonseptated hyphae, making them more susceptible to damage during tissue manipulation. Recovery from tissue can be improved by mincing (not homogenizing) tissue and using culture techniques that simulate in vivo growth, including incubation at 35° to 37°C in semianaerobic conditions.[164,165]

Differentiating Mucormycosis From Other Molds

The importance of early differentiation of mucormycosis from other mold infections has generated interest in the development of non–culture-dependent or non–histopathology-dependent diagnostic tests, such as detection of specific antigens or nucleic acid by PCR. Unfortunately, molecular techniques are in use only in research laboratories for the diagnosis of mucormycosis but have shown some promise when evaluated retrospectively in histopathologic or culture-confirmed cases.[166,167] Antigen tests for *Aspergillus* (galactomannan) and other fungal species (β-D-glucan) are not useful for mucormycosis. Several studies have attempted to improve early diagnosis through detecting nucleic acid in serum by using PCR assays or in situ hybridization techniques.[161,168–170] To date these techniques have shown the greatest utility as adjunctive diagnostics, for molecular typing in epidemiologic studies, or for confirming the presumptive genus of the pathogen when histopathology is positive, but cultures are negative.[171] In a prospective study of CT-guided percutaneous lung biopsy samples from nonthrombocytopenic patients with suspected fungal pneumonia, Lass-Florl and colleagues[105] demonstrated that the rapidity of diagnosis and differentiation of mucormycosis from aspergillosis could be improved by using a three-step analysis approach for biopsy specimens: (1) calcofluor white staining to rapidly distinguish septate versus aseptate hyphae, (2) *Aspergillus* galactomannan and PCR testing for rapid identification, and (3) PCR testing of DNA in select biopsy specimens in which aseptate hyphae were observed or *Aspergillus* markers were negative. These promising results will require further confirmation in a wider range of patients before PCR becomes a standard-of-care adjunctive diagnostic test for mucormycosis.

Antifungal Susceptibility Testing

Susceptibility testing of Mucorales isolates is not routine in most clinical microbiology laboratories. Although standardized methods applicable for testing Mucorales isolates have been proposed by both the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST), minimal inhibitory concentration (MIC) results obtained using these two methods often differ.[172,173] Moreover, MIC results often increase by at least two dilution steps with incubation beyond 24 hours.[174] Agar-based methods, such as the Etest, have shown relatively good reproducibility and agreement (70%–100%) with broth microdilution methods, depending on the isolate and Mucorales tested.[175] Nevertheless, the performance of many commercially available tests has not been systematically compared with reference methods for Mucorales. Interpretive MIC breakpoints have not been defined for the Mucorales. Therefore the role of MIC testing in the management of mucormycosis remains uncertain.

Amphotericin B is considered the most active drug against Mucorales and is active in vitro against most species within this order with MIC values of 0.03 to 2 μg/mL (Table 258.3).[174] However, susceptibility does vary by species, with higher MICs observed for *Cunninghamella* spp.[176] Epidemiologic cut-off values (ECVs) for amphotericin B have

TABLE 258.3 Summary of in Vitro Antifungal Activity of Different Antifungal Drugs Against Mucorales (Data Are Adapted From Dannaoui)

CLASS	MOLECULE	MIC_{50} (μg/mL)	INTERPRETATION
Allylamines	Terbinafine	0.06 to >16	Variable activity, depending on species
Pyrimidine analogues	Flucytosine	>16	Inactive
Polyenes	Amphotericin B	0.03 to 2	Active
Azole	Fluconazole	>16	Inactive
	Itraconazole	0.25 to 16	Variable activity depending on species
	Voriconazole	2 to >16	Not active
	Posaconazole	0.03 to 2	Active, depending on species
	Isavuconazole	0.25 to 8	Active, depending on species
Echinocandins	Caspofungin	>8	Inactive
	Micafungin	>8	Inactive
	Anidulafungin	>8	Inactive

been proposed using CLSI methods for *Lichtheimia corymbifera*, *M. circinelloides*, and *Rhizopus microsporus* (2 μg/mL) and for *R. arrhizus* (4 μg/mL).[176] The frequency of non–wild-type isolates based on these ECVs ranges from 0%–2.15% depending on the species. One small case series reported that Mucorales infections associated with an amphotericin B MIC <0.5 μg/mL was significantly associated with better 6-week outcomes.[177]

Posaconazole and, perhaps, isavuconazole are considered to have clinically useful anti-Mucorales activity (see Table 258.3). The MIC_{50} of posaconazole ranges from 0.25 to 2 μg/mL for *Rhizopus* spp., 0.5 to 2 μg/mL for *Mucor* spp., and 0.125 to 1 μg/mL for *Lichtheimia* spp.[172,173,178] When evaluated in neutropenic murine model of invasive pulmonary mucormycosis, the posaconazole serum area under the concentration-time curve (AUC)/MIC ratio was most predictive of antifungal effect, with the greatest reduction in lung fungal burden measured at an AUC/MIC greater than 100.[179] For *R. arrhizus* (MIC, 2 μg/mL), an AUC/MIC target >100 was achieved when serum trough posaconazole exposures in the animals exceeded 4 μg/mL.[179]

