

Pulmonary Aspergillosis: Recent Advances

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ABSTRACT

Aspergillosis remains a significant cause of morbidity and mortality. The spectrum of disease is diverse and ranges from noninvasive disease with an excessive immune response, such as in allergic bronchopulmonary aspergillosis (ABPA), to a lack of an immune response as seen in patients with quantitative or qualitative granulocyte deficits and subsequent invasive pulmonary aspergillosis.

Noninvasive diagnostic testing has improved the time to initiation of effective antifungal therapy, and numerous agents in different therapeutic classes are now available as treatment options. Voriconazole remains the preferred agent in the treatment of invasive pulmonary aspergillosis, and recent data have increased interest in the potential of combination therapy against this often lethal infection. The role of host genetics in selecting patients that may benefit from more aggressive antifungal prophylaxis or treatment practices remains unclear but is likely to guide therapeutic choices as newer data become available.

KEYWORDS: Aspergillosis, chronic cavitary aspergillosis, aspergilloma

Aspergillosis refers to the clinical spectrum of disease caused by *Aspergillus* spp. Clinical manifestations vary widely and depend upon the immune status of the host. Pulmonary manifestations range from noninvasive disease, such as an allergic response seen in allergic bronchopulmonary aspergillosis (ABPA) (covered in detail by Drs. Hogan and Denning in this issue), or saprophytic colonization as is seen in aspergilloma, to semi-invasive or invasive infections such as chronic necrotizing pneumonia and invasive pulmonary aspergillosis.^{1–3} Infection of virtually all organs has been described, with disseminated disease found almost exclusively in those with profound or prolonged granulocytopenia. Despite advances in both diagnosis and therapy, mortality rates remain high, especially in severely immunosuppressed hosts. This review provides an update on recent important advances for the management of this infection.

MYCOLOGY AND EPIDEMIOLOGY

With the use of molecular techniques, over 250 distinct species of *Aspergillus* have been described in several subgenera and multiple “sections” (previously known as groups).^{4,5} The most common species in invasive infection include *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger* (Table 1)⁶; however, less common species, including less pathogenic species, have been reported to cause infection, especially in highly immunosuppressed patients. Identification of the genus *Aspergillus* in cultures, particularly the most common pathogenic species, is not usually difficult, but identification at a species level can be challenging, especially for poorly sporulating isolates, so that misidentification of these “cryptic” members of the *Aspergillus* sections is common.⁷ Phenotypic methods and DNA internally transcribed spacer sequencing only identify isolates within a section so that it is recom-

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Table 1 Frequency of *Aspergillus* spp. Received in a Fungal Reference Laboratory⁶

<i>A. fumigatus</i>	57%
<i>A. flavus</i>	12%
<i>A. niger</i>	10%
<i>A. terreus</i>	12%
Others	9%
<i>A. nidulans</i>	2%
<i>A. claidoustus</i>	2%
<i>A. sydowii</i>	2%
<i>A. versicolor</i>	2%

mended that clinical laboratories report species as members of a "species complex."⁵ Most species of *Aspergillus* reproduce asexually, but a teleomorph, or sexual form, has been identified for several pathogenic species, including *Aspergillus fumigatus* with its teleomorph *Neosartorya fumigata*. However, despite the usual priority given to the sexual form in taxonomy, the generic term *Aspergillus* is generally retained rather than utilizing new and unfamiliar species.⁸⁻¹⁰

The incidence of infection with *Aspergillus* spp. has increased in recent years, primarily due to the expanding hematopoietic stem cell (HSCT) and solid organ transplant populations and the increasing number of immunosuppressed patients encountered in clinical practice with the use of corticosteroids and other immune-modulating drugs, and in patients with advanced acquired immunodeficiency syndrome (AIDS). Undoubtedly, improvements in *Aspergillus* diagnostics have also played a role in the increasing incidence of this often lethal infection.

Exposure to *Aspergillus* spp., which is a ubiquitous organism found in water, food, air, and soil, is unavoidable. No geographic predilection to infection has been thought to exist, although recent evidence has called this into question and correlated regional differences in the incidence of infection with geoclimatic patterns.¹¹ Traditionally, patients at highest risk for infection and invasive disease have been those with either profound or prolonged neutropenia or those with qualitative neutrophil defects such as chronic granulomatous diseases. Evolving hematology and stem-cell transplantation practices have decreased both the duration and the frequency of neutropenia, and these periods of highest risk may be further reduced with the use of granulocyte colony stimulating factors.

Following these changes, the temporal association of infection has shifted to a bimodal distribution. The highest risk is still seen early after transplantation (<20 days) with a second peak in incidence occurring more than 100 days posttransplant. This later peak in incidence is related to the use of high-dose corticosteroid therapy in the treatment of acute or chronic graft versus host disease. It is also important to recognize that

recurrent neutropenia, which can occur with repeated courses of chemotherapy or with the long-term relative neutropenia in conditions such as myeloproliferative syndromes, also increases the risk of invasive aspergillosis. This concept of a relative prolonged deficiency of neutrophils is a concept described by Portugal and colleagues that substantially increases risk of invasive aspergillosis.¹²

