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# Invasive pulmonary aspergillosis

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In susceptible patients, invasive aspergillosis has a high incidence and a mortality of up to 80%. The diagnosis of this condition is difficult, especially in the early stages of the disease and, as a consequence, antifungal therapy, despite its expense and toxicity, is often initiated empirically. Until recently, there were very few effective antifungal agents for established invasive aspergillosis, but the introduction of two new drugs, voriconazole and caspofungin, has increased the treatment options. These newer antifungal therapies, combined with improved early diagnosis due to the introduction of newer microbiologic techniques, offer the hope that there will be a significant improvement in the substantial morbidity and mortality associated with invasive aspergillosis over the next 5 years.

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*Aspergillus* spp. are ubiquitous fungal saprophytes present in air, soil and water [1]. On average, approximately 600 *Aspergillus* spores (termed conidia) are inhaled per day, but this varies with the seasons and increases dramatically in areas adjacent to building work [2]. Inhaled conidia can colonize the airways, especially in patients with pre-existing lung disease, or cause invasive disease if host defenses are impaired [3]. Over 20 strains of *Aspergillus* are known to cause disease in humans, of which four account for the majority: *Aspergillus fumigatus*, *Aspergillus terreus*, *Aspergillus niger* and *Aspergillus flavus*. *A. fumigatus* accounts for over 90% of infections [1]; however, recent epidemiologic data suggest that the other strains may be more common than initially thought [4]. This carries particular relevance when choosing antifungal therapy, as sensitivity to antifungal therapy varies between *Aspergillus* spp. (e.g., *A. terreus* is resistant to amphotericin) [5]. This paper will review the current diagnostic strategies and therapeutic options available for invasive infections due to *Aspergillus*, as well as discuss the likely advances in the management of this difficult clinical problem over the next 5 years. The lung is the most commonly affected organ by invasive forms of *Aspergillus* infection, resulting in invasive pulmonary aspergillosis (IPA), but infection can also affect the sinuses or

disseminate from the respiratory tract to other organs (TABLE 1). This review will not cover diseases associated with *Aspergillus* colonization of the respiratory tract or an allergic response to fungal antigens such as aspergilloma or allergic bronchopulmonary aspergillosis.

## Epidemiology & risk factors

IPA is an opportunistic infection, and *Aspergillus* spp. only rarely cause invasive infection in an immunocompetent host. The predominant risk factors for developing IPA are prolonged neutropenia and treatment with high-dose corticosteroids [6,7]. The depth and duration of neutropenia is directly related to the risk of developing invasive disease, and it is unusual for IPA to develop unless neutropenia has persisted for over 10 days [8]. As a consequence, the main categories of patients who develop IPA are those with hematologic malignancies, in particular acute leukemias, following allogeneic hematopoietic stem cell transplantation (within the first month due to prolonged neutropenia, and associated with graft-versus-host disease [GvHD] and its treatment with high-dose corticosteroids at approximately 3 months post transplantation), patients with aplastic anemia and recipients of solid organ transplantation, especially lung and heart–lung transplants [8–10]. The susceptibility to IPA

associated with immune suppression is probably exacerbated by mucosal damage due to radiotherapy and chemotherapy regimens. The risk factors for hematology patients for developing IPA and the associated incidence and mortality due to invasive fungal infections for different groups of patients are discussed by O'Brien and colleagues [11]. In addition, IPA is also associated with immunosuppressive treatment of autoimmune diseases [12,13] and chronic granulomatous disease (CGD) [14], a rare genetic disorder in which phagocytes are unable to generate an oxidative burst and have an impaired ability to kill fungal hyphae. Patients with lesser degrees of immunosuppression such as diabetes, pre-existing lung damage or cystic fibrosis can sometimes develop a more indolent form of invasive aspergillosis termed chronic necrotizing pulmonary aspergillosis (CNPA) [15]. A similar condition may arise when a ball of *Aspergillus* colonizing a pre-existing lung cavity (termed an aspergilloma) starts to invade the surrounding lung.

With the increasing incidence of allogeneic hematopoietic stem cell transplantation, as well as nonmyeloablative transplantation giving rise to new therapeutic options in more elderly and debilitated patients, invasive aspergillosis is a growing problem, with rates of infection in high-risk patients of up to 20% [4,7].

#### Pathophysiology

Once *Aspergillus* conidia have entered the respiratory tract, the clinical presentation for the affected patient largely depends on the level of immunosuppression, in particular the degree of neutropenia. Classically, IPA has a relatively indolent presentation as a small number of focal lung lesions

that gradually increase in size. The disease may remain localized in the respiratory tract, resulting in tracheobronchitis, bronchiolitis or focal pneumonia, but patients with severe neutropenia can develop rapidly progressing infiltrates with hematogenous dissemination to other organs such as the kidneys, brain or even the eye [16–18]. Occasionally, as the neutrophil count recovers, IPA lesions cavitate and involute to form a nidus of *Aspergillus* contained in thin-walled lung cavities, and these patients are at high risk of reactivation of IPA during further episodes of neutropenia [19]. Angioinvasion by fungal hyphae results in hemorrhagic tissue infarction and a risk of fatal hemoptysis due to rupture of a major vessel [20].

#### Diagnosis of invasive pulmonary aspergillosis

At present, diagnosis of IPA remains difficult. The clinical presentation can be vague, and although symptoms of cough, hemoptysis and pleuritic chest pain are common, IPA can present with just fever and no localizing symptoms. Examination findings are frequently absent or subtle and are not specific for IPA. The necessary diagnostic tests are often negative or difficult to perform in an ill patient, but as the treatment for IPA is frequently prolonged and toxic, establishing as firm a diagnosis as possible is highly important. The Invasive Fungal Infections Cooperative Group of the European Organisation for Research and Treatment of Cancer (EORTC) together with the Mycoses Study Group (MSG) of the USA drew up guidelines to standardize the definition of fungal infection and aid research in this field (TABLE 2) [21]. These criteria include categories of proven, probable and possible invasive fungal infection. Histologic

Table 1. Clinical spectrum of invasive *Aspergillus fumigatus* infections.

Disease process	Clinical features	Radiology	Notes
IPA	Fever, cough, hemoptysis, dyspnea, chest pain	Nodular infiltrates, cavitation, consolidation, air-crescent or halo sign	Most common organ involved in aspergillosis
<i>Aspergillus</i> tracheobronchitis	Fever, cough, hemoptysis	Bronchial wall thickening	Improved diagnostic yield from bronchoscopy compared with IPA
Chronic necrotizing pulmonary aspergillosis	Malaise, fever, cough, weight loss	Focal lesion with or without cavitation	Associated with mild immune deficiency
<i>Aspergillus</i> sinusitis	Fever, cough, nasal discharge, headache, confusion, ulcers on septum, turbinates or palate, orbital apex syndrome	Sinusitis MRI may help to show CNS involvement	Progressive destruction and local invasion Surgical debridement necessary
Cerebral aspergillosis	Fever, headache, confusion, focal neurologic abnormalities, seizures	Localized abscesses ± surrounding edema or mass effect	May be coexistent with extracerebral involvement Mortality >90%
Disseminated aspergillosis	Fever, multiple organ involvement giving rise to many clinical symptoms and signs	CT, MRI, US and echo may all demonstrate multiple lesions	Mortality >90%

CT: Computed tomography; IPA: Invasive pulmonary aspergillosis; MRI: Magnetic resonance imaging; US: Ultrasound.

**Table 2. Amended EORTC/MSG criteria for invasive aspergillosis.**

Type of criteria	Criteria
Proven	Histo-/cytopathologic demonstration of fungal hyphae in tissue sample, or positive culture from blood or sterile site (e.g., CSF) with clinical evidence for infection
Probable	1 host factor AND 1 microbiologic criterion AND 1 major OR 2 minor clinical criteria
Possible	1 host factor AND 1 microbiologic OR 1 major OR 2 minor clinical criteria
<i>Host factors</i>	
	Neutropenia (<0.5/ml for >10 days)
	Fever for >96 h despite antibiotics in high-risk patients
	Fever AND neutropenia >10 days in previous 60 days OR immunosuppressive therapy in last 30 days OR previous aspergillosis OR symptomatic AIDS
	>3 weeks of corticosteroids in last 60 days
	Graft-versus-host disease
<i>Microbiologic</i>	
	Positive culture or cytology for <i>Aspergillus</i> from sputum, BAL or sinus aspirate
	Positive galactomannan in BAL, CSF or >2 blood samples
<i>Clinical</i>	
Major	New CT signs: halo, air-crescent, cavitating consolidation
	Radiologic evidence of invasive infection in sinuses
	Radiologic evidence suggesting CNS infection
	Papular/nodular skin lesions
Minor	Cough, chest pain, hemoptysis, dyspnea, pleural rub or effusion
	New radiologic infiltrate
	Nose ulceration, periorbital swelling, maxillary tenderness, necrosis of the hard palate
	Focal neurologic symptoms/signs, cognitive change
	Abnormalities in CSF biochemistry and cell count

BAL: Bronchoalveolar lavage; CSF: Cerebrospinal fluid; CT: Computed tomography; EORTC/MSG: European Organisation for Research and Treatment of Cancer/Mycoses Study Group.

demonstration of hyphae invading tissue or a positive culture from a normally sterile site (e.g., blood or cerebrospinal fluid [CSF]) constitutes a proven diagnosis of IPA. Establishing the diagnosis in other circumstances depends upon the results of a combination of investigations, including radiology, microscopy and culture of *Aspergillus* or newer diagnostic tests such as galactomannan detection. An understanding of how a diagnosis of IPA can be established is essential for a proper understanding of therapeutic strategies, in particular at what level of clinical suspicion empirical therapy for IPA should be initiated. Confirmation of the diagnosis in early IPA is rare, yet treatment is less likely to be effective in extensive disease when the diagnosis is more easily made. A balance has to be achieved between initiating early treatment of IPA and a reasonable probability of the disease to avoid unnecessary drug toxicity and expense.

### Radiology

Chest x-rays are carried out frequently in the routine care of patients at risk of infection whilst neutropenic, but plain radiographs have low sensitivity and specificity for fungal infection until late in the disease course when the air-crescent sign may become apparent [22]. The development of high-resolution computed tomography (HRCT) has considerably improved the ability to identify new pulmonary shadowing possibly caused by IPA, and abnormalities are often present whilst the chest radiograph remains normal. Characteristic diagnostic features for IPA on CT scans are present in up to 60% of patients and include air-crescents, halo signs (representing an area of hemorrhage surrounding the fungal infection) or cavitation within an area of consolidation [23]. Halo signs occur usually within the first week of infection, and the air-crescent sign usually appears in the third week [24]. The

presence of compatible CT findings in association with a positive microscopy or culture result (e.g., sputum) from the affected tissue in a high-risk patient is very suggestive of IPA [25]; what is more contentious is whether suggestive CT findings for IPA in a high-risk patient is adequate evidence for initiating antifungal therapy without obtaining further microbiologic, molecular or histologic evidence of fungal infection. CT imaging of the sinuses or brain is also very helpful in identifying lesions that could be due to invasive aspergillosis, but does not often have diagnostic appearances.

#### Microscopy & culture of *Aspergillus*

*Aspergillus* can be cultured from sputum, blood, urine and, if appropriate, sinonasal aspirate, CSF and biopsy samples and a positive culture from a high-risk patient strongly supports a diagnosis of IPA. However, the yield from noninvasive samples is very low [26] and as culture of bronchoalveolar lavage (BAL) fluid has a higher yield, varying in the literature between 21 and 83%, bronchoscopy is usually necessary if a positive culture is to be obtained [27]. As well as helping to confirm the diagnosis, a positive culture for *Aspergillus* provides reassurance that the disease is not due to other filamentous fungal pathogens such as *Zygomycetes* or *Penicillium*, which have similar clinical presentations to IPA but may be resistant to standard antifungal therapy.

#### Biopsy

The gold standard for the diagnosis of IPA is an open or video-assisted thoracoscopic (VATS) lung biopsy, as these allow the demonstration of hyphae within damaged tissue and a positive culture from lung tissue. However, due to the risks of bleeding or other postoperative complications, open lung biopsy remains a last resort for confirming the diagnosis of IPA [28]. Radiologically-guided lung biopsy offers an alternative method of obtaining tissue for histology and culture from within the possible IPA lesion, but has been relatively underexplored. A recent retrospective study of 17 CT-guided percutaneous biopsies from hematologic patients at risk from IPA reported that 12 biopsies demonstrated histologic evidence of fungal infection, eight due to *Aspergillus* and four due to *Mucor* [29]. Four patients had nonspecific findings, and in one patient a diagnosis of bronchoalveolar cell carcinoma was made. The complication rate was low – one patient suffered a pneumothorax requiring intercostal drain insertion. These data support the wider use of image-guided percutaneous biopsy for the diagnosis of IPA. Transbronchial biopsy is not recommended due to the risk of hemorrhage and poor yield. In contrast, in patients with *Aspergillus* tracheobronchitis, the bronchial mucosa is visibly affected and bronchial biopsies of abnormal tissue have a very high positive yield [30].

#### Newer diagnostic tests

The inability of existing diagnostic methods to obtain an early diagnosis of IPA has stimulated the development of molecular and other newer diagnostic tests. Two main methods have been

investigated: amplification of *Aspergillus* DNA from respiratory secretions or blood [31], or detection of the *Aspergillus* cell wall constituent galactomannan from blood. PCR may also help monitor response to treatment as levels of fungal DNA decline in patients with a clinical response to antifungal therapy [32]. The application of real-time PCR may improve the sensitivity and specificity of PCR for *Aspergillus* DNA [33]. However, due to the lack of standardization, the high level of technical skill required and lack of knowledge on the sensitivity and specificity of PCR for *Aspergillus*, as yet this technique is not routinely widely used.

Galactomannan is a constituent of the cell wall of *Aspergillus* that can be detected during invasive infection in blood, urine and BAL fluid using enzyme immunoassays (EIA) [34,35] or latex agglutination techniques [36]. The most consistent success has been with the sandwich enzyme-linked immunosorbent assay (ELISA) (Platelia® *Aspergillus*, Bio-Rad Laboratories) [35,37], which can detect galactomannan levels of approximately 1 ng/ml [38]. The antigen may be detectable in blood before clinical or radiologic abnormalities are present, and the detection of blood galactomannan may allow screening for IPA in high-risk patients [39]. As the significance of an isolated positive result is unclear, a positive result should lead to consecutive samples being analyzed over the next few days. Detection of galactomannan is sensitive and specific in blood, but a positive result for either galactomannan or PCR in BAL might represent environmental contamination or simple colonization by *Aspergillus* rather than frank invasive disease. The galactomannan assay has been approved for the diagnosis of IPA by the US Food and Drug Administration (FDA), and is now widely used in the USA and in many centers in Europe. More recently, there has been evidence to suggest that the use of certain penicillins, most notably piperacillin–tazobactam, have been associated with false positivity for galactomannan ELISA [40,41]. It is also intuitive that the use of antifungal therapy would have some influence on the detection of galactomannan. It seems likely that most antifungal agents decrease the sensitivity of the test [42], whilst possibly due to their mechanism of action, the echinocandin class of antifungals may increase galactomannan release and subsequent detection by causing increased hyphal fragmentation [43,44]. This finding was not supported in a retrospective analysis of galactomannan ELISA results in the caspofungin trial of salvage therapy [45]. Further trials are needed to assess the impact of a variety of treatment regimens on the assay's sensitivity and false positivity.

#### Treatment of invasive pulmonary aspergillosis

The number of antifungal agents that are effective in IPA is limited, and many questions remain on what the optimal regimen is (TABLE 3). Even with adequate therapy, the mortality of IPA is very high, and often medical treatment will fail unless there is some recovery of the patient's immune function. The relative low incidence of IPA, the subacute nature of the disease and the difficulties in achieving a certain diagnosis make clinical trials assessing treatment of IPA difficult to perform, and

Table 3. Antifungal agents utilized for invasive aspergillosis.

Agent	Oral bioavailability	Time to peak plasma concentration	Metabolism	Plasma elimination half-life	Route of elimination	Mechanism of action	Major side effects	Significant interactions
Conventional amphotericin B	No	1 h	Not fully known	15 days	Urine Feces	Binding to ergosterol	Infusional toxicity, nephrotoxicity	Cyclosporin and aminoglycosides increase nephrotoxicity
Lipid formulations of amphotericin B	No	~2 h	Not fully known	2 h	Urine Feces	Binding to ergosterol	Infusional toxicity	Cyclosporin and aminoglycosides may increase nephrotoxicity
Itraconazole	Poor	2–5 (oral) h	Hepatic	30–40 h	Urine (intravenous)	Inhibition of ergosterol biosynthesis	Gastrointestinal Elevated hepatic enzymes	Drugs subject to cytochrome P450 metabolism
Voriconazole	High	2 h	Hepatic	6 h	Urine 80% Feces 20%	Inhibition of ergosterol biosynthesis	Gastrointestinal Elevated hepatic enzymes Photopsia Photosensitivity	Drugs subject to cytochrome P450 metabolism
Caspofungin	No	1 h	Hepatic	12–16 h	Feces	Inhibition of $\beta$ [1,3]-D-glucan synthesis	Elevated hepatic transaminases	Cyclosporin Tacrolimus

until recently amphotericin B was the only effective treatment. The introduction of the newer azole drugs and echinocandins has considerably improved the range of available treatment options, but as a consequence, more research is necessary to define the exact roles of each of these drugs for the treatment of IPA.

#### Amphotericin B

Despite being introduced in 1958, amphotericin and its lipid formulation derivatives have until recently formed the mainstay of treatment for IPA. The active component, amphotericin deoxycholate, is a polyene that binds to ergosterol, an important component of the fungal cell membrane [46]. This disrupts membrane structure and modifies its permeability, and cell death is thought to occur through leakage of essential nutrients [1]. The conventional form, amphotericin B, is mainly administered by slow intravenous infusion at a dose of 1–1.5 mg/kg/day. In the blood, amphotericin B is mainly bound to plasma proteins and reaches peak levels approximately 1 h after a 4-h infusion. There is a progressive accumulation of the drug in the tissues with ongoing administration. Concentrations in the blood do not reflect tissue concentrations accurately, and these may well exceed the minimum inhibitory concentration (MIC). After an initial half-life of 24–48 h due to uptake of amphotericin B by lipids, the half-life is 15 days [47]. Amphotericin B has a wide spectrum of activity and, despite having been in use for nearly 50 years, very little resistance has developed in susceptible strains.

However, of note is that some fungal species are intrinsically resistant to amphotericin B – notably *A. terreus*, some strains of *A. flavus*, *Scedosporium apiospermum*, *Trichosporon* spp. and *Candida lusitanae* [46].

Amphotericin B binds to cholesterol in human cells and this probably accounts for its relative toxicity. During the infusion, up to 90% of patients experience fever or chills, possibly mediated by tumor necrosis factor (TNF)- $\alpha$  or interleukin (IL)-1, as well as pain at the injection site and thrombophlebitis [48,49]. Other documented side effects include gastrointestinal disorders, bronchospasm and cardiac dysrhythmias [50]. These effects are more common in the elderly and with rapid infusions, but do decrease with continued administration [46]. Nephrotoxicity is a common serious side effect related to dose, and results in hypokalemia, hypomagnesemia and uremia [48]. Concomitant use of other nephrotoxic drugs, such as cyclosporin and aminoglycosides, increases the risk of significant renal damage, whereas saline loading prior to administration or administration as a 24-h infusion can reduce nephrotoxicity [51].

Early trials demonstrated a role for conventional amphotericin B as empirical therapy for febrile neutropenic patients unresponsive to broad-spectrum antibiotics, resulting in defervescence of fever; however, there was no overall effect on mortality [52,53]. Treatment success has more recently been calculated on a combined outcome score, including defervescence of fever, mortality, rate of breakthrough invasive fungal infection and discontinuation of therapy due to adverse

effects. Based on these criteria, treatment success of conventional amphotericin B for IPA is approximately 50% [54]. In other forms of invasive aspergillosis, such as disseminated disease or cerebral aspergillosis, amphotericin B is less efficacious and mortality approaches 90% [55]. Local or endobronchial instillation treatment has shown some potential in IPA, but its use is limited practically by thrombocytopenia and the risk of pneumothorax [56]. Nebulized amphotericin is unlikely to be effective in established IPA.

#### *Lipid formulations of amphotericin B*

Lipid formulations of amphotericin B were developed to reduce its nephrotoxic effects. There are three main formulations available: amphotericin in colloidal dispersion (ABCD; Amphocil® or Amphotec®), amphotericin B lipid complex (ABLC; Abelcet®) and liposomal amphotericin B (AmBisome®). Lipid formulations of amphotericin B undoubtedly cause considerably less infusion-related side effects and nephrotoxicity than conventional amphotericin B [57,58], and as a consequence, these drugs can be administered in much higher doses than conventional amphotericin B (i.e., 3–10 mg/kg/day). Why lipid formulation reduces the toxicity of amphotericin B is not fully understood, but it is thought that association with lipids prevents amphotericin B from binding to host cholesterol [59]. In animal studies, the efficacy of the lipid formulations of amphotericin B is less than conventional amphotericin B [59,60], but in clinical practice this may be offset by increased concentrations of lipid-formulation amphotericin B in reticuloendothelial tissue, the brain [61] and the lung [62]. Existing trial data suggests that lipid formulations of amphotericin B are not less efficacious than conventional amphotericin B, and may have a slight advantage in treating IPA, probably as the lower toxicity profile allows more complete treatment [63,64]. Whether high doses of liposomal amphotericin B are more effective than lower doses is unclear [65,66]. A EORTC randomized trial into 1 or 4 mg/kg/day of AmBisome for the treatment of invasive aspergillosis in neutropenic patients with hematologic cancers, or following bone marrow transplantation, found no differences in response rate or survival [66]. However, the statistical power of this study may not have been sufficient to identify smaller differences, and higher doses are often used in clinical practice. Unfortunately, lipid formulations of amphotericin B are expensive and so are usually only used if a patient is unable to tolerate conventional amphotericin B or develops nephrotoxicity.

#### *Pyrimidine analogs: flucytosine*

The pyrimidine analog 5-flucytosine is incorporated into DNA and therefore terminates fungal cellular replication. *In vitro* studies suggest a possible additive effect for combining flucytosine and amphotericin B [67], but there is very little clinical data on the efficacy of 5-flucytosine for treating IPA, either alone or in combination with other antifungal treatment [59]. 5-flucytosine may suppress bone marrow function

and is therefore avoided in patients with hematologic malignancies, thus limiting the patients at risk of IPA in whom it may be used.

#### *Azoles*

Azoles inhibit the fungal cytochrome P450 enzymes 14 $\alpha$ -demethylase and 24-methylene dihydrolanosterol demethylase, which catalyze late steps in ergosterol production. Azoles therefore inhibit biosynthesis of ergosterol, and so affect fungal membrane permeability [68]. Earlier azoles (clotrimazole, ketoconazole or fluconazole) have no effect on *Aspergillus* and are not used to treat IPA. The two newer azoles, itraconazole and voriconazole, are effective against *A. fumigatus*, and have the added advantage of being the only antifungal treatment for IPA that can be given orally.

#### *Itraconazole*

Itraconazole is available in three formulations; intravenous, tablet and oral solution. The tablet form is poorly absorbed through the gut, leading to the development of the oral solution, which is better absorbed. Patients with achlorhydria or those taking histamine receptor antagonists have significantly reduced gastric absorption of itraconazole [69]. Itraconazole levels can be measured by specialist centers, and should be considered for patients having prolonged oral therapy. The intravenous formulation is relatively well tolerated. Itraconazole exhibits nonlinear pharmacokinetics, and has significant drug interactions due to its effect on the human cytochrome P450 system in the liver. Concomitant administration of itraconazole with rifampicin or phenytoin results in decreased serum levels of itraconazole [70,71], whereas itraconazole raises cyclosporin and tacrolimus levels by up to 50% [72,73]. The main side effects of itraconazole are gastrointestinal symptoms and elevated hepatic transaminases. However, the efficacy of itraconazole for the initial control of IPA in immunocompromised patients is probably poor and it is generally used either in combination with amphotericin B or as maintenance therapy after initial treatment with amphotericin B [74]. It is likely that the introduction of voriconazole will substantially reduce the use of itraconazole for treating IPA.

#### *Voriconazole*

Voriconazole is a new synthetic derivative of fluconazole that has a much higher affinity for the *Aspergillus* 14 $\alpha$ -methylase and 24-methylene dehydrolanosterol demethylase [75]. As a result, voriconazole is both fungicidal and fungistatic for *Aspergillus* spp. and represents a considerable step forward for antifungal therapy for IPA. Voriconazole has activity against most *Aspergillus* species, even those such as *A. terreus*, which are frequently resistant to amphotericin B [75]. However, unlike amphotericin B, voriconazole is not active against some fungal species, such as the *Zygomycetes* that cause a similar clinical picture to IPA [76], underlining the need to establish an accurate microbiologic diagnosis for invasive fungal infections, if at all possible. The increased use of voriconazole instead of

amphotericin B may unmask infections due to *Zygomycetes* and there have been several reports of breakthrough infection with *Zygomycetes* during treatment with voriconazole [77,78]. Unlike itraconazole, voriconazole has excellent oral bioavailability and achieves its maximum plasma concentration within 2 h [75]. Voriconazole is mainly metabolized by the liver with an elimination half-life of approximately 6 h, and is usually administered as a twice-daily dose or as an intravenous infusion over 1 h. Metabolites are excreted in the urine and feces, and patients with moderate hepatic insufficiency have reduced clearance of the drug [79]. Like other azoles, voriconazole has nonlinear pharmacokinetics, so small changes in dose can lead to large changes in plasma concentrations. The intravenous formulation of voriconazole (and itraconazole) contains the nephrotoxic compound cyclodextrin, and it is therefore recommended that it is used with caution in patients with renal dysfunction [80].

There have been several studies assessing the efficacy of voriconazole in the setting of invasive fungal infection [81,82]. The National Institute of Allergy and Infectious Diseases Mycoses Study Group carried out a multicenter, open-label equivalence trial of voriconazole and liposomal amphotericin B as empirical therapy for neutropenic fever [81]. Treatment response and mortality was similar for both drugs. For patients given liposomal amphotericin B, there was an increase in documented breakthrough infections, as well as higher levels of toxicity related to infusions and more frequent nephrotoxicity, whereas photopsia and visual hallucinations were features of those treated with voriconazole. Rates of hepatotoxicity did not differ between groups. Somewhat surprisingly, despite the advantage of the oral preparation, there was no significant reduction in length of hospital stay overall for the voriconazole treatment arm, although once clinicians become more familiar with its use, this may become an advantage in the future. Treatment was terminated early due to perceived treatment failure more frequently in those treated with voriconazole, perhaps because the open-label design of the trial encouraged clinicians to be more cautious with the newer and less familiar drug.

Another large prospective clinical trial examined the role of voriconazole as primary therapy in invasive aspergillosis [82]. Patients with definite or probable invasive aspergillosis (based on EORTC/MSG criteria) were randomized to receive either conventional amphotericin B or voriconazole. Most of those included in the trial had an underlying hematologic malignancy or had undergone hematopoietic stem cell transplantation; over 60% were at high risk for IPA. Partial or complete response to treatment occurred in 52.8% of the voriconazole group versus 31.6% of the amphotericin B group, with the majority of the difference due to an increased frequency of a partial response to treatment in the voriconazole group. Overall mortality at 12 weeks was reduced in the voriconazole arm – 29.2 versus 42.1% for amphotericin B. Again, the voriconazole group had significantly less toxicity related to infusions and renal impairment than the amphotericin B group. However, there was more visual disturbance and a trend towards more hepatic toxicity for patients receiving voriconazole. However,

interpretation of these encouraging results is confounded by two major factors. First, in the voriconazole treatment arm, 24% of patients also received amphotericin B therapy, leading to some uncertainty as to whether the benefits can be solely attributed to the study drug. Second, patients in the voriconazole group received treatment for a median duration of 77 days (range 2–84), whilst the amphotericin B treatment group received a median duration of 10 days of amphotericin B therapy (range 1–84). Hence, the improvement in clinical outcome could reflect more prolonged treatment rather than an intrinsic benefit of voriconazole over amphotericin. However, overall, these trials suggest voriconazole is at least as effective as amphotericin B without the high toxicity, and also has the advantage of oral preparation. Voriconazole is therefore a very useful addition to the treatment options for IPA.

#### Posaconazole & ravuconazole

Posaconazole is closely related to itraconazole and is still under development. *In vitro*, posaconazole has good activity against *Aspergillus* spp. [83], and case reports have described the successful use of posaconazole for rescue therapy for patients with invasive aspergillosis who have not responded to other antifungal agents [84,85]. Posaconazole is expected to carry similar advantages to voriconazole with respect to a broad spectrum of action and oral formulation, but has a much longer half-life of 18–24 h. Ravuconazole is related to fluconazole and itraconazole, is well absorbed as an oral preparation and has good activity against IPA in animal models [86]. No clinical data are as yet available on its efficacy.

#### Echinocandins

##### Caspofungin

Caspofungin is the first drug to become available for clinical use of a new class of antifungal agents known as echinocandins. This class of drugs noncompetitively inhibits fungal  $\beta$ [1,3]-D-glucan synthase and therefore prevents  $\beta$ [1,3]-D-glucan synthesis, an important component of the fungal cell walls. As a result, the cell wall loses integrity and the cell lyses. Caspofungin has activity against a range of *Aspergillus* spp., including *A. fumigatus* and species that are intrinsically resistant to amphotericin such as *A. terreus* and some strains of *A. flavus*. This latter property suggests that it may be a useful drug for second-line therapy in disease unresponsive to amphotericin B. Caspofungin has poor oral bioavailability and is administered intravenously. Following intravenous administration in animals, it can be detected in the kidneys, liver, spleen and lungs [87]. Caspofungin has linear pharmacokinetics and a relatively long half-life of 9–11 h. Importantly, caspofungin is safe in moderate renal failure, with no nephrotoxicity in patients with normal renal function. However, it has been recommended that reduced doses are administered in moderate hepatic impairment. Excretion is in the urine and feces, where only 2% is present in a nonmetabolized form. It is not metabolized by the cytochrome P450 enzyme system, but does reduce tacrolimus levels by up to 20% [88]. When healthy volunteers were given both caspofungin and cyclosporin, transient reversible elevation of alanine aminotransferase was noted.

Few trials have been carried out assessing the efficacy of caspofungin in IPA. When given as salvage therapy for patients with proven or probable IPA refractory to treatment with amphotericin or triazoles, 45% of 87 patients had a complete or partial response to caspofungin [89]. In general, caspofungin was well tolerated, with severe adverse effects forcing discontinuation of treatment for only two patients in this study. Caspofungin has also been compared with liposomal amphotericin as empirical treatment for neutropenic patients with persistent fever [90]. Compared with patients treated with amphotericin B, patients treated with caspofungin had less nephrotoxicity or infusion-related side effects, but a similar treatment success rate, early mortality and rates of breakthrough fungal infection. These results suggest that caspofungin is also a potentially valuable new treatment for patients with IPA.

#### Micafungin & anidulafungin

Two other echinocandins are currently under investigation but are not yet widely available for clinical use – micafungin and anidulafungin. They have a similar spectrum of activity as caspofungin, although possibly with less hepatotoxicity when used in combination with cyclosporin [79].

#### Combination chemotherapy

Although large trials have not concluded a definite place for combination therapy for IPA, in practice the high mortality of the disease and the poor efficacy of first-line therapy often results in the patients receiving combinations of antifungal treatment. As amphotericin, the azoles and caspofungin have different modes of action, dual therapy is appealing, and there is some laboratory data supporting synergy of effect for the combinations of voriconazole with caspofungin [91], amphotericin B with flucytosine [92] and amphotericin B with caspofungin [93]. As with treatment of tuberculosis, employment of multiple antimicrobials could also reduce the risk of emergent resistant *Aspergillus* strains. However, there is very little clinical data available on combination therapy [94], and animal studies may not be truly representative of the pathogenesis of human IPA [95]. There is also a potential risk of antagonism; for instance, there have been case reports of reduced efficacy of amphotericin B when used after azoles, perhaps due to reduced ergosterol availability [96]. In addition, although combination therapy may allow reduced doses of individual antifungal agents, the majority of patients requiring treatment are subject to polypharmacy and any additional drugs may result in increased toxicity. Despite these caveats, the introduction of voriconazole and caspofungin will probably stimulate further controlled trials of the efficacy of combinations of these drugs with each other or amphotericin versus single-agent therapy. Indeed, the combination of voriconazole and caspofungin has been reported to improve survival compared with historical controls treated with voriconazole alone [97], and combination therapy may become standard in the future.

#### Immunomodulatory therapies

As IPA is rare in the immunocompetent, an attractive therapeutic strategy would be to boost the immune response to *Aspergillus* using immunotherapy to either increase the number of host phagocytes or their efficacy. However, case reports of the response of patients with IPA to granulocyte infusions have not universally shown an improvement in the clinical condition of the patient, perhaps since donor granulocytes can cause lung injury and so offset any improvement in the control of IPA [98]. In patients with neutropenia following chemotherapy or bone marrow transplantation, the number of circulating polymorphonuclear leukocytes can also be increased using granulocyte colony-stimulating factor (G-CSF). Animal studies have suggested synergy between G-CSF and amphotericin for treating IPA [99], but these results have yet to be replicated in humans. Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation and activation of monocyte/macrophages, and case reports have suggested that treatment with GM-CSF can assist treatment of IPA [100]. However, it is possible that overstimulation of the immune response could have negative consequences, and this is reinforced by a case report of a patient given GM-CSF who died following bone marrow recovery from lung cavitation and massive hemoptysis [101]. Interferon- $\gamma$  has been proposed for the treatment of IPA unresponsive to antifungal therapy in patients with chronic granulomatous disease [102]. Its success in the patient described was attributed to an increase in macrophage activation that mirrored the clinical response. However, this treatment cannot be readily applied to neutropenic patients with IPA. In general, immunomodulation has not yet been a successful treatment strategy for IPA, probably because the immune deficit that allows IPA to develop is generally too severe.

#### Surgery

In most centers, surgery is usually reserved as a method of managing patients at risk of life-threatening hemoptysis due to IPA, or perhaps to remove localized residual *Aspergillus* lesions prior to another period of immunosuppression [103]. However, as IPA often presents with localized lesions and medical treatment is difficult and prolonged, surgical resection has also been considered as a curative treatment for IPA. Some groups have reported considerable success in lung resection as treatment for a localized pulmonary focus of disease, either by wedge resection or lobectomy, with relatively low postoperative complication rates and mortality [104,105]. Reichenberger and colleagues described a case series of 27 neutropenic patients (58% of whom were also thrombocytopenic) who underwent lung resection as diagnosis and treatment of localized pulmonary nodules thought to be due to IPA [25]. Postoperative complications included persistent air leak, hemothorax or recurrent pneumothorax, but only occurred in a minority of patients and in general blood loss was low considering that many patients had thrombocytopenia. IPA recurred in 14% of patients (one patient each with orbital, renal and pleural aspergillosis). Similar results were obtained from a more recent retrospective cohort study



comparing patients who were managed surgically with those who received medical therapy alone for localized IPA [106]. At 6 months, the surgical group had higher rates of survival (70 vs. 42%) and lower rates of progression of IPA (17 vs. 52%). In the published literature, the postoperative mortality for patients undergoing resection for IPA seems to be approximately 10%, which compares favorably with an overall mortality of 60–80% for IPA. Whether these encouraging results for the surgical treatment of IPA reflect a strong patient selection bias requires more evaluation. However, surgery does seem to have a potentially important role for the treatment of selected patients with localized IPA.

### *Prophylaxis*

IPA is a nosocomial infection caused by a ubiquitous environmental microorganism, and prevention of infection by preventing exposure to *Aspergillus* is very difficult to achieve. Rigorous reverse barrier nursing, exemplary hand hygiene by medical and nursing staff, and wearing high-efficiency particulate masks will probably have little effect in preventing the patient inhaling *Aspergillus* spores [107]. Hence, some centers nurse patients at high risk of IPA in rooms with high-efficiency particulate air (HEPA) filtration. However, the cost of HEPA filtration units is substantial and there is little good prospective data on their efficacy at preventing IPA [108,109].

Given that the patients at risk of IPA can be readily identified, antifungal prophylaxis is an attractive option and has become routine in clinical practice. However, unlike fluconazole prophylaxis against candidiasis, the efficacy of prophylaxis against invasive aspergillosis and other mold infections remains unclear. Prophylaxis with liposomal amphotericin (AmBisome, at a dose of 2 mg/kg/day) in high-risk patients decreased fungal colonization but, despite this, there was no difference in the rate of invasive fungal infection compared with placebo [110]. In a large multicenter study of allogeneic HSCT recipients, prophylaxis with itraconazole resulted in a reduction in rates of IPA when compared with fluconazole, although at the cost of a higher incidence of gastrointestinal side effects [111]. Mortality remained similar at approximately 40% in both study arms, but fewer deaths were attributable to fungal infection in the itraconazole arm. There is also some evidence to support its use as prophylaxis in chronic granulomatous disease, where there was a trend towards reduced rates of invasive fungal infection in the treatment group [112]. A meta-analysis of 38 randomized controlled trials of antifungal prophylaxis in chemotherapy and bone marrow transplant recipients demonstrated reductions in the order of 40% or more for the use of parenteral antifungal agents, incidence of invasive fungal infection, and fungal infection-related mortality. These encouraging results did not translate into a clear overall benefit on survival [113]. Nonetheless, many hematology patients use antifungal prophylaxis routinely for high-risk patients until bone marrow function has recovered. Currently, trials are underway to assess newer azoles as prophylaxis, such as voriconazole. Secondary prophylaxis is less contentious, as patients who have had proven or probable

IPA and are about to undergo further immunosuppressive therapy are at high risk of reactivation or a second episode of IPA. Many clinicians would treat these patients with a full dose of antifungal chemotherapy, one example of the pre-emptive treatment strategy discussed below.

### *Timing of therapy for invasive pulmonary aspergillosis*

The incidence of IPA in high-risk patients is substantial and the mortality high, but for the majority of patients with IPA the diagnosis is never fully confirmed, and the decision to start antifungal treatment is empirical. However, at what threshold of clinical suspicion empirical therapy is started probably varies between hospitals, and even between individual clinicians at the same hospital. Earlier empirical treatment could prevent advanced disease, but might lead to potentially unnecessary drug-related toxicity and expense, whereas delaying treatment may increase the chance of treatment failure or prolong the course of treatment required. Commonly, antifungal therapy is started for neutropenic patients with a fever that persists for over 96 h despite broad-spectrum antibiotics [52,64]. However, the clinical picture is often confused, and a low threshold for starting therapy for IPA can result in treatment being started in many situations where IPA is an unlikely diagnosis. The introduction of voriconazole and caspofungin may exacerbate this problem, as these drugs are less toxic than amphotericin B and clinicians may feel less inhibited in prescribing them. Hence, potentially toxic and expensive antifungal treatment may be given unnecessarily to patients at low risk of disease, and monitoring response to treatment and deciding the duration of antifungal therapy in these circumstances is difficult. Furthermore, excessive antifungal agent use could lead to resistant strains developing. Conversely, waiting for good evidence for IPA from CT scan appearances and/or microbiologic specimens before starting antifungal therapy may adversely affect prognosis, particularly in the presence of severe neutropenia, when IPA may progress rapidly. Improved diagnostic techniques such as PCR or measuring galactomannan may resolve some of these difficulties and provide good evidence for IPA at an early stage of disease and so allow pre-emptive therapy.

### *Pre-emptive therapy*

A pre-emptive treatment strategy has been advocated for certain patients who are at very high risk of IPA. This treatment strategy involves commencing these patients on full-dose antifungal therapy immediately as they are immunocompromised [114,115]. Patients who have had a previous episode of IPA and are perhaps left with continuing radiologic change, or patients who have undergone lung transplantation and subsequently isolate *Aspergillus* from respiratory secretions without signs of infection, would probably benefit from this strategy [113]. However, the identification of other high-risk patients is less clear-cut. As the risk of IPA is related to the duration of neutropenia, patients with prolonged neutropenia could be one high-risk group. Another could be hematology patients known to be colonized with *Aspergillus* before transplantation or chemotherapy

who would then receive antifungal treatment during periods of immunosuppression. To identify these patients would require regular screening using sputum microscopy and culture, but the low sensitivity of sputum culture for *Aspergillus* in the respiratory tract would inhibit the effectiveness of this strategy, and using BAL or tracheal aspirates as alternative screening methods is probably not justified.

An alternative strategy is surveillance of patients using the newer noninvasive diagnostic tests such as PCR or galactomannan during their period of risk for IPA. Those patients developing a consistent positive result even in the absence of clinical evidence of infection would be treated with full-dose antifungal therapy. This may allow treatment to be given to patients early in the course of the disease, but with a reasonable certainty that IPA is developing. Further assessment is required for the clinical and resource effectiveness of this strategy, preferably by randomized controlled trials versus standard treatment.

#### *Duration of therapy*

There is no definitive data regarding when treatment can be safely discontinued. Due to the high risk of recurrent disease in IPA, particularly in the case of hemato-oncology patients undergoing further immunosuppressive chemotherapy and subsequent neutropenia, therapy should ideally not be stopped until complete resolution of changes on the HRCT. With the advent of more efficacious oral preparations, this is more practical – patients can now be discharged from the ward on antifungal medication. Therapy may often be needed for many weeks.

#### *Expert commentary*

Despite advances in the treatment of IPA, major changes to the high mortality of this disease will probably only be achieved by prevention or early detection of the disease. This requires the rigorous use of protocols to identify high-risk patients, stratification of clinical presentations for the probability of a diagnosis of invasive aspergillosis and the rapid use of CT scanning and bronchoscopy (preferably the same day) for those likely to have IPA. Patterns of care need to be organized to prevent delays in obtaining CT scans and invasive tests, otherwise patients will start empirical therapy without good evidence for IPA. Unnecessary empirical therapy is likely to be an increasing problem now that less toxic treatments are available, and will increase drug costs and cause patients unnecessary drug toxicity. Newer diagnostic techniques such as galactomannan detection and *Aspergillus* PCR are promising methodologies that can help confirm the diagnosis of IPA at an early stage and so reduce the need for invasive tests and empirical therapy. However, these newer diagnostic techniques still require further investigation into the significance of a positive result, their sensitivity, and which sample source is the most useful. As *Aspergillus* can be identified by histology or cytology, the authors believe that CT-guided percutaneous biopsy of focal lung lesions in high-risk patients should be increasingly utilized to confirm or exclude the diagnosis of IPA.

With the addition of caspofungin and voriconazole to conventional and lipid-formulation amphotericin B, there are now four reasonably effective treatment options for invasive aspergillosis. Although voriconazole has been shown to have a treatment advantage over amphotericin B in one controlled trial, the existing data are not convincing enough at present to suggest that any of these drugs are significantly more effective than the others. However, it is clear that voriconazole and caspofungin are less toxic than lipid-formulation amphotericin B, and that lipid-formulation amphotericin B is less toxic than conventional amphotericin B. Unfortunately, with the exception of conventional amphotericin B these drugs are all very expensive. As a result, for patients who are at low risk of nephrotoxicity, conventional amphotericin B may still be used as a first-line therapy and the patient then switched to one of the other treatments if toxicity develops. However, many of the patients at risk of IPA are also at risk of nephrotoxicity due to existing therapy or comorbidity and will need to be treated with lipid-formulation amphotericin B. Which of the newer treatments is used in these circumstances would depend on which side effects and drug interactions need to be avoided for that particular patient. Where voriconazole and perhaps itraconazole do have an important role is oral therapy for the majority of patients who will require prolonged therapy after initial control of IPA with intravenous therapy. Further clinical trials are urgently required to fully assess the efficacy of caspofungin versus voriconazole or amphotericin B for established IPA, when treatment can be stopped, and the role of combination therapy. Furthermore, the role of surgery and the best therapeutic options for breakthrough infection require detailed assessment.

#### *Five-year view*

Over the next 5 years, the role of the newer diagnostic tests for invasive aspergillosis will be clarified. If galactomannan detection or PCR in urine or blood prove to be highly specific in clinical practice for early invasive aspergillosis, this would allow regular screening of high-risk patients and pre-emptive therapy in those with positive results. The latter strategy probably holds out the best hope for reducing mortality from IPA, along with prophylaxis for high-risk patients with voriconazole or one of the azoles in development. Diagnostic techniques such as exhaled breath condensate, which is a noninvasive test that allows sampling of airway lining fluid for evidence of increased airway inflammation, may also be introduced [116], and could be used for earlier diagnosis and monitoring the response to therapy. The results of new trials will define the best treatment options for patients with IPA, and combination treatment with caspofungin and voriconazole or an amphotericin B preparation may become first-line therapy. The echinocandins and azoles currently under development are likely to increase the available treatment options, but perhaps not provide a major advantage over the introduction of voriconazole and caspofungin. Overall, although invasive aspergillosis remains a serious and difficult problem, substantial progress has been made over recent years and control of this problem is likely to improve in the near future.

## Key issues

- Although invasive aspergillosis is a rare disease in immunocompetent patients, it is a common and frequently fatal complication for patients with certain types of severe immunosuppression.
- Newer diagnostic tests such as galactomannan antigen testing or PCR could lead to the earlier diagnosis of invasive aspergillosis and therefore more effective treatment.
- The accurate diagnosis of invasive aspergillosis is difficult but essential to ensure effective therapy is given and to avoid patients receiving toxic and expensive treatment unnecessarily.
- Voriconazole and caspofungin are valuable new drugs which are effective in treating invasive aspergillosis.
- More research is required to identify the exact roles of the different forms of amphotericin, caspofungin and voriconazole for treating invasive aspergillosis.
- Newer azoles and echinocandins are being developed and will increase the range of available treatments for invasive aspergillosis.
- In the future, prophylaxis with voriconazole and combined therapy for established disease may lead to a reduction in the incidence of invasive aspergillosis and its mortality.

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