Isavuconazole susceptibility is species dependent, with MIC_{50s} ranging from 0.25 to 1 μg/mL for *Lichtheimia* spp. and *Rhizomucor* spp., 1 μg/mL for *Rhizopus* spp., but significantly higher for *M.* circinelloides (8 μg/mL).[172] Although MICs in general are one to three dilutions higher for isavuconazole versus posaconazole, slightly higher serum drug exposures with isavuconazole might possibly compensate for these modest differences in potency.[172]

Itraconazole exhibits variable species-specific antifungal activity in Mucorales, similar to isavuconazole, but itraconazole's unpredictable absorption does not make it a reliable triazole for treatment of invasive mucormycosis. Fluconazole lacks activity against Mucorales (see Table 258.3), and voriconazole lacks sufficient activity in vitro and in vivo to treat mucormycosis at clinically achievable concentrations.[172,173,178] The lack of sufficient voriconazole activity in vivo is also reflected by frequent cases of breakthrough mucormycosis in patients on voriconazole prophylaxis or therapy.[51,115,121]

Although 1,3-β-D-glucan synthase target is present in Mucorales, 1000-fold higher echinocandin concentrations are required to inhibit the enzyme complex in *R. arrhizus* compared with *Candida albicans* or *Aspergillus fumigatus*.[180] In consequence, echinocandins are not considered effective agents in vivo and have demonstrated only limited activity in animal models as monotherapy at lower but not higher doses.[180–182]

Combination Therapy

In vitro and preclinical in vivo studies of antifungal combination therapy (lipid amphotericin B formulations, triazoles, echinocandins, flucytosine, and terbinafine, as well as non-antifungal agents) have revealed mostly indifferent interactions with occasional synergy but no reports of antagonism for combinations of triazoles, amphotericin B, echinocandins, terbinafine, and, on occasion, non-antifungal agents.[174,183] A combination of lipid amphotericin B plus an echinocandin demonstrated improved survival in *R. arrhizus* rodent infection models compared with lipid amphotericin B alone.[181,184] Combinations of posaconazole or isavuconazole with either echinocandins or lipid amphotericin B formulations generally display indifferent results with respect to either triazole or lipid amphotericin B monotherapy.[174,185,186]

Deferasirox, the iron chelator with antifungal effects against Mucorales, has been evaluated in combination with L-AMB[68] and in triple combination with micafungin.[184] Both of these combination regimens, which were administered in diabetic mice, were more effective at prolonging survival compared with L-AMB alone. Modest synergistic effects were also noted in a neutropenic murine models of *R. arrhizus* invasive pulmonary mucormycosis with a combination of posaconazole and deferasirox.[179] However, combination regimens (posaconazole plus L-AMB, posaconazole plus an echinocandin, or posaconazole plus deferasirox) were only as effective as maximally dosed posaconazole.[179] Nevertheless, when deferasirox combined with L-AMB was evaluated in a double-blind multicenter study of neutropenic patients with mucormycosis, no survival benefit was found in the combination therapy group.[70]

TREATMENT

Successful treatment of mucormycosis relies on timely diagnosis, reversal of underlying predisposing factors, early surgical débridement of infected tissue, and rapid initiation of effective high-dose systemic antifungal therapy.[187] Early diagnosis is critical to the outcome of mucormycosis because small focal lesions can be surgically resected before the lesions progress to involve critical structures or distal organs.[50,121] Patients often have an indolent clinical presentation until extensive invasion or dissemination of the infection. Delays in the administration of systemic antifungal therapy increase the probability of patient death due to disseminated infection.[106]

Patients with suspected rhinocerebral mucormycosis should undergo a thorough examination and "staging" of their disease, including CT of the paranasal sinuses and lungs as well as endoscopic examination of nasal turbinates with biopsy of any suspicious lesions or necrotic eschars. Patient outcome can be improved if initial treatment decisions are based on frozen tissue samples from biopsy rather than waiting for tissues to be fixed and stained for histology.[188] Rapid correction of predisposing conditions, such as control of hyperglycemia, reversal of ketoacidosis, and rapid tapering of glucocorticoid therapy, are critical for reversing conditions that favor fungal virulence and dissemination.[59] In rare cases, correction of diabetic ketoacidosis was sufficient to allow recovery from cavitary pulmonary mucormycosis without antifungal treatment.[6] In neutropenic patients granulocyte transfusions may be beneficial as a temporary approach until granulocyte recovery.[150]

Antifungal Therapy

No prospective randomized studies of the primary treatment of mucormycosis have been performed, owing to the rarity and heterogeneous nature of this mycosis. Most evidence concerning the activity of existing antifungals has come from retrospective small case series, case registries, prospective open-label studies, and animal models of infection. Because both surgical and medical interventions are simultaneously or sequentially performed, it is difficult to ascertain the relative efficacy of drug therapy alone.[17] Historically, the drug of choice for the treatment of mucormycosis was conventional amphotericin B deoxycholate (ABD), administered