Patients undergoing solid organ transplantation are also at increased risk for invasive aspergillosis, particularly those undergoing lung transplantation, with an incidence of around 10 to 15% in most series.^{13,14} The reasons for higher rates of aspergillosis in lung transplant recipients as compared with some other solid organ transplant recipients include previous colonization with *Aspergillus* spp. in the native or transplanted lung, a reduction in mucociliary clearance capability, and the continued exposure to the environment of the transplanted tissue.¹⁵

Critically ill patients without traditional risk factors have also recently been recognized as at risk for invasive aspergillosis.^{16,17} Receipt of corticosteroids during treatment of the acute respiratory distress syndrome (ARDS) or chronic obstructive pulmonary disease (COPD), impaired mucociliary clearance following the resolution of bacterial or viral pneumonia, cirrhosis, and underlying autoimmune disease have all been postulated as additional risk factors within the intensive care setting.¹⁸

Invasive aspergillosis has also been described in other immunosuppressed populations. Infection with advanced AIDS, hereditary immunodeficiencies, or biological agents such as tumor necrosis factor- α inhibitors such as infliximab all appear to predispose to invasive disease.^{2,19,20}

HOST SUSCEPTIBILITY

In the environment, *Aspergillus* spp. exist as filamentous molds producing both vegetative and aerial hyphae. Aerial hyphae produce unicellular conidia (2 to 3 μ m) subjecting the passerby to a large inhalational burden on a daily basis. In the immunocompetent host conidia are readily engulfed by alveolar macrophages with little to no inflammatory response.²¹ However, a stage-specific regulation of the immune response to *Aspergillus* spp. has been recognized, and germinating conidia (presenting an imminent risk for the formation of hyphae with subsequent invasive disease) trigger a robust proinflammatory response by engaging Dectin-1, the major β -glucan cell surface receptor present on macrophages, and Toll-like receptors 2 and 4 (TLR2 and TLR4).^{22,23}

Despite numerous single-nucleotide polymorphisms within these cell surface receptors few immunogenetic risk factors have been recognized. An early stop codon polymorphism (Y238X) within Dectin-1 has been

associated with *Candida* colonization, recurrent mucocutaneous candidiasis, and murine models of infection have demonstrated an essential role for Dectin-1 in host defense against invasive aspergillosis.²⁴ However this polymorphism showed only a trend toward host susceptibility in HSCT recipients and did not alter the clinical course of infection. In the non-HSCT population, this polymorphism does appear to be a moderate risk factor favoring the development of invasive pulmonary aspergillosis.²⁵

Common polymorphisms in TLR genes have also been associated with susceptibility to several infections, and evidence suggests two donor TLR4 haplotypes (S3 and S4) increase the risk of invasive aspergillosis in stem-cell transplant recipients.²⁶ Clearly from these studies it is possible that the presence of two or more different polymorphisms may impose a “cumulative risk,” and further work is needed to develop effective prevention strategies in high-risk populations.

CLINICAL MANIFESTATIONS

Aspergillosis may present with diverse symptoms, and the extent of disease is largely dependent upon the site and severity of involvement and the host immune status. Although infection with *Aspergillus* has been reported involving virtually every organ, the upper airways, lungs, and surrounding structures are those most frequently involved.

Pulmonary aspergillosis exists as a spectrum and includes allergic manifestations of infection (ABPA, see article 3, this issue), and noninvasive saprophytic infection (aspergillus fungus ball or aspergilloma), semi-invasive syndromes such as chronic cavitary pneumonia and chronic necrotizing aspergillosis, and frankly invasive forms (invasive pulmonary aspergillosis).

Invasive Disease

Traditionally, invasive pulmonary aspergillosis (IPA) was seen 10 to 14 days after hematopoietic stem cell transplantation and was associated with significant granulocytopenia.²⁷ Recent reports, however, document the shifting epidemiology of infection, with less than one third of patients neutropenic at the time of diagnosis impacting the clinical presentation and period of high risk for infection.²⁸

Pleuritic chest pain, dyspnea, hemoptysis, dry cough, and fever are the most common manifestations of IPA; however, the neutropenic host often exhibits a paucity of symptoms, underscoring their frequent inability to mount an immunologic or febrile response to infection. For this reason a careful history should be taken in those at risk for IPA because symptoms of hemoptysis and pleuritic chest pain occur secondary to angioinvasion of the organism.

Radiographic imaging may be suggestive of IPA, particularly in high-risk patients, yet findings are not diagnostic and may be seen in other invasive pulmonary infections, including both fungal and nonfungal etiologies of pulmonary disease. Diffuse nodular infiltrates, pleural-based densities, and cavitary lesions and pleural effusions are common radiographic manifestations.²⁹ The presence of a “halo” sign, an area of low-attenuation surrounding a pulmonary nodule, has classically been associated with IPA. These areas of consolidation may later cavitate and give rise to the “air-crescent” sign.³⁰

It is important to recognize the radiographic evolution of IPA. When serial computed tomographic (CT) images are obtained the volume of lesions often increases over the first 7 days, even with early initiation of antifungal therapy.³¹ In fact, the calculated radiographic volume of IPA increased three- to fourfold over this time period and was correlated with bone marrow recovery, further illustrating the necessity of granulocytes to manifest an inflammatory response to IPA.³²

Histopathological features of aspergillosis are useful to document tissue invasion and can show angioinvasion typical of disease. *Aspergillus* is suggested by the presence of dichotomous branching hyphae, but it is important to recognize that the pathological appearance is not specific for *Aspergillus*, and similar features are observed in other hyaline molds, including *Fusarium*, *Scedosporium*, *Penicillium* spp., and others, and culture verification remains the gold standard for definitive identification.

