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REVIEW

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Invasive Aspergillosis in 2002: An Update

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Abstract Despite significant advances in the management of immunosuppressed patients, invasive aspergillosis remains an important life-threatening complication. In the past two decades, the incidence of invasive aspergillosis in this population has continued to increase. Factors that predispose patients to develop invasive aspergillosis include prolonged granulocytopenia, the development of graft-versus-host disease, immunosuppressive therapy, the use of adrenal corticosteroids, and the prolonged impairment of host defenses associated with diseases such as chronic granulomatous disease. Environmental factors also play a key part in the pathogenesis of this infection, and therefore, infection control measures play a critical role in reducing exposure of patients to *Aspergillus*. New exciting developments in the early diagnosis of invasive aspergillosis and the acceleration of antifungal drug discovery offer promise for the future.

Introduction

In this new millennium, invasive aspergillosis (IA) has emerged as the major clinical problem of modern mycology. There are many reasons why IA is such a challenging fungal infection to manage. First, the population at risk for the disease is increasing in number due to an expanding population of immunosuppressed patients [1]. IA is currently a major direct or contributory cause of death in leukemia patients as well as a common cause of compromised chemotherapy and failure of remission-induction chemotherapy. It is a major cause of mortality among bone marrow and stem cell transplant recipients, not only during the early post-transplant period but also later when graft-versus-host disease occurs. Second, IA is difficult to diagnose, and its therapy is suboptimal, especially in immunocompromised hosts. In addition, because IA has long been known to result in residual defects due to tissue infarcts, it has a propensity to reactivate, especially in the setting of continuous immunosuppression. Finally, IA is frequently a subacute or even chronic infection, resulting in mounting hospital costs. Therefore, building upon our review of IA that was published in this journal 10 years ago [2], we herein summarize some of the new developments in the epidemiology, pathogenesis, diagnosis, and management of this disease and offer some perspectives for the future.

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The Impact of Invasive Aspergillosis: Some Sobering Facts

There is no doubt that since it was first described in the 1940s, IA has emerged as a common mycosis. For example, there was a 14-fold rise in its prevalence upon autopsy over a 12-year period in a European study [3]. The relatively high frequency of IA was also suggested in a recent large nationwide study of unselected autopsies in Japan, which reported a rate of 1 to 2% [4]. Furthermore, IA has actually surpassed invasive candidiasis as the most frequent fungal infection found at autopsy at some tertiary care centers [5, 6, 7; unpublished data]. In patients at high risk for invasive mycoses in particular (e.g., allogeneic bone marrow transplantation [BMT] recipients), the routine prophylactic use of fluconazole over the past decade has resulted in a dramatic decrease in the incidence of invasive candidiasis and emergence of IA as the most common mycosis [6]. Finally, an incidence of 12.4 cases/million/year was reported in a large U.S. population-based surveillance study [8]. That was probably an underestimation of the true incidence, though, as detection of IA in that study was based only on a positive bronchoalveolar lavage (BAL) culture.

Additionally, the crude mortality rate of IA remains high [9, 10, 11]. Ten percent of all deaths in patients who undergo allogeneic BMT are attributed to IA, which has

a mortality rate of approximately 90% in that setting [9, 10, 11]. From our own experience at the University of Texas M.D. Anderson Cancer Center (1993–1998), IA was found at autopsy in about 20% (95/484) of patients with hematologic malignancies. Failure of antifungal therapy (given over at least 10 days) was seen in 85% of these patients who had IA, the majority of whom died within 6 weeks [12]. Furthermore, the financial burden of IA-associated hospitalization is enormous: U.S. data from 1996 estimated the total cost of IA treatment to be $633 million, with an average cost per case of $65,000 [13].

Groups at Risk for Invasive Aspergillosis: An Expanding List

In addition to the "classic" groups of patients at risk for IA, such as those with prolonged, profound neutropenia due to a hematologic malignancy (5–25% risk) or aplastic anemia, those who have received allogeneic BMT, stem cell transplantation (5–30% risk), or lung transplantation (17–26% risk), those with acquired immunodeficiency syndrome, severe combined immunodeficiency, or chronic granulomatous disease (25–40% lifetime risk), burn patients, and chronic steroid users [1, 2, 14, 15], IA has been described increasingly in other groups of patients who share the same predisposing factors. Particularly, IA was found to be a relatively common cause of death (15%) in patients with systemic lupus erythematosus in a recent autopsy study from Brazil [16] and has been recognized as a common cause of infectious death in patients with multiple myeloma who receive high-dose steroids [17]. Similarly, the susceptibility of premature neonates to severe IA was recently reviewed by Groll et al. [18]. Even within groups of patients at high risk for IA, subgroups at very high risk have emerged. For example, matched, unrelated, allogeneic BMT recipients are at higher risk of fungal infection than recipients of stem cell transplants [19]. Even the use of high-potency inhaled steroids may predispose some seemingly normal hosts to invasive pulmonary aspergillosis [20].

Pathogenesis: Moving Toward a Better Understanding of Immune Responses

Despite a growing body of evidence in the literature, the immunologic mechanisms of host resistance to IA are not completely understood [21]. It is generally accepted that neutrophils and macrophages represent the first two lines of (innate) host defense against IA, even though recognition of the role of T cells in immune response against IA is increasing [21, 22]. Specifically, pulmonary alveolar macrophages ingest and kill inhaled conidia, while polymorphonuclear neutrophil leukocytes and monocytes are fungicidal to the hyphal form of *Aspergillus* spp. [21, 23]. How these innate host defenses interact with *Aspergillus* spp. to shape the subsequent cognate immune response orchestrated by T cells is less well known, however. Animal studies have been useful in studying the pathogenesis of IA [24], and some exciting recent developments have been noted. In particular, researchers have found that the ability of neutrophils to kill hyphal elements is upregulated in animals that survive IA but severely suppressed in animals that die of the disease [25]. These quantitative changes in neutrophil activity were most evident 3 to 5 days after infection, which suggests the appearance of factors having both positive and negative effects on neutrophil antifungal effector functions.

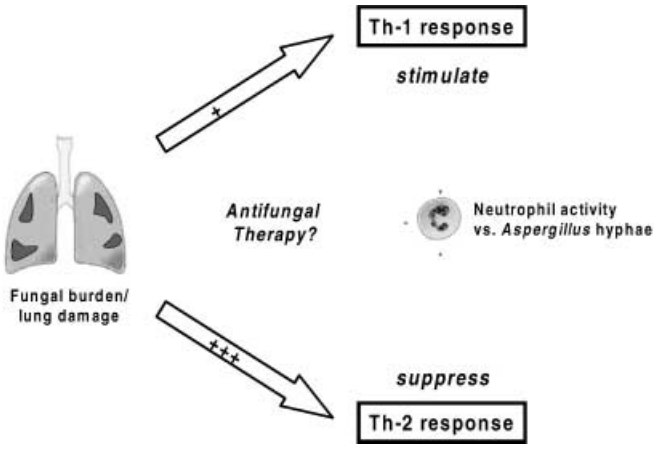

Fig. 1 Interplay between antifungal therapy and adaptive immune response

It is likely that neutrophils, through regulation of the fungal burden in tissues, actively participate in the generation of a subsequent adaptive T-helper (Th) cell response that in turn modulates their antifungal activity (Fig. 1) [1, 26, 27]. These Th-cell responses can be broadly classified into two categories according to cytokine production. First, the Th-1 type of response, characterized by elevation in the production of the proinflammatory cytokines interferon-γ, tumor necrosis factor, interleukin-2, and interleukin-12, is associated with enhanced phagocyte effector cell function, which is necessary for elimination of *Aspergillus* hyphae. Second, the Th-2 type of response is characterized by increased production of the cytokines interleukin-4 (IL-4), interleukin-5 IL-5), and interleukin-10 (IL-10); stimulation of mast-cell/eosinophil-mediated reactions; and suppression of macrophage/neutrophil phagocytic activity [26]. The role of additional cell populations, such as dendritic cells [28] or platelets [29], in regulating host response to *Aspergillus fumigatus* has been suggested in recent studies.

Recent animal studies have also suggested that Th-1/Th-2 dysregulation and a switch to a Th-2-predominant response contributes to the development and unfavorable outcome of IA. Several investigators have demonstrated that administration of Th-1-type cytokines such as interferon-γand tumor necrosis factor has a protective role in mice with IA [30]. The beneficial effect of such administration has also been shown in patients with chronic

granulomatous disease and IA [31]. In contrast, neutralization of the Th-2-type cytokines IL-4 and IL-10 has been shown to augment resistance to *Aspergillus* infection in a murine model of disseminated infection [32, 33]. Similarly, susceptibility to IA has been correlated with impaired conidial killing and hyphal damage as well as increased IL-4 and IL-10 production [32]. Administration of IL-4 and IL-10 to mice with IA increases their susceptibility to this challenging fungus and reduces their length of survival after an infectious challenge. However, administration of the soluble IL-4 receptor, which is a blocking agent for IL-4, enhances the resistance of mice to *Aspergillus fumigatus*, improving survival in mice with active pulmonary disease.

It appears that *Aspergillus* infection may also trigger a Th-2 response in humans that correlates with progression of invasive disease. Recently, in a pilot study, Roilides et al. [34] reported an association between Th-2 response as measured according to serum IL-10 levels and progression of IA in non-neutropenic hosts. Two patterns of correlation between serum IL-10 concentrations and the outcome of IA were observed. In the first pattern, a favorable outcome or stabilization of disease was seen in patients that had an undetectable level of IL-10 or a low or high baseline level that decreased to an undetectable level during the course of the disease. In the second pattern, IL-10 levels increased during the progression of disease until death, despite the use of antifungal therapy.

Furthermore, *Drosophila melanogaster* has recently provided profound insights into the nonadaptive innate response against *Aspergillus fumigatus* [35]. In one particular study, *Drosophila* mutants having loss-of-function mutations in their Toll receptors (the homologues of the mammalian CD14 receptors in immune effector cells) were found to be very susceptible to lethal *Aspergillus fumigatus* infection; moreover, the involvement of CD14 and Toll-like receptors in the responses of monocytes against *Aspergillus fumigatus* hyphae was shown [36].

Epidemiology of Invasive Aspergillosis: Will the Molecular Tools Shed Light on the Many Controversies?

Many controversies still exist regarding the epidemiology of IA [37, 38, 39, 40, 41, 42]. Among those, the definition of nosocomial versus community-acquired IA predominates. Most cases of IA are considered to be nosocomial, which implies that the incubation period is known and that the patient acquired the infection because he or she was in the hospital, both of which are difficult to ascertain The nature of the underlying disease and its treatment is such that these patients require hospitalization; hence, the risk of infection if the patient remains at home or elsewhere is unknown but most likely is higher than if the patient is hospitalized. Exposure to *Aspergillus* spp. within modern hospitals is usually lower than that outside, but that was not the case in one hospital epidemic associated with internal facility renovations [40]. A sizeable number of IA cases in allogeneic BMT recipients occur not while the patients are in the hospital and neutropenic but rather after they leave the hospital; such cases may be associated with adrenal corticosteroid administration and graft-versus-host disease [38]. However, a lack of uniform, reliable methodology for environmental sampling is a key reason for the lack of a consistent pattern in the various studies (most of which were small) that tried to correlate, with varying results, the density of *Aspergillus* spores in the air with subsequent cases of IA [37].

A substantial number of nosocomial clusters of IA have been reported over the past 3 decades. There have been sufficient numbers of clusters associated with hospital construction and defects in air-handling equipment to conclude that these associations are valid, but there are many pitfalls in tracing the source of an IA outbreak. Air and environmental surfaces have been the focus of most investigations, which have neglected other possible sources. In addition, only small volumes of air are collected over relatively brief periods of time in only a few locations and at infrequent intervals; thus, bursts of spore dissemination may be missed. This may be important, because it is reasonable to assume that the risk of infection is related to the concentration of inhaled spores. In addition, based on the predisposing factors present, different patients have different degrees of risk of infection. Because the incubation period of IA is unknown, patients may be exposed prior to hospitalization or develop the first symptoms of infection after being discharged.

What constitutes sufficient evidence of true nosocomial IA? Ideally, the same *Aspergillus* sp. should be recovered from all patients included in the epidemic and from some environmental source providing common exposure, with a close temporal relationship between infected patients and the source. Is it valid to conclude that a nosocomial source has been identified because the same species isolated from infected patients is recovered from a distant site in the hospital several months after the cluster of infections? As concluded by VandenBerg et al. [37], “With the relatively low frequency of invasive aspergillosis seen at many hospitals, even small changes in the number of cases may appear to be a cluster when in fact it is not”. It is hoped that some of these difficulties will be overcome when some of the typing techniques discussed below are perfected, although the evidence to date suggests that this may be an overly optimistic assumption.

Moreover, a survey of *Aspergillus* contamination of hospital air suggested that different *Aspergillus* spp. have different capabilities of colonizing patients, but no attempt was made in that survey to geographically or temporally link the air sample results with colonized patients [42]. Interesting new potential sources of *Aspergillus* contamination have been reported, however. A recent study found that cotton fabric harbors and disperses *Aspergillus* spores more readily than other types of fabric [43]. Hence, it is possible that cotton clothing worn by

hospital personnel and visitors may serve as a source of IA exposure to susceptible patients. Finally, there is controversy about whether hospital water reservoir systems account for some episodes of IA [44]. The available epidemiologic evidence implicating the acquisition of nosocomial IA through such systems is poor [45]. More careful studies are needed to address this hypothesis.

In recent years, various DNA fingerprinting systems (e.g., restriction fragment length polymorphism with repetitive probes, random amplified polymorphic DNA analysis, microsatellite length polymorphism) have been developed to identify individual strains of *Aspergillus* spp. [46, 47, 48, 49, 50]. For example, Debeaupuis et al. [51] analyzed Southern blot hybridization patterns to determine whether cases of IA were caused by strains of *Aspergillus fumigatus* with unique characteristics. They compared 136 isolates collected from 115 infected neutropenic patients with 97 isolates collected from cystic fibrosis patients and 115 random isolates collected from environmental sources. All told, 2–15 isolates were collected from 34 infected patients over a period lasting from 1 day to 6 months and from 22 cystic fibrosis patients over 12–32 months. They found that none of the cystic fibrosis patients were colonized by a single strain of *Aspergillus fumigatus*, while 18 of the 34 infected patients were colonized. They also found that the genetic variability of *Aspergillus fumigatus* was extremely high and that discrimination between strains was not related to geographic origin. This study suggests that every strain of *Aspergillus fumigatus* in the environment has the potential to cause infection in a susceptible host.

In addition, Chazalet et al. [52] collected clinical and environmental isolates of *Aspergillus fumigatus* from air and surface samples and clinical isolates in four different hospitals over 1 to 2 years and conducted DNA fingerprinting using a dispersed repeated DNA sequencing methodology. At hospital 1, they recovered 276 unique genotypes from 376 environmental samples. Only 17% of these genotypes were recovered more than once (2–13 times) over the 2-year study period, at different times and from different locations. Moreover, no single strain was isolated repeatedly from any site in hospital 1. In hospital 2, 157 different genotypes were recovered from 252 isolates. Only 12% of these genotypes were isolated more than once, but two genotypes accounted for 58% of the isolates recovered on multiple occasions, mainly from different sites on the same day. In total, isolates collected from 73 patients at the four hospitals were studied. Chazalet et al. [52] found that only 11 of 27 patients having multiple isolates had a single genotype. Among the 73 patients, the same genotype was recovered in only two patients or in one patient and the environment in 30 cases.

In another study, Radford et al. [53] used a polymerase chain reaction (PCR)-based method for discrimination of *Aspergillus fumigatus* types. They identified 11 DNA types among 119 isolates (59 collected from 32 patients and 60 collected from the environment at hospital sites). Eight of the 11 types were isolated from both patients and the environment, 7 of which were isolated from the patient first and the environment 2 days to 156 months later. Also, multiple types were isolated from three patients on different occasions, and different types were isolated from the same specimens collected from two patients. Furthermore, multiple types were isolated from autopsy specimens collected from several patients. Although the authors suggested that their findings implied that the patients underwent nosocomial acquisition of infections, their data are not conclusive.

Unfortunately, for many reasons, the epidemiology of IA is complex. For instance, not all typing methods are sufficiently precise for epidemiologic studies. The methodologic pitfalls of the various genotypic methods used for epidemiology were recently reviewed by Soll [46]. Furthermore, the numerous potential sources of contamination make it difficult to identify the correct source, and large volumes of air and numerous surface samples must be collected to obtain reliable information [37]. Chazalet et al. [52] estimated that they typed less than 20% of the genotypes present in the hospitals they studied. Additionally, many different genotypes may be present in the environment simultaneously, and some patients are infected by multiple genotypes. Future studies are needed to define the most vigorous methodology for addressing all of these complexities.

Early Diagnosis of Invasive Aspergillosis: Promises and Challenges

IA is often diagnosed late, when currently used drugs are less likely to be effective. Many problems contribute to the lack of timely diagnoses of IA. For example, the clinical manifestations of IA are subtle and typically occur late in the course of the disease [2]. Histopathologic studies, which constitute the gold standard of diagnostic modalities for IA, are in reality performed in a minority of patients who are not at excessive risk for surgery due to their morbidity or underlying thrombocytopenia. In addition, microbiology is of limited overall value. More specifically, blood cultures have no utility because they represent contamination in the overwhelming majority of cases [54]. Similarly, the sensitivity of respiratory secretion cultures is low [55] and has not changed particularly over the past 30 years [56]. Moreover, the yield of both premortem and autopsy cultures in autopsy-proven IA cases is exceedingly low and almost identical to that reported in earlier studies [56, 57, 58].

The implementation of routine screening using high-resolution chest computed tomography (CT) scanning, despite its relative lack of specificity, has been helpful for the early detection of IA. A recent study based on autopsy data showed that lesions indicative of pulmonary aspergillosis on chest radiographs or CT scans have a 90% probability of being due to IA [59]. Since the late 1980s, it has been shown that such strategies result in earlier administration of systemic antifungal therapy and could have an impact on outcome. More specifically,

Caillot et al. [60] compared two cohorts of neutropenic cancer patients at risk for IA. The patients who underwent systematic screening by chest CT followed by aggressive surgical and antifungal therapy had a survival advantage over those who did not, due to better control of IA.

However, the excitement regarding early diagnosis of IA has come from the development of non-culture-based diagnostic methods, such as serodiagnosis and PCR [61]. Additionally, the detection of galactomannan (GM) has the potential to be a clinically useful marker for early diagnosis [62, 63]. A sandwich enzyme-linked immunosorbent assay (ELISA) method is commercially available for detecting GM, which is a component of the cell wall of *Aspergillus* spp. found in plasma and other sterile body fluids. The GM test involves using the rat monoclonal antibody EB-A2, which recognizes the 1->5-D-galactofuranoside side chains of the GM molecule, to both capture and detect as little as 1 ng/ml GM.

In a previous study, GM detection in serum was reported to have a sensitivity of about 93% and a specificity of 95% in a retrospective cohort of autopsy-proven IA [62]. More importantly, diagnosis of IA in that study, based on the GM detection, was reached 1 to 2 weeks before other diagnostic clues appeared. These same investigators recently published their latest experience in prospective validation of screening for circulating GM as a noninvasive diagnostic tool for IA in 191 prolonged neutropenia patients and stem cell transplantation recipients [63]. These subjects had 362 treatment episodes that were surveyed for the presence of sinusal and pulmonary signs of IA and were subjected to a standard diagnostic work-up for invasive fungal infective disease. The results were not used to diagnose IA prospectively or determine which therapy to use, however. Instead, IA was defined according to the criteria proposed recently by the EORTC/MSG [64]. Use of these criteria allowed cases to be classified on the basis of premortem data. In addition, GM was considered to be present if it was detected in two consecutive serum samples. Diagnostic use of the GM-ELISA test was assessed both using premortem data and after incorporating all of the available data. Not 1 of the 30 proven IA cases was missed, and GM was detected in at least two consecutive serum samples. However, GM was also detected in 5 (55.5%) of the 9 probable IA cases, in 4 (7.4%) of the 54 possible IA cases, and in 5 (2%) of the 264 non-IA cases. Hence, while the sensitivity and negative predictive value of this test regarding proven aspergillosis were both 100%, the specificity was 96%, and the positive predictive value was only 68%. Furthermore, transiently positive GM was not noted. Empiric antifungal therapy was used for 43% of the episodes, whereas reliance on the GM-ELISA test would have reduced the use of empiric therapy to a more moderate 12%. The researchers had to test serum regularly (an average of 12 samples per patient were collected), because a single test was not sufficiently informative.

Fewer data exist for the detection of GM in BAL specimens. This procedure is more cumbersome but probably more sensitive than BAL culture and could be useful [60]. However, there are no good comparative data on the performance of serum versus BAL GM tests. Therefore, some caution is required when using this procedure. In addition, detection of GM in serum has not been evaluated outside the setting of allogeneic BMT. For other patients at high risk for IA, such as those having leukemia, the data are scant. Furthermore, there are some problematic issues with this method, including transient positivity, false-positive results, the kinetics of antigenemia in the presence of antifungal therapy, high cost, and a lack of comparative trials. In our view, this method is unlikely to be sufficient by itself, but it will probably be an important element in aggressive combined future diagnostic strategies. Finally, the detection of another fungal metabolite, 1, 3-D-glucan (G test), is less vigorous, as it requires some degree of immune response for good performance; therefore, it appears to be less promising.

The detection of *Aspergillus* DNA using PCR is also promising, yet only somewhat at earlier stages of development [65, 66, 67, 68]. Several issues remain unresolved, such as the best source of material (e.g., whole blood, serum, plasma, BAL specimens), the amplification protocol (e.g., real-time PCR, sample volume, extraction methods), and primer selection (“panfungal” PCR, 18S rRNA, 28S rRNA, mitochondrial DNA) [65, 66, 67, 68]. PCR diagnostics is a rapidly evolving field in which there are difficulties in comparing studies. However, some conclusions can be made. First, the sensitivity of PCR using serum as a source of material is very good, because it depends on the degree of angioinvasion. Therefore, its negative predictive value should be very good, although its specificity may still be in question.

In addition, PCR using BAL to obtain material seems to be less promising when compared with that using serum or plasma, due to the higher number of false-positive results [69], even though a recent small study by Buchheidt et al. [70] suggested that this method has an acceptable positive predictive value. Nested PCR using serum is a very sensitive method [65] but, at least theoretically, is more prone to contamination than other methods. So far, results of PCR using blood appear to be very promising for both early diagnosis of IA and assessment of therapeutic response [65, 66, 71]. In addition, a recently reported study showed that the use of real-time PCR has promise [67]. Specifically, this approach, although technically more difficult than other PCR methods, appears to be beneficial in terms of sensitivity, specificity, and accurate quantitation of *Aspergillus* DNAemia.

There have been no good studies examining how PCR performs in comparison with GM detection for the early diagnosis of IA. However, there has been a suggestion that PCR is inferior to serum GM assay in animal models [72] and in some but not all human studies [67, 73]. More and better studies are needed, as this is a fertile area for future investigation.

There is no question that non-culture-based methods of early IA diagnosis are the future. Much more work,

though, needs to be done to validate surrogate markers for both diagnosis and management of IA [74]. More specifically, some urgent questions should be addressed, and the impact of these methods on mortality should be the "hard" endpoint. First, is detection of antigenemia (GM) better than detection of DNAemia (PCR)? Second, is it preferable to combine detection of antigenemia with that of DNAemia (with or without the use of high-resolution CT) for early detection? Third, what is the independent prognostic significance of GM detection and PCR and the two methods' comparative ability to "quantify" the fungal burden, especially in the presence of antifungal agents?

Finally, novel molecular tools, such as an *Aspergillus*-specific monoclonal antibody-tagged radioactive isotope, assays for detecting fungal metabolites, and reverse transcriptase-PCR for *Aspergillus* tissue-specific mRNA detection (in situ reverse transcriptase-PCR), are exciting areas of further investigation. In our view, it will be important to vigorously pursue the use of autopsy in future studies aiming to validate the usefulness of new tests. This will allow a more accurate estimate of the incidence of proven cases than would be possible otherwise, as invasive procedures have not been used in thrombocytopenic patients.

Treatment of Invasive Aspergillosis: Still Too Many Uncertainties

IA is a complex, heterogeneous, frequently multifocal infection [1, 2]. Furthermore, there is a paucity of well-conducted, controlled clinical studies of the treatment of IA. The lack of uniform definitions (until recently) for the diagnosis and response of IA, the relatively small patient numbers in IA studies, differences in the IA patient populations, and institutional and publication biases have created uncertainty about the optimal management of this frequently lethal infection [75, 76, 77, 78, 79, 80]. Many factors other than antifungal therapy per se, such as failure to recover from neutropenia, delayed therapy, and the site of involvement, affect outcome and have not been clearly controlled in many studies. Prospective open-labeled trials compared with historical cases have been the most common trial design for salvage therapy using new investigational drugs for IA. However, this approach may introduce multiple biases (e.g., information, temporal, and selection biases) that could overestimate the efficacy of a new antifungal agent in comparison with historical controls. Thus, the need for alternative trial designs and evaluation strategies has been emphasized lately [81, 82]. The recent introduction of uniform diagnostic criteria for proven, probable, and possible IA by the EORTC Invasive Fungal Infections Cooperative Group/Mycoses Study Group consortium has been a major advance [64]. These criteria are practical, validated, standardized, and reproducible and consist of host-risk, microbiologic, clinical/radiologic, PCR, and/or GM criteria. Standardization of definitions for diagnosing and assessing the effectiveness of antifungal therapy holds promise for addressing the many controversies that currently exist in the management of IA. Some of these controversies are discussed below.

Thus far, no studies have directly compared amphotericin B (AMB) with one of the lipid formulations of AMB for IA. Moreover, it is highly unlikely that any such studies will be conducted in the future. The lipid formulations of AMB have been shown in uncontrolled studies to have an efficacy rate of about 40–60% in IA patients whose disease was refractory to AMB or who were intolerant of it [83]. There is a consensus that the lipid products of AMB result in lower nephrotoxicity and infusion-related toxicity, but the daily acquisition prices are much higher than those of AMB deoxycholate. This nephrotoxicity frequently appears to be clinically significant, especially in the most heavily immunosuppressed patients [84]. Furthermore, a recent pharmacoeconomic analysis of a randomized, double-blind comparative trial of AMB and liposomal AMB as empiric therapy in febrile neutropenic patients showed that nephrotoxicity, which was caused mainly by AMB, increased hospital costs. When modeled in an analysis of sensitivity, the higher acquisition costs of liposomal AMB neutralized the increased hospital costs associated with nephrotoxicity [85].

An even more controversial issue is whether there are clinically meaningful differences between the various lipid formulations of AMB. Most of the available data have been derived from indirect comparisons and suggest that all lipid formulations of AMB, when given using the standard dosage of 5 mg/kg/day, appear to have comparable efficacy. This therapeutic equivalence was suggested in the only comparative study of AMB lipid complex and liposomal AMB, which was recently published [86]. However, this study may not have been sufficiently powