Aspergillosis

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Introduction

Aspergillosis is caused by *Aspergillus,* a hyaline mold responsible not only for invasive aspergillosis but also for a variety of noninvasive or semi-invasive conditions. These syndromes range from colonization to allergic responses to *Aspergillus,* including allergic bronchopulmonary aspergillosis (ABPA), to semi-invasive or invasive infections, spanning a spectrum from chronic necrotizing pneumonia to invasive pulmonary aspergillosis.

The genus *Aspergillus* was first recognized in 1729 by Micheli*,* in Florence. He described the resemblance between the sporulating head of an *Aspergillus* species and an aspergillum used to sprinkle holy water. In 1856, Virchow published the first complete microscopic descriptions of the organism [1].

The frequency and severity of invasive fungal infections in immunocompromised patients have increased steadily over the past three decades with the growing population of patients undergoing transplantation and the persistent challenges in preventing, diagnosing, and treating these infections [2]. Mortality due to documented invasive aspergillosis approaches 80–100 % in high-risk patients, including those with underlying hematologic malignancy, bone marrow, or solid organ transplantation, and may be related to several factors, including diagnostic and therapeutic inadequacies [2–5]. Apart from organ transplant recipients, individuals with AIDS and patients hospitalized with severe illnesses, major increases in invasive fungal infections have been observed in patients with hematologic malignancies who receive induction or consolidation chemotherapy and those who undergo hematopoietic stem cell transplantation (HSCT) [5].

Successful therapy depends not only on an early diagnosis—which is often difficult to establish—but, even more importantly, on reversal of underlying host immune defects, such as neutropenia or high-dose immunosuppressive therapy [2]. Nonculture-based tests and radiological approaches can be used to establish an early diagnosis of infection and may result in improved outcomes of infection [2, 6, 7]. Even when a therapy begins promptly, efficacy of the therapy is poor, particularly in patients with disseminated or central nervous system disease [2, 3, 5]. Recent developments include more widespread use of newer diagnostic approaches and improved understanding of how best to use available antifungal agents [8].

Etiologic Agents

Aspergillus fumigatus is one of the most ubiquitous of the airborne saprophytic fungi [9]. *A. fumigatus* has emerged worldwide as a frequent cause of nosocomial infection and may be regarded as the most important airborne pathogenic fungus [9]. As *Aspergillus* species can be readily found in the environment, invasive aspergillosis is widely believed to occur as a consequence of exogenous acquisition of the conidia (spores) of the species [9]. The most common route of transmission of *Aspergillus* infection is the airborne route. *Aspergillus* conidia are resilient and may survive for long periods in fomites (any substance that can absorb, retain, and transport infectious species, e.g., woolen clothes or bedding) [10]. *Aspergillus* infection occurs less frequently through damaged mucocutaneous surfaces (e.g., following surgery or through contaminated dressings). However, the sources of *Aspergillus* may be broader than have traditionally been thought, as waterborne transmission of *Aspergillus* conidia through contaminated aerosols has been suggested [11].

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D. R. Hospenthal, M. G. Rinaldi (eds.), *Diagnosis and Treatment of Fungal Infections,* Infectious Disease,
DOI 10.1007/978-3-319-13090-3_10, © Springer International Publishing Switzerland 2015

The most common species causing invasive aspergillosis include: *A. fumigatus* (Fig. 10.1), by far the most common, *A. flavus, A. terreus,* and, less commonly for invasive infection, *A. niger* [5] (Table 10.1). Recent studies have shown emergence of less common species, including *A. terreus* (which is frequently resistant to polyenes) and other unusual less pathogenic species as the etiologic agents of invasive infection [12].

Epidemiology

The incidence of invasive aspergillosis has increased substantially during the past few decades because of the use of more intensive cytotoxic anticancer chemotherapy and the introduction of novel immunosuppressive therapies for organ transplant recipients, both of which have prolonged the period of risk for many individuals. The increasing number of patients undergoing solid organ, bone marrow, and hematopoietic stem cell transplantation, and the implementation of aggressive surgical interventions has also contributed to the increased incidence [9]. The changes in epidemiology of invasive aspergillosis may also be the result of growing awareness of aspergillosis among clinicians, the introduction of noninvasive diagnostic tools and improved microbiological laboratory techniques.

Invasive fungal infections are an important cause of morbidity and mortality among patients with severely compromised immune systems. Although there have been significant advances in the management of immunosuppressed patients, invasive aspergillosis remains an important life-threatening complication, and is the leading cause of infection-related mortality in many immunocompromised individuals [13].

Immunosuppression and breakdown of anatomical barriers, such as the skin, are the major risk factors for fungal infections [7]. Individuals at risk for invasive aspergillosis include those with severely comprised immune systems as a result of anticancer chemotherapy, solid organ or bone marrow transplantation, AIDS, or use of high-dose corticosteroids. Patients with hematological disorders, such as prolonged and severe neutropenia, those undergoing transplantations, and those treated with corticosteroids and newer immunosuppressive therapies such as the tumor necrosis factor-α antagonists (e.g., inflixamab) are considered to be at highest risk for invasive aspergillosis [7, 14].

Pathogenesis and Immunity

Invasive aspergillosis most frequently originates via inhalation of *Aspergillus* conidia into the lungs, although other routes of exposure, such as inhalation of water aerosols contaminated with *Aspergillus* conidia have been suggested [11].

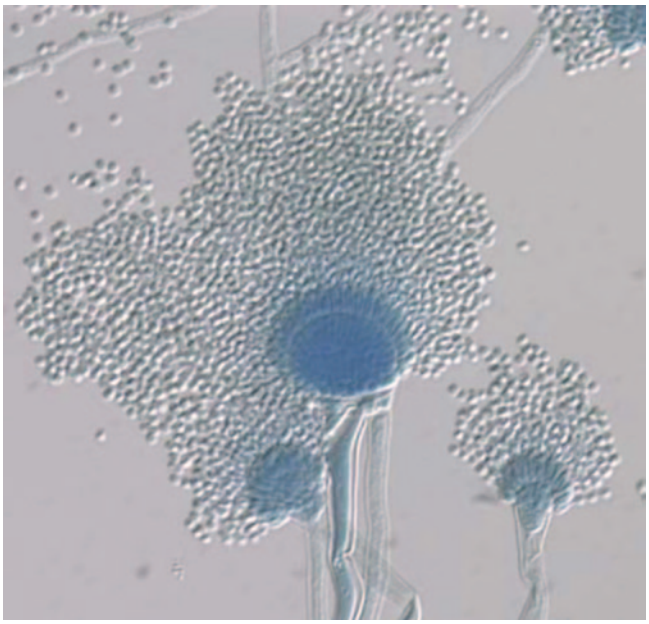

Fig. 10.1 Microscopic morphology of *Aspergillus fumigatus* showing a single role of phialides (uniseriate) bearing smooth conidia in a columnar fashion. (Courtesy of www.doctorfungus.org)

In the absence of effective pulmonary host defenses, the inhaled small resting conidia enlarge and germinate, then transform into hyphae with subsequent vascular invasion and eventual disseminated infection. The incubation period for conidial germination in pulmonary tissue is variable, ranging from 2 days to months [15]. Hydrocortisone significantly increases the growth rates of *Aspergillus*; likely one of the reasons corticosteroids pose a risk factor for invasive disease [16].

Although infection in apparently normal hosts can occur, invasive aspergillosis is extremely uncommon in immunocompetent hosts [5]. Normal pulmonary defense mechanisms usually 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 conidia size. This feature is one reason for the increased pathogenicity of *A. fumigatus* as compared with other species of *Aspergillus* [16]. Once conidia reach the alveoli, pulmonary macrophages are generally capable of ingesting and killing *Aspergillus* conidia [17]. When macrophages fail to kill the conidia (e.g., high-fungal inoculum, decreased number or function of macrophages), conidia germinate and begin to form hyphae. Polymorphonuclear leukocytes are recruited via complement activation and production of neutrophil chemotactic factors and extracellularly kill both swollen conidia and hyphae [18]. Antibodies against *Aspergillus* are common due to the ubiquitous nature of the organism, although they are not protective nor

Table 10.1 Characteristics of common *Aspergillus* species

Aspergillus species	Mycological characteristics	Clinical significance	Mycoses
A. flavus	Olive to lime green colonies	Second most common species, produces aflatoxin, may be less susceptible to polyenes	Sinusitis, cutaneous infection, pulmonary, and disseminated disease
A. fumigatus	Smoky, blue- or gray-green, small, smooth conidia (2–2.5 μm)	Most common species causing invasive infection	Invasive pulmonary aspergillosis, disseminated infection, CNS, others
A. niger	Typically black colonies, radiate conidial head, large rough conidia	Common cause of otomycosis, produces oxalate crystals which may be seen in host	Otomycosis, cutaneous, endophthalmitis, aspergilloma, invasive pulmonary, or disseminated disease less common
A. terreus	Beige to buff colonies, globose accessory conidia along hyphae	Increasing frequency, associated with soil, usually resistant to polyenes	Pulmonary, disseminated, cutaneous, keratitis, CNS
A. lentulus	Poorly sporulating variant of A. fumigatus	May be multidrug resistant, recently described variant, may be underdiagnosed	Invasive pulmonary, disseminated, other sites

CNS central nervous system

are they useful in the diagnosis of infection in high-risk patients due to the lack of consistent seroconversion following exposure or infection [19].

Corticosteroids play a major role in increasing susceptibility to *Aspergillus* by decreasing oxidative killing of the organism by pulmonary macrophages and by increasing the linear growth rate by as much as 30–40% and cell synthesis by more than 150% [16].

Many *Aspergillus* species produce toxins including aflatoxins, ochratoxin A, fumagillin, and gliotoxin. Gliotoxin works in several ways to help evade host defenses:

- Inhibition of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation (key in host defense (versus filamentous fungi)
- Inhibition of macrophage ingestion of *Aspergillus*
- Suppression of functional T cell responses [20, 21]

In tissues, invasive aspergillosis causes extensive destruction across tissue planes via vascular invasion with resulting infarction and necrosis of distal tissues.

Clinical Manifestations

The clinical syndromes associated with aspergillosis are diverse, ranging from allergic responses to the organism including allergic bronchopulmonary aspergillosis (ABPA), asymptomatic colonization, superficial infection, and acute or subacute, and chronic invasive disease. The clinical presentation generally corresponds to the underlying immune defects and risk factors associated with each patient group, with greater immune suppression correlating with the increased risk for invasive disease. Although this chapter focuses on invasive aspergillosis, a brief description of other presentations follows. The reader is encouraged to refer to other sources for more in-depth discussion of those conditions [1].

Allergic Bronchopulmonary Aspergillosis

ABPA is a chronic allergic response to *Aspergillus* characterized by transient pulmonary infiltrates due to atelectasis. The incidence of ABPA is estimated to range from 1 to 2% in patients with persistent asthma and approximately 7% (with a range from 2 to 15%) of patients with cystic fibrosis [22]. Specific criteria are used to establish the diagnosis of ABPA as no single finding is diagnostic for the condition, although some presentations, like central bronchiectasis in patients with asthma highly suggest the diagnosis [22–24]. ABPA typically progresses through a series of remissions and exacerbations but can eventually lead to pulmonary fibrosis, which is associated with a poor long-term prognosis [24]. 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 [25]. The role for antifungal therapy was evaluated with a randomized, double-blind, placebo-controlled trial that showed itraconazole at 200 mg per day for 16 weeks significantly reduced daily corticosteroid use, reduced levels of immunoglobulin E (IgE), and improved exercise tolerance and pulmonary function [23, 26].

Aspergilloma

A pulmonary fungus ball, due to *Aspergillus* or "aspergilloma," is a solid mass of hyphae growing in a previously existing pulmonary cavity, typically in 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 AIDS [27, 28]. On chest radiograph,

a pulmonary aspergilloma appears as a solid round mass in a cavity. In many patients, the fungus ball due to *Aspergillus* remains asymptomatic, but in a significant number, hemoptysis occurs and can be fatal [29]. Surgical resection is considered as the definitive therapy, but the dense pleural adhesions adjacent to the fungus ball and the poor pulmonary reserve of most patients with this condition, makes 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 or an Eloesser procedure, further compromising lung function [29].

Aspergillus can also be associated with fungal balls of the sinuses without tissue invasion [28]. The maxillary sinus is the most common site for a sinus aspergilloma to occur [28]. Clinical presentation is similar to that for any chronic sinusitis. Management is usually directed at surgical removal and a generous maxillary antrostomy for sinus drainage, along with confirmation that invasive disease has not occurred.

Other Superficial or Colonizing Syndromes

Other superficial or colonizing syndromes of aspergillosis include otomycosis, a condition of superficial colonization typically due to *A. niger* [30]; onychomycosis which, although rare, can become chronic and respond poorly to antifungal agents [31]; and keratitis, particularly following trauma or corneal surgery [32].

Chronic Pulmonary Aspergillosis

Denning et al. have described three distinct syndromes of chronic pulmonary aspergillosis in order to better characterize those patients who develop chronic pulmonary disease related to *Aspergillus* [33]. These conditions include chronic cavitary pulmonary aspergillosis, which is characterized by the formation and expansion of multiple cavities, which may contain fungus balls; chronic fibrosing aspergillosis, which as its name suggests involves extensive fibrosis; and chronic necrotizing aspergillosis or subacute aspergillosis, in which slowly progressive infection occurs usually in a single thin-walled cavity. In all of these conditions, the diagnosis is suggested by radiological and clinical features and the role for therapy remains speculative, although it appears that long-term antifungal therapy may be beneficial in a subset of patients, perhaps even with the extended spectrum triazole antifungals [33, 34].

Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis is the most common form of invasive aspergillosis in immunocompromised patients. This infection occurs following approximately 2 weeks of neutropenia [35] or during the course of graft versus host disease, now the most common risk factor in hematopoietic stem cell transplant recipients [36]. Symptoms include fever (may be absent in the presence of high-dose corticosteroid therapy), dry cough, shortness of breath, pleuritic chest pain, hemoptysis, as well as pulmonary infiltrates all of which lag behind disease progression. In lung transplant patients and those with AIDS, *Aspergillus* tracheobronchitis can present with cough, wheezing, and shortness of breath and chest radiographs show normal lungs with or without atelectasis [37].

Disseminated Aspergillosis

A variety of signs and symptoms are seen with disseminated invasive aspergillosis, based on the organs involved. The organs involved include kidneys, liver, spleen, and central nervous system (CNS; signs and symptoms of stroke or meningitis) most frequently, followed by the heart, bone, skin, and other organs [8]. Aspergillosis of the skin can occur either as a manifestation of disseminated disease or by direct extension from a local inoculation, for example, from an intravenous catheter [38].

Sinusitis

Aspergillosis of the sinuses presents clinically like rhinocerebral mucormycosis, but is more common in neutropenic patients than in those with diabetic ketoacidosis, and inflammatory signs may thus be less frequent. Fever, nasal congestion, facial pain can progress to visual changes, proptosis, and chemosis if the infection spreads to the orbit. Posterior extension to the brain can lead to cranial nerve palsies, other focal neurologic deficits, as well as a depressed level of consciousness [39].

Endocarditis

Aspergillus endocarditis is the second most common form of fungal endocarditis after that caused by *Candida* species and occurs in prosthetic valve recipients and in native cardiac valves in intravenous drug users and patients with indwelling central venous catheters [40]. Clinically, these patients present with fever and embolic complications. Blood cultures are rarely positive even with extensive disease [41].

Table 10.2 Diagnosis of invasive aspergillosis

Diagnostic method	Comment
Respiratory culture	Not frequently positive early in course of infection; positive result in high-risk patient (bone marrow transplant, neutropenia) highly correlates with infection; may indicate colonization in other populations (chronic pulmonary diseases, lung transplant)
Galactomannan	*Aspergillus* Platelia system (BioRad, Redmond, WA) with variable sensitivity—low (~40 %) with single samples or prior antifungal therapy, or prophylaxis; better yield with reduced threshold for positivity (>0.5), serial samples, testing on BAL samples. False positives historically with pipercillin–tazobactam, certain foods, neonates
1,3-β-D-glucan	Nonspecific detection of cell wall glucan. Commercially available Fungitell™ assay (Associates of Cape Cod, Falmouth, MA), limited validation and availability
PCR	Remains investigational due to lack of standardized reagents and methods, both false positives and negatives may occur, some recent studies have suggested less sensitive than other assays
Computed tomography	In high-risk patient, "halo" sign and/or pulmonary nodules without other documented cause may be a frequent and early sign of invasive pulmonary aspergillosis

BAL bronchoalveolar lavage, *PCR* polymerase chain reaction

Diagnosis

Current diagnostic modalities are limited and the clinician must rely on the combination of knowledge of risk factors, a high index of suspicion, clinical judgment, and the finding of fungi in tissue specimens and/or cultures from the presumed site of infection (Table 10.2). The diagnosis of proven invasive aspergillosis requires both tissue biopsy demonstrating invasion with hyphae and a culture positive for *Aspergillus* species [42]. *Aspergillus* produce hyaline, 3–6 μm wide septate hyphae that typically branch at acute angles [43] (Fig. 10.2). In tissue, these features can often distinguish *Aspergillus* from agents of mucormycosis, but they cannot distinguish *Aspergillus* from a large number of other opportunistic molds, including *Fusarium* and *Scedosporium* (*Pseudallescheria*). Thus, culture is needed to confirm the diagnosis [43]. Unfortunately, invasive, or even less invasive procedures like bronchoscopy, are often contraindicated in immunosuppressed patients, many of whom have low platelets due to chemotherapy and other complications. In this setting, positive culture can support the diagnosis of invasive aspergillosis.

Plain chest radiography is of limited utility in invasive aspergillosis as it has low sensitivity and specificity in this disease [6]. In contrast, chest CT scans have proven useful in early diagnosis of invasive pulmonary aspergillosis as the "halo sign" of low attenuation surrounding a pulmonary nodule, has successfully been used as a marker for early initiation of therapy in high-risk patients with neutropenia or who have undergone HSCT [44–46]. Of note, these radiographic findings are also consistent with other infections such as *Nocardia* species, and may increase over the first week of therapy even when the patient in improving; follow-up scans should be ordered and interpreted cautiously with full attention to the clinical progress of the patient [44].

Nonculture diagnostic tests have also been used to diagnose aspergillosis and in attempts to preempt difficult-to-treat proven disease. A sandwich enzyme immunoassay (EIA) that utilizes a monoclonal antibody to *Aspergillus* galactomannan (Platelia *Aspergillus*, BioRad, Redmond, WA) is approved for serum and bronchoalveolar lavage (BAL) fluid and is being used with varying success around the world [47–49]. Questions remain regarding the value of routine surveillance testing, frequency of testing, role of false-positive results (seen in solid organ transplant recipients, patients treated with piperacillin–tazobactam and other medications, and neonates), importance of prior antifungal therapy, and correlation of serum galactomannan results with clinical outcome [50].

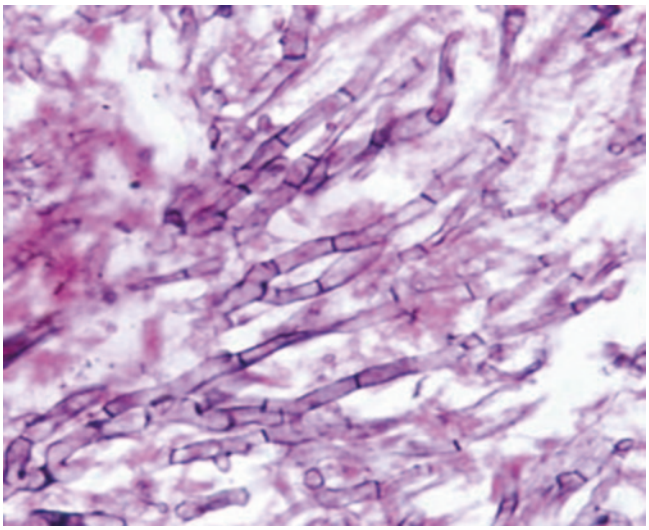

Fig. 10.2 Periodic acid–Schiff (PAS) stained tissue section of lung showing dichotomously branched, septate hyphae of *Aspergillus fumigatus*. (Courtesy of www.doctorfungus.org)

Several reports demonstrate the potential for using polymerase chain reaction (PCR) as an early diagnostic marker, which appears more sensitive than other methods including galactomannan [51, 52]. These assays may be associated with false-positive results due to the ubiquitous nature of *Aspergillus* conidia, are not standardized, and remain investigational at the present time [53–56].

Other nonculture-based methods for the diagnosis of invasive aspergillosis include detection of the nonspecific fungal marker 1,3-β-D-glucan using a variation of the *Limulus* amebocyte assay. This assay (Fungitell™, Associates of Cape Cod, Falmouth, MA) has been approved for diagnostic purposes by the Food and Drug Administration (FDA) and is a colorimetric assay that can indirectly determine the concentration of 1–3, β-D-glucan in serum samples [57]. The test appears promising as an indicator of infection due to many fungi, including *Aspergillus* and *Candida* but not *Cryptococcus* or Mucorales (which contain little or no β-D-glucan). One study suggested the utility of the assay in early diagnosis of invasive fungal infection in a leukemic population, but validation remains limited [58].

Interpretation of results is complicated with frequent false-positive β-D-glucan results, as well as reports of "interfering substances," hemodialysis with cellulose membranes, intravenous immunoglobulin, albumin, gauze packing of serosal surfaces, intravenous amoxicillin–clavulanic acid (not available in the USA) [59], and bloodstream infections with certain bacteria, such as *Pseudomonas aeruginosa* [60, 61]

Treatment

The goals of treatment of patients with invasive aspergillosis are to control infection and to reverse any correctable immunosuppression. Patients at high risk of developing invasive aspergillosis should be treated based on clinical or radiological criteria alone if microbiological or histological diagnosis would significantly delay treatment [2].

Treatment of *Aspergillus* infection is challenging due to difficulty in diagnosis, the presence of advanced disease in many by the time of diagnosis, and the presence of severe, often irreversible, immunosuppression. Mortality rates are high in patients with invasive aspergillosis and the efficacy of currently available treatments is limited by spectrum of activity, extensive drug–drug interactions, and serious toxicity. Treatment failure with currently available antifungal medication in patients with invasive aspergillosis has been reported to be 40% or higher in some series [3, 4]. Antifungal therapies with activity against *Aspergillus* include broad-spectrum triazoles (voriconazole, posaconazole, and isavuconazole), lipid formulations of amphotericin B, and the echinocandins (caspofungin, micafungin and anidulafungin), all of which offer options for therapy of this disease [62, 63] (Table 10.3). Guidelines developed by the Infectious Diseases Society of America and the American Thoracic Society provide summaries of existing data as well as recommendations. Of note, relatively few randomized trials of therapy for invasive aspergillosis have been completed so many recommendations stem from nonrandomized and noncomparative studies, as well as expert consensus [2].

Azoles

Voriconazole is a potent, broad-spectrum triazole that has fungicidal activity against many *Aspergillus* species, including *A. terreus*, is approved for therapy of invasive aspergillosis, and has replaced amphotericin as the recommended primary therapy for patients with invasive aspergillosis [2, 62, 64]. This recommendation is based on data from a randomized trial that compared voriconazole to conventional amphotericin B for the primary treatment of invasive aspergillosis, with each agent followed by other licensed antifungal therapy if needed for intolerance or progression of disease, in severely immunocompromised patients with invasive aspergillosis [45]. In this trial, voriconazole was superior to amphotericin B with successful outcomes in 52% of patients as compared to only 31% in those receiving amphotericin B. In addition, voriconazole demonstrated a survival advantage to amphotericin B with an absolute 13% difference in mortality between treatment groups.

In clinical trials, voriconazole has been adequately tolerated and the drug exhibits a favorable pharmacokinetic profile. There are a number of issues to consider, including important drug interactions, especially those with immunosuppressive agents, such as cyclosporine, tacrolimus, and sirolimus, the latter of which is contraindicated for use with voriconazole, and intolerance to the drug. The most common adverse event has been a transient and reversible visual disturbance described as an altered perception of light which has been reported in approximately 30% of the treated patients, but has not been associated with pathological changes [45]. Other adverse events include liver function test abnormalities in 10–15%, and skin rash in 6% (sometimes associated with sun exposure). Long-term voriconazole therapy has been associated with skin cancer and periostitis related to high fluoride levels [65–67].

Both toxicity (e.g., liver function abnormalities and CNS side effects increase with higher levels) and efficacy (i.e., poorer outcomes with lower levels) have been associated with voriconazole concentrations. As voriconazole metabolism varies between patients and is affected by so many relevant drug–drug interactions, many advocate the use of therapeutic drug monitoring. In the pivotal aspergillosis trial, serum concentrations between 2 and 5.5 mcg/mL were associated with successful outcomes. A more recent study suggests achieving serum trough concentrations of >1 mcg/mL and <5.5 mcg/mL [68, 69]. In patients with documented invasive aspergillosis, we recommend concentrations between 2 and 5.5 mcg/mL.

Itraconazole is approved for use as salvage therapy of aspergillosis. Its utility has been limited due to the fact that the only reliably absorbed formulation is an oral solution as its intravenous formulation is no longer marketed in the USA [70]. For these reasons, itraconazole is more frequently used

Table 10.3 Antifungal agents for treating invasive aspergillosis

Agent	Typical dose/route of administration	Comments
Azole		
Voriconazole	6 mg/kg IV q12 h x 2 doses, then 4 mg/kg IV q12 h; 200 mg PO bid (weight-based dosing should be considered)	Better efficacy and improved survival compared with amphotericin B deoxycholate; current recommended primary therapy for invasive aspergillosis; drug interactions common, hepatic toxicity (10–15 %) may be dose limiting; visual effects common (~30 %) but not usually dose limited and no long-term toxicity reported [98]
Itraconazole	200 mg tid for 3 days, then 200 mg PO bid (oral solution)	Second-line agent for invasive aspergillosis; erratic bioavailability, improved with oral solution; drug interactions including chemotherapeutic agents; intravenous formulation no longer available [2]
Posaconazole	Oral solution—200 mg PO qid loading, 400 PO bid maintenance; extended release tablets—300 mg bid x 2 doses, then 300 mg daily; intravenous—300 mg bid x 2 doses, then 300 mg daily	Recommended for salvage therapy; FDA approved for prophylaxis; P450 drug interactions; limited metabolism with favorable tolerance in clinical studies [2, 99]
Isavuconazole	Investigational	Full clinical development underway
Ravuconazole	Investigational	In vitro activity, but limited clinical development at present [63]
Polyene		
Amphotericin B deoxycholate	1.0–1.5 mg/kg IV daily	Prior "gold standard"; associated with significant toxicity and limited efficacy in severely immunosuppressed patients [100]
Liposomal amphotericin B	3–6 mg/kg IV daily	Alternative primary therapy; well tolerated; limited nephrotoxicity or infusion-related reactions; anecdotal reports of efficacy with higher doses (7.5 mg/kg/d or more)
Amphotericin B lipid complex	5 mg/kg IV daily	Indicated for salvage therapy or intolerance to standard agents, generally well tolerated [101]
Amphotericin B colloidal dispersion	3–6 mg/kg IV daily	Less nephrotoxic than amphotericin B deoxycholate, but associated with more infusion-related and pulmonary toxicity than other lipid formulations [81]
Echinocandin		
Caspofungin	70 mg x 1 dose, then 50 mg IV daily	Indicated for salvage therapy of aspergillosis, experimental and clinical data for use in combination therapy; well tolerated [84]
Micafungin	Investigational for aspergillosis (IV)	Used in doses of 100 mg/d in salvage studies; 50 mg/d for prophylaxis; well tolerated [102]
Anidulafungin	Investigational for aspergillosis (IV)	In vitro activity; studied at doses of 100 mg/d after 200 mg loading dose in other fungi; well tolerated [103]

IV intravenous, *PO* orally, *bid* twice daily, *qid* four times daily

in less immunosuppressed patients who are able to take oral therapy and for use as sequential oral therapy [5].

Posaconazole is FDA approved for prophylaxis of fungal infections in neutropenic patients and for the treatment of mucocutaneous candidiasis. It has also been studied in patients who failed to tolerate or had fungal infections refractory to standard therapy [71]. In 2005, Posaconazole was approved in the EU for salvage therapy of invasive aspergillosis. Initially available only as an oral suspension, in 2013, the FDA approved delayed release tablets with higher absorption and less dependency on having a full stomach, and in 2014 an intravenous formulation was approved. Gastrointestinal side effects are common, including stomach upset. Currently, posaconazole is recommended as a consideration in salvage therapy.

Other second-generation triazoles, including isavuconazole and ravuconazole, were developed with an expanded spectrum of activity to include *Aspergillus* [64, 72]. Isavuconazole is in phase 3 development and studies of aspergillosis have been completed. Ravuconazole has been evaluated in early phase clinical trials and has also shown activity in animal models of invasive aspergillosis [73].

Polyenes

Amphotericin B deoxycholate was the previous "gold standard" therapy in patients with invasive aspergillosis [2]. A number of studies documented the limited efficacy and substantial toxicity with amphotericin B deoxycholate in high-risk patients [45, 74, 75]. The overall response rates of amphotericin B deoxycholate are less than 25 %, with responses of only 10–15 % in more severely immunosuppressed patients [5, 45]. Wingard et al. documented increased morbidity and mortality associated with conventional amphotericin B (amphotericin B deoxcholate) in patients receiving bone marrow transplantation and those receiving concomitant nephrotoxic agents [75]. Similar

findings were documented by Bates et al. who found that renal toxicity occurred in approximately 30% of the patients receiving conventional amphotericin B and that this toxicity was associated with sixfold increase in mortality as well as a dramatic increase in hospital costs [74]. These unacceptably high mortality rates and significant toxicities highlighted the need for new therapeutic approaches in this disease.

The lipid formulations of amphotericin B were developed to decrease toxicity and allow the administration of higher doses of drug [76, 77]. To date, few comparative studies of the efficacy of lipid formulations of amphotericin B in treating invasive aspergillosis have been conducted though studies of these drugs as salvage therapy led to the approval of three lipid formulations [78]. Clinical experience has nevertheless been favorable, which is consistent with preclinical studies in animal models [79]. One small study by Leenders et al. compared liposomal amphotericin B at 5 mg/kg/d to standard amphotericin B at 1.0 mg/kg/d for proven or suspected invasive mycoses [80]. Overall outcomes of both groups in this small study were similar but analysis of patients with proven invasive aspergillosis favored the lipid preparation of amphotericin B. Another study evaluated amphotericin B colloidal dispersion for primary therapy for invasive aspergillosis [81]. In this study of severely immunosuppressed patients with invasive aspergillosis, success rates with the lipid formulation were not better than those for conventional amphotericin B although toxicity was minimally decreased. While lipid formulations of amphotericin B are dramatically more expensive than standard amphotericin B, hidden costs of standard amphotericin B in terms of morbidity and mortality as well as resource utilization justify the use of lipid formulation of amphotericin B in most patients with invasive infection except in resource-limited settings where the lipid formulations are cost prohibitive [74].

The optimal dose of lipid formulations of amphotericin B remains controversial. A small observational study suggested that using higher doses of lipid formulations of amphotericin B results in better response rates [82]. A double-blind trial in patients with confirmed aspergillosis, most with hematologic malignancy and neutropenia, compared the efficacy of 10 mg/kg per day versus 3 mg/kg per day dosing for the first 14 days of treatment, followed by receipt of 3 mg/kg/day [83]. Patients treated with higher initial doses experienced more nephrotoxicity and success rates were similar. Based on these data, liposomal amphotericin B at 3 mg/kg/day is recommend as alternative primary therapy for those patients unable to tolerate voriconazole or in whom voriconazole is contraindicated because of drug interactions or other reasons. Amphotericin B lipid complex (usually at initial doses of 5 m/kg once daily) is also a reasonable alternative [2].

Echinocandins

Echinocandins are natural cyclic hexapeptide antifungal compounds that noncompetitively inhibit 1,3 β-D-glucan synthase, an enzyme complex that is unique to a number of fungi, that forms glucan polymers in the fungal cell wall [63]. These agents are active against *Candida* species and *Pneumocystis*. Specific modifications to the N-acyl aliphatic or aryl side chains expand the antifungal spectrum to include *Aspergillus* [63]. These agents are all poorly bioavailable and produced in intravenous formulation only.

Caspofungin is approved for treating patients refractory to or intolerant of standard therapies for invasive aspergillosis based on an open-label trial that demonstrated therapeutic efficacy in 22 of 54 (41%) patients studied [84]. Caspofungin has been very well tolerated in clinical trials; in the aspergillosis study, only approximately 5% of patients discontinued therapy. Drug interactions with cyclosporine may occur, but have not been a significant issue [84, 85]. In March 2005, micafungin was approved for the treatment of esophageal candidiasis and prevention of *Candida* infections. In the one prophylaxis study, used to support this approval, micafungin may have reduced the number of *Aspergillus* infections as compared to standard prophylaxis with fluconazole [86]. Micafungin also demonstrated efficacy when used as salvage therapy and in prevention of invasive fungal infection in patients with hematologic malignancy at high risk due to neutropenia or graft versus host disease [86–88]. Anidulafungin is another echinocandin with activity against *Aspergillus* spp. that appears to have a favorable toxicity profile similar to the other echinocandins. It was approved by the FDA in February 2006 for candidemia and other *Candida* infections (including abdominal abscess, peritonitis, and esophagitis). Notably, these agents are neither classically fungicidal nor fungistatic for *Aspergillus,* but exert their effect on the growing hyphal tips where the glucan synthase target is located [89]. For this reason, they have not frequently been used for primary therapy where outcomes have been poor, and have been more frequently used as salvage therapy or more recently in combination regimens [90–92].

Combination Therapies and Therapeutic Approaches

Outcomes for patients with invasive aspergillosis remain poor despite the advent of newer antifungal agents. This together with the availability of several antifungal drugs and drug classes against *Aspergillus* has increased interest in combination antifungal therapy for this infection [93, 94]. Marr et al. reported on a historical control study of caspofungin and voriconazole compared with voriconazole alone in patients who failed amphotericin formulations in 2004.

In this study, the use of combination salvage therapy was associated with an improved 3-month survival rate [92]. In 2012, the same investigators presented results of a randomized trial of voriconazole versus voriconazole with anidulafungin for the treatment of invasive aspergillosis in patients with hematologic malignancies and/or hematopoietic cell transplant in abstract form [95]. Among the 277 patients with proven or probable invasive aspergillosis, 6-week mortality was 19.3% for combination therapy patients and 27.5% for those treated with voriconazole monotherapy (95% CI −19.0 to 1.5). A post-hoc analysis of patients with probable invasive aspergillosis showed a significant difference in mortality (16% with combination therapy versus 27% with voriconazole monotherapy; 95% CI −22.7 to −0.4). Most current guidelines do not recommend initial combination therapy, but these results suggest that some subgroups of patients may benefit from such an approach. Based on these data, current recommendations are to consider combination therapy in patients who fail to respond to initial therapy and in select patients as primary therapy.

Preventative strategies include prophylaxis and targeted preemptive therapy in high-risk patients. Two large randomized clinical trials in patients with graft versus host disease and in acute leukemia or myelodysplastic syndromes showed the benefit of posaconazole prophylaxis in those patients, with improved survival and decreased invasive mycoses, including aspergillosis [87, 88]. Other strategies include intensive use of diagnostic tools in conjunction with early antifungal therapy in order to reduce the number of invasive fungal infections. A full discussion is beyond the scope of this chapter.

Adjuvant therapies, including surgical resection or use of granulocyte transfusions and growth factors, in invasive aspergillosis can augment antifungal therapy, although their utility has not been established in randomized trials. In older studies, surgical resection of isolated pulmonary nodules prior to additional immunosuppressive therapies was shown to improve outcome of the infection. With the use of newer, more effective therapies, like voriconazole, resection may not be necessary or indicated [6, 96]. Recent studies also suggest that the majority of patients will have bilateral infection when the diagnosis is first made, limiting the utility of this approach. Surgical resection may be most appropriate in patients with severe hemoptysis or with lesions near the hilar vessels or pericardium.

Summary

In summary, prompt diagnosis and aggressive initial therapy remain critical in improving the outcome of this infection [97]. Radiography and use of galactomannan EIA may facilitate an early detection of aspergillosis in high-risk patients, for whom outcomes are especially poor [46]. Primary therapy with voriconazole is recommended in most patients [2, 45]. In patients who are intolerant of voriconazole, have a contraindication to the drug, or have progressive infection, alternative agents include lipid formulations of amphotericin B. The echinocandins or another triazole is available for salvage therapy [76, 79, 84]. Primary use of combination therapy is not recommended at the present, but the addition of another agent in a salvage setting may be considered, due to the poor outcomes of a single agent in progressive infection [92]. Sequential therapy with oral azoles after initial intravenous therapy may be a useful option [5]. Although the optimal duration of antifungal therapy is not known, improvement in underlying host defenses is crucial to successful therapy. While substantial advances have recently been made in the management of invasive aspergillosis, newer approaches to therapy including the potential of more targeted combination therapy and newer diagnostic tools are needed to improve the outcome of this disease.

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