Mortality of IPA ranges from 50 to 90%,³³ and attempts to initiate early antifungal therapy should be undertaken. Studies examining the reduction in mortality with early therapy are limited; however, one such study reports a 50% reduction in mortality with prompt initiation of antifungal therapy.³⁴

Tracheobronchial Aspergillosis

The presence of *Aspergillus* spp. in the large airways may range from colonization to tracheobronchitis typical of infection in patients with AIDS or lung transplant recipients.^{35,36} Nonspecific symptoms predominate, with cough, chest pain, fever, and hemoptysis the most common. Bronchoscopic findings include pseudomembranes and ulcerative lesions. Infection of the anastomotic site in lung transplant patients has been reported as an unusual cause of dehiscence.³⁶

Chronic Forms of Pulmonary Aspergillosis

Aspergillosis of the lower respiratory tract includes other “semi-invasive” forms (formerly chronic necrotizing pulmonary aspergillosis).^{37,38} However, it is likely that these semi-invasive forms exist on a continuum with invasive disease (Fig. 1).³⁹

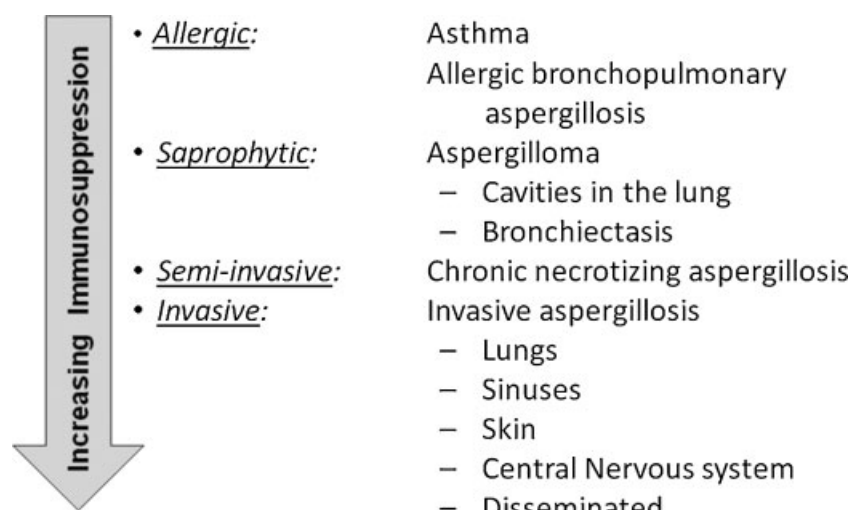


Figure 1 Clinical syndromes due to aspergillosis.

Chronic Necrotizing Pulmonary Aspergillosis (CNPA), Chronic Cavitary Pulmonary Aspergillosis (CCPA)

Chronic necrotizing pulmonary aspergillosis (CNPA) is defined by the presence of cavitary lung lesions, chronic respiratory symptoms, and serum precipitating antibodies to *Aspergillus* spp. Several reports depict direct invasion of *Aspergillus* spp. into the lung parenchyma, with CNPA described as a subacute or non-angioinvasive form.³⁹ Several of these cases, however, report progressive damage to the lung parenchyma without clear evidence of tissue invasion. The term *chronic cavitary pulmonary aspergillosis* (CCPA) has been applied to the formation and expansion of multiple pulmonary cavities (Fig. 2).⁴⁰ Left untreated the survival rate for chronic pulmonary aspergillosis is 50% at 1 year, although if antifungal therapy is administered mortality is reported to improve to 50% at 5 years.⁴¹

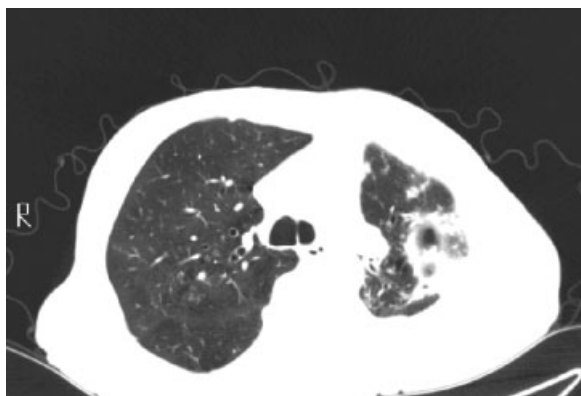


Figure 2 Chronic cavitary pulmonary aspergillosis on computed tomography of lung.

NONINVASIVE DISEASE

Aspergilloma

An aspergilloma, or aspergillus fungus ball, forms within an existing pulmonary cavity caused by COPD, carcinoma, or prior pulmonary tuberculosis. Often asymptomatic, an aspergilloma may present with life-threatening hemoptysis requiring emergent bronchial artery embolization or surgical resection. The radiographic finding of a freely moveable, solid round mass within a cavitary lung lesion is characteristic (Figs. 3A, B). Surgical resection is curative, although underlying lung disease often precludes definitive treatment and long-term antifungal therapy is controversial, although recent studies have suggested that triazole therapy is beneficial in that setting.⁴⁰

DIAGNOSIS

Definitive diagnosis of aspergillosis requires direct visualization of the characteristic branching septate hyphae on microscopic examination. Percutaneous needle aspiration, bronchoscopy, or thoracoscopic biopsy is often required to obtain a tissue diagnosis; however, these procedures can be difficult to perform in the neutropenic patient with concurrent thrombocytopenia. *Aspergillus* spp. grow well on standard media, although optimization of growth is achieved by the use of fungal-specific media. False-negative results do occur, typically as a consequence of previous or concurrent antifungal therapy.^{42,43}

Nonculture-based diagnostics have been developed to improve diagnosis and perhaps also assessment of response with serial measurement. The galactomannan enzyme immunoassay (EIA) has been proven reliable in patients with hematologic malignancy; however, the sensitivity varies greatly (44 to 90%) depending on several

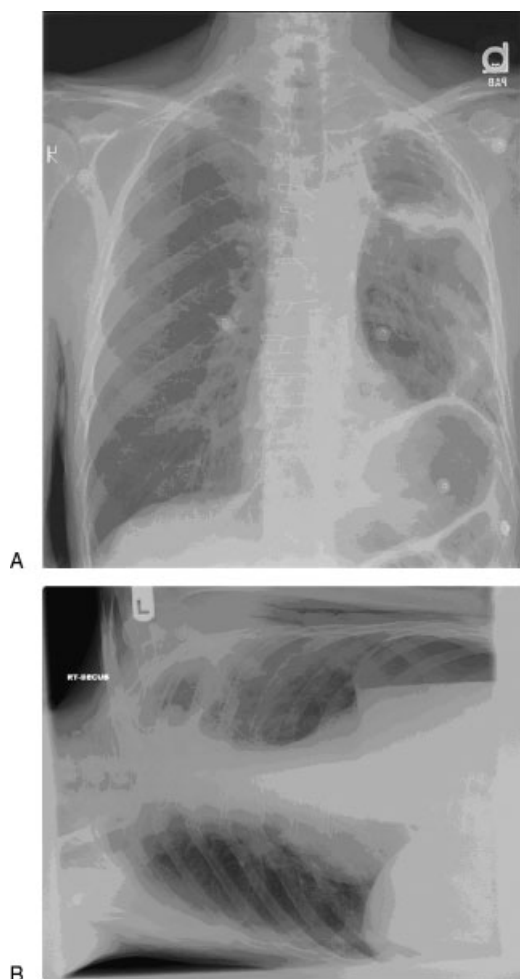


Figure 3 (A) Chest radiograph of pulmonary fungus ball due to *Aspergillus* (aspergilloma). (B) Demonstrating mobile lesion on lateral decubitus view.

factors, including number of samples taken, extent of infection, underlying host immunosuppression, and, importantly, the receipt of prior antifungal therapy, which dramatically reduces levels of circulating galactomannan.^{44,45} False-positive results have been reported in patients receiving semisynthetic penicillins (piperacillin-tazobactam and amoxicillin-clavulanate) and in those infected with the dimorphic fungi *H. capsulatum* or *B. dermatitidis* due to the presence of galactomannan in these species as well.⁴⁶ Serial EIA testing of high-risk patients is recommended, although the testing frequency remains unclear, and murine models have demonstrated the utility of daily screening given the rapid tissue destruction of IPA in neutropenic hosts.⁴⁷ Serum galactomannan determination also provides prognostic information, with declining values associated with improvements in patient outcomes.⁴⁸ Recently testing of bronchoalveolar lavage fluid has been cleared by the FDA for clinical use, which seems to substantially increase the yield of positive results as compared with serum testing.⁴⁹

Serum (1→3)- β -D-glucan (a component of the fungal cell wall) determination may also be helpful, although this marker is not specific for *Aspergillus* and its presence may indicate several invasive fungal infections.⁵⁰ The negative predictive value of (1→3)- β -D-glucan has generated interest in its use as a screening test in high-risk patients.⁵¹

PCR-based methods have also been evaluated and appear promising, yet testing is not commercially available in the United States for the diagnosis of *Aspergillus*.⁵² The use of multiple concurrent modalities has not been prospectively evaluated but may be a significant advance in noninvasive diagnostic testing for *Aspergillus* spp.

TREATMENT

The availability of additional classes of antifungal agents with several agents having activity against *Aspergillus* spp. has dramatically increased the treatment options for this disease. Voriconazole has become the mainstay of therapy, with a large clinical trial supporting its use as compared with the previous standard of amphotericin B deoxycholate.⁵³ Voriconazole has been associated with improved outcomes even in severely immunosuppressed patients and is recommended as primary therapy in most guidelines, including those of the Infectious Diseases Society of America.¹ Nevertheless, important questions remain regarding treatment of invasive aspergillosis, including selection of antifungal agents, management of toxicities, and need for therapeutic drug monitoring and the role of combination therapy. Finally, management of breakthrough infections is impeded by lack of evidence-based data, and the role of combination therapy remains controversial.

Triazoles

The triazoles inhibit ergosterol synthesis through inhibition of the fungal cytochrome P450 enzyme. The side effects of this class are thus predominantly hepatic, related to their inhibition of human P450 enzyme, and the potential for drug-drug interactions with other medications metabolized by this system is high. Resistance of *Aspergillus* to triazoles has been reported, although no species has predictable antifungal resistance to this class.

Voriconazole

Voriconazole is available in both oral and intravenous (IV) formulations. The IV form exists in a cyclodextrin vehicle that may accumulate in patients with renal impairment, and only limited evidence is available examining the potential side effects of such accumulation.⁵⁴ Liver function test abnormalities occur in as many as 15% of patients and can be severe and dose limiting;

nausea, vomiting, diarrhea, skin rashes, hallucinations, and other adverse events may complicate its use. The complaint of photopsia (flashing lights) is common and associated with higher serum concentrations; although often disconcerting to the patient, photopsia is transient and self-limited.⁵⁵ Voriconazole is administered as a loading dose of 6 mg/kg intravenously every 12 hours for two doses, followed by 4 mg/kg every 12 hours in the treatment of invasive aspergillosis. Oral therapy can be maximized by the use of 4 mg/kg/dose twice daily, although levels vary significantly, even with weight-based dosing.¹ Pediatric patients clear voriconazole more rapidly than adults, and a maintenance dosage of 7 mg/kg is therefore recommended. Due to both inter- and inpatient variability of serum voriconazole concentrations some experts recommend that all patients undergo therapeutic drug monitoring to maximize efficacy and avoid toxicity.

In a prospective, double-blind trial comparing amphotericin B deoxycholate to voriconazole, voriconazole was significantly more effective in clinical response and survival rates.⁵³ Following this, the IDSA recommended voriconazole as primary therapy for most patients with invasive pulmonary aspergillosis (Table 2).⁵⁶

Posaconazole

Posaconazole is another triazole with excellent in vitro activity against *Aspergillus* species. It is structurally similar to itraconazole and is generally well tolerated; it differs from other triazoles in that it is not hepatically metabolized so that it appears to have less hepatic toxicity. However, posaconazole is an inhibitor of cytochrome P450 enzyme 3A4 so that clinically significant drug interactions can occur. Available only in an oral solution, steady-state levels are not obtained for up to 7 days, limiting its use as primary therapy. Other

toxicities include diarrhea, nausea, and dyspepsia. Recommended serum drug concentrations can be difficult to obtain without attempts to maximize absorption conditions.⁵⁷ Posaconazole prophylaxis has been evaluated in two studies in high-risk patients undergoing hematopoietic stem cell transplantation with graft versus host disease and in patients with acute myelogenous leukemia or myelodysplastic syndrome.^{58,59} Posaconazole was shown to reduce breakthrough aspergillosis and improved survival compared with that of standard prophylaxis regimens in the later study.⁵⁹ The concurrent intake of food has been found to boost serum posaconazole levels up to fourfold.⁶⁰ The oral suspension preparation should be given as 200 mg three times daily when used for prophylaxis and as 800 mg in two to four divided doses when used as salvage therapy.⁵⁶

Itraconazole

Itraconazole is currently available in only oral capsules and an oral solution. Absorption of the capsules is variable and increased in the presence of food or an acidic beverage. Side effects of itraconazole include elevation of hepatic transaminases, hypertriglyceridemia, edema, hypokalemia, nausea, and vomiting.⁶¹ Oral doses of 400 mg/day (capsules) or 2.5 mg/kg twice a day (solution) are currently recommended for use in aspergillosis, but itraconazole has a very limited role in therapy for acute invasive infection due to significant toxicities and erratic bioavailability; however, it may have a role in saprophytic infection and in allergic disease.⁶²

Echinocandins

The echinocandins, caspofungin, micafungin, and anidulafungin, inhibit synthesis of 1,3- β -D-glucan, a polysaccharide present in the cell wall of fungi. Adverse events associated with these agents are minimal, and clinically relevant drug interactions are uncommon.⁶³ Caspofungin is approved by the FDA for salvage therapy of invasive aspergillosis, but all the clinically available agents have in vitro activity against *Aspergillus* species. As a single agent these drugs inhibit growth of the organisms but are not completely fungicidal. In a clinical study of severely immunosuppressed patients, caspofungin produced successful outcomes in 33% of patients so that these agents are not recommended as sole agents for primary therapy.⁶⁴ Combinations of voriconazole and echinocandins have demonstrated improved efficacy in preclinical models and in a retrospective study; the results of a prospective randomized trial are eagerly awaited.⁶⁵

Amphotericin B

Amphotericin B (AMB) deoxycholate was the traditional antifungal of choice against invasive aspergillosis given its

Table 2 Antifungal Therapy for Aspergillosis of the Lower Respiratory Tract

Disease	Treatment
Allergic bronchopulmonary aspergillosis	Itraconazole $\pm$ corticosteroids
Chronic aspergillosis including <i>Aspergillus</i> fungus ball	No therapy, or itraconazole/voriconazole
Chronic cavitary or necrotizing pulmonary aspergillosis	Voriconazole preferred agent
Tracheobronchial aspergillosis	Voriconazole preferred agent
Invasive pulmonary aspergillosis	Voriconazole; Alternatives: amphotericin B, caspofungin, itraconazole, or posaconazole after stabilization of disease

fungicidal activity against *Aspergillus* spp. with the exception of *A. terreus*, which is inherently resistant. However, substantial toxicities and poor outcomes associated with AMB deoxycholate, especially in highly immunosuppressed hosts, have resulted in this agent being no longer recommended for use in this disease.^{2,66,67}

The lipid formulations of AMB have shown reduced nephrotoxicity in comparison to traditional AMB deoxycholate and are preferred when AMB-based therapies are needed. When used for the treatment of invasive aspergillosis, AMB lipid complex (ABLC), is given in doses of 5 mg/kg/day. Liposomal amphotericin B (L-AMB) has been used for salvage therapy of invasive aspergillosis at doses of 3 to 5 mg/kg/d. Higher doses of L-AMB (10 mg/kg/d) as compared with initial doses of 3 mg/kg/d were compared in a clinical trial of invasive pulmonary aspergillosis. Results showed similar efficacy and greater toxicity in the group of patients receiving higher initial L-AMB doses, suggesting that higher doses are not necessarily beneficial, at least in patients receiving early therapy for pulmonary infection.⁶⁸ From the results of this study, L-AMB is recommended as alternative primary therapy in those patients for whom voriconazole is contraindicated or cannot be used.

BREAKTHROUGH INFECTIONS

The development of invasive fungal infections during receipt of prophylactic or therapeutic antifungal therapy poses several diagnostic and therapeutic challenges.

Establishment of a definitive diagnosis can be difficult in the patient receiving antifungal agents if some degree of drug efficacy remains. These patients may possess a low burden of disease, and thus the sensitivity of noninvasive markers may be reduced. Additionally, more invasive procedures such as bronchoscopy, needle biopsy, or a wedge resection are often relatively contraindicated due to additional host factors such as thrombocytopenia.

The development of drug resistance in *Aspergillus* spp. despite antifungal therapy or subtherapeutic serum drug levels has also been found to predispose to breakthrough infection. In HSCT recipients on long-term posaconazole, breakthrough infections were more likely in those with suboptimal serum posaconazole levels (<400 ng/mL).⁶⁹

Drug resistance in *Aspergillus* spp. has also recently increased. Prior to 2004 less than 1% of isolates recovered exhibited in vitro resistance to itraconazole, yet since that time up to 8% of all isolates have been found to be itraconazole resistant. These isolates are additionally cross-resistant to voriconazole (65%) and posaconazole (74%).⁷⁰ Some species are known to have intrinsic antifungal resistance such as the reduced susceptibility of *A. terreus* to polyenes, although other species, including *A. flavus* and *A. calidoustus*, have

elevated minimal inhibitory concentrations (MICs) to AMB in the majority of isolates tested.⁶ Even in *Aspergillus fumigatus* complex, "cryptic" species such as *A. lentulus* may exhibit decreased antifungal susceptibility, which may explain in part the poor response of some patients to polyene therapy.^{71,72} While increased MICs to other classes of antifungals can occur in some of these species (such as *A. calidoustus*) some isolates may have better susceptibility to other classes of antifungals, such as the azoles, so that identification of the isolates and testing antifungal susceptibility may be beneficial in some cases.⁶

Several different mutations within the azole target enzyme CYP51A have been described conferring antifungal resistance.⁷⁰ Patients receiving long-term antifungal therapy for chronic cavity aspergillosis appear at highest risk for developing or harboring resistant organisms, and in this population resistance has been associated with treatment failure.^{41,73} Additionally, current guidelines recommend determination of serum drug levels and attempts to ensure optimal drug exposure in patients experiencing therapeutic failure.¹

CONCLUSIONS

Cases of aspergillosis continue to climb as a consequence of the growing immunosuppressed population. Diagnostic and treatment practices are also evolving but are complicated by the poor immunologic response to invasive infection in most patients. Early therapy remains critical in attempts to improve outcomes, and the growing number of antifungal medications offers the potential for more effective and less toxic therapeutic options. Ongoing trials will hopefully delineate the role of combination therapy in the treatment of invasive pulmonary aspergillosis and provide further insight on genetic factors increasing host susceptibility.

REFERENCES

1. Walsh TJ, Anaissie EJ, Denning DW, et al; Infectious Diseases Society of America. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2008;46(3):327-360
2. Patterson TF, Kirkpatrick WR, White M, et al. Invasive aspergillosis: disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine (Baltimore) 2000;79(4):250-260
3. Thompson GR III, Patterson TF. Pulmonary aspergillosis. Semin Respir Crit Care Med 2008;29(2):103-110
4. Summerbell R. Ascomycetes: *Aspergillus*, *Fusarium*, *Sporothrix*, *Piedraia*, and their relatives. In: Howard DH, ed. Pathogenic Fungi in Humans and Animals. 2nd ed. New York: Marcel Dekker; 2003:237-498
5. Balajee SA, Houben J, Verweij PE, et al. Aspergillus species identification in the clinical setting. Stud Mycol 2007;59:39-46

6. Sutton DA, Fothergill AW, Rinaldi MG. *Aspergillus* in vitro antifungal susceptibility data: new millennium trends. In: Abstracts of Advances Against Aspergillosis, 9–11 September, San Francisco, CA, 2004. Abstract 16
7. Balajee SA, Nickle D, Varga J, Marr KA. Molecular studies reveal frequent misidentification of *Aspergillus fumigatus* by morphotyping. *Eukaryot Cell* 2006;5(10):1705–1712
8. Klich M, Pitt J. A Laboratory Guide to Common *Aspergillus* Species and Their Teleomorphs. North Ryde, New South Wales, Australia: Commonwealth Scientific and Industrial Research Organization; 1988
9. O’Gorman CM, Fuller HT, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* 2009;457(7228):471–474
10. Pitt JI, Samson RA. Nomenclatural considerations in naming species of *Aspergillus* and its teleomorphs. *Stud Mycol* 2007;59:67–70
11. Panackal AA, Li H, Kontoyiannis DP, et al. Geoclimatic influences on invasive aspergillosis after hematopoietic stem cell transplantation. *Clin Infect Dis* 2010;50(12):1588–1597
12. Portugal RD, Garnica M, Nucci M. Index to predict invasive mold infection in high-risk neutropenic patients based on the area over the neutrophil curve. *J Clin Oncol* 2009;27(23):3849–3854
13. Patterson JE, Peters J, Calhoon JH, et al. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2000;2(1):22–28
14. Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. *Clin Microbiol Rev* 2005;18(1):44–69
15. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. *Medicine (Baltimore)* 1999;78(2):123–138
16. Denning DW. Aspergillosis in “nonimmunocompromised” critically ill patients. *Am J Respir Crit Care Med* 2004;170(6):580–581
17. Lat A, Bhadelia N, Miko B, Furuya EY, Thompson GR III. Invasive aspergillosis after pandemic (H1N1) 2009. *Emerg Infect Dis* 2010;16(6):971–973
18. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Wijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. *Am J Respir Crit Care Med* 2004;170(6):621–625
19. Warris A, Bjørneklett A, Gaustad P. Invasive pulmonary aspergillosis associated with infliximab therapy. *N Engl J Med* 2001;344(14):1099–1100
20. Cohen MS, Isturiz RE, Malech HL, et al. Fungal infection in chronic granulomatous disease: the importance of the phagocyte in defense against fungi. *Am J Med* 1981;71(1):59–66
21. Gersuk GM, Underhill DM, Zhu L, Marr KA. Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. *J Immunol* 2006;176(6):3717–3724
22. Hohl TM, Van Epps HL, Rivera A, et al. *Aspergillus fumigatus* triggers inflammatory responses by stage-specific beta-glucan display. *PLoS Pathog* 2005;1(3):e30
23. Luther K, Torosantucci A, Brakhage AA, Heesemann J, Ebel F. Phagocytosis of *Aspergillus fumigatus* conidia by murine macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. *Cell Microbiol* 2007;9(2):368–381
24. Werner JL, Metz AE, Horn D, et al. Requisite role for the dectin-1 beta-glucan receptor in pulmonary defense against *Aspergillus fumigatus*. *J Immunol* 2009;182(8):4938–4946
25. Chai LY, de Boer MG, van der Velden WJ, et al. The Y238X stop codon polymorphism in the human β -glucan receptor dectin-1 and susceptibility to invasive aspergillosis. *J Infect Dis* 2011;203(5):736–743
26. Bochud PY, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* 2008;359(17):1766–1777
27. Gerson SL, Talbot GH, Hurwitz S, Strom BL, Lusk EJ, Cassileth PA. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann Intern Med* 1984;100(3):345–351
28. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 1997;175(6):1459–1466
29. Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998;26(4):781–803; quiz 804–805
30. Greene R. The radiological spectrum of pulmonary aspergillosis. *Med Mycol* 2005;43(Suppl 1):S147–S154
31. Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001;19(1):253–259
32. Miceli MH, Maertens J, Buvé K, et al. Immune reconstitution inflammatory syndrome in cancer patients with pulmonary aspergillosis recovering from neutropenia: proof of principle, description, and clinical and research implications. *Cancer* 2007;110(1):112–120
33. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis* 2010;50(12):1559–1567
34. von Eiff M, Roos N, Schulten R, Hesse M, Zühlsdorf M, van de Loo J. Pulmonary aspergillosis: early diagnosis improves survival. *Respiration* 1995;62(6):341–347
35. Denning DW, Follansbee SE, Scolaro M, Norris S, Edelstein H, Stevens DA. Pulmonary aspergillosis in the acquired immunodeficiency syndrome. *N Engl J Med* 1991;324(10):654–662
36. Kramer MR, Denning DW, Marshall SE, et al. Ulcerative tracheobronchitis after lung transplantation: a new form of invasive aspergillosis. *Am Rev Respir Dis* 1991;144(3 Pt 1):552–556
37. Binder RE, Faling LJ, Pugatch RD, Mahasaen C, Snider GL. Chronic necrotizing pulmonary aspergillosis: a discrete clinical entity. *Medicine (Baltimore)* 1982;61(2):109–124
38. Gefter WB, Weingrad TR, Epstein DM, Ochs RH, Miller WT. “Semi-invasive” pulmonary aspergillosis: a new look at the spectrum of aspergillus infections of the lung. *Radiology* 1981;140(2):313–321
39. Hope WW, Walsh TJ, Denning DW. The invasive and saprophytic syndromes due to *Aspergillus* spp. *Med Mycol* 2005;43(Suppl 1):S207–S238
40. Denning DW, Riniotis K, Dobrashian R, Sambatakou H. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. *Clin Infect Dis* 2003;37(Suppl 3):S265–S280
41. Felton TW, Baxter C, Moore CB, Roberts SA, Hope WW, Denning DW. Efficacy and safety of posaconazole for chronic pulmonary aspergillosis. *Clin Infect Dis* 2010;51(12):1383–1391

42. Muñoz P, Alcalá L, Sánchez Conde M, et al. The isolation of *Aspergillus fumigatus* from respiratory tract specimens in heart transplant recipients is highly predictive of invasive aspergillosis. *Transplantation* 2003;75(3):326–329
43. Horvath JA, Dummer S. The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. *Am J Med* 1996;100(2):171–178
44. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002;20(7):1898–1906
45. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001;97(6):1604–1610
46. Verweij PE, Mennink-Kersten MA. Issues with galactomannan testing. *Med Mycol* 2006;44(Suppl):179–183
47. Hope WW, Petraitis V, Petraitiene R, Aghamolla T, Bacher J, Walsh TJ. The initial 96 hours of invasive pulmonary aspergillosis: histopathology, comparative kinetics of galactomannan and (1->3) β -D-glucan and consequences of delayed antifungal therapy. *Antimicrob Agents Chemother* 2010;54(11):4879–4886
48. Koo S, Bryar JM, Baden LR, Marty FM. Prognostic features of galactomannan antigenemia in galactomannan-positive invasive aspergillosis. *J Clin Microbiol* 2010;48(4):1255–1260
49. Maertens J, Maertens V, Theunissen K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* 2009;49(11):1688–1693
50. Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004;39(2):199–205
51. Obayashi T, Yoshida M, Mori T, et al. Plasma (1->3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995;345(8941):17–20
52. Donnelly JP. Polymerase chain reaction for diagnosing invasive aspergillosis: getting closer but still a ways to go. *Clin Infect Dis* 2006;42(4):487–489
53. Herbrecht R, Denning DW, Patterson TF, et al; Invasive Fungal Infections Group of the European Organisation for Research and Treatment of Cancer and the Global Aspergillus Study Group. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;347(6):408–415
54. Alvarez-Lerma F, Allepuz-Palau A, Garcia MP, et al; Voriconazole Study Group in Critically Ill Patients. Impact of intravenous administration of voriconazole in critically ill patients with impaired renal function. *J Chemother* 2008;20(1):93–100
55. Eiden C, Peyrière H, Cociglio M, et al; Network of the French Pharmacovigilance Centers. Adverse effects of voriconazole: analysis of the French Pharmacovigilance Database. *Ann Pharmacother* 2007;41(5):755–763
56. Steinbach WJ, Stevens DA. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. *Clin Infect Dis* 2003;37(Suppl 3):S157–S187
57. Thompson GR III, Rinaldi MG, Pennick G, Dorsey SA, Patterson TF, Lewis JS II. Posaconazole therapeutic drug monitoring: a reference laboratory experience. *Antimicrob Agents Chemother* 2009;53(5):2223–2224
58. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007;356(4):335–347
59. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;356(4):348–359
60. Courtney R, Wexler D, Radwanski E, Lim J, Laughlin M. Effect of food on the relative bioavailability of two oral formulations of posaconazole in healthy adults. *Br J Clin Pharmacol* 2004;57(2):218–222
61. De Beule K, Van Gestel J. Pharmacology of itraconazole. *Drugs* 2001;61(Suppl 1):27–37
62. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009;53(1):24–34
63. Bennett JE. Echinocandins for candidemia in adults without neutropenia. *N Engl J Med* 2006;355(11):1154–1159
64. Viscoli C, Herbrecht R, Akan H, et al; Infectious Disease Group of the EORTC. An EORTC Phase II study of caspofungin as first-line therapy of invasive aspergillosis in haematological patients. *J Antimicrob Chemother* 2009;64(6):1274–1281
65. Marr KA, Boeckh M, Carter RA, Kim HW, Corey L. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004;39(6):797–802
66. Patterson TF, Boucher HW, Herbrecht R, et al; European Organization for Research and Treatment of Cancer (EORTC) Invasive Fungal Infections Group (IFIG); Pfizer Global Aspergillus Study Group. Strategy of following voriconazole versus amphotericin B therapy with other licensed antifungal therapy for primary treatment of invasive aspergillosis: impact of other therapies on outcome. *Clin Infect Dis* 2005;41(10):1448–1452
67. Bates DW, Su L, Yu DT, et al. Mortality and costs of acute renal failure associated with amphotericin B therapy. *Clin Infect Dis* 2001;32(5):686–693
68. Cornely OA, Maertens J, Bresnik M, et al; AmBiLoad Trial Study Group. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007;44(10):1289–1297
69. Winston DJ, Barton K, Territo MC, Schiller GJ. Efficacy, safety, and breakthrough infections associated with standard long-term posaconazole antifungal prophylaxis in allogeneic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2011;17(4):507–515
70. Howard SJ, Cerar D, Anderson MJ, et al. Frequency and evolution of Azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009;15(7):1068–1076
71. Balajee SA, Gribskov JL, Hanley E, Nickle D, Marr KA. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* 2005;4(3):625–632
72. Balajee SA, Weaver M, Imhof A, Gribskov J, Marr KA. *Aspergillus fumigatus* variant with decreased susceptibility to multiple antifungals. *Antimicrob Agents Chemother* 2004;48(4):1197–1203
73. Denning DW, Park S, Lass-Flörl C, et al. High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis* 2011;52(9):1123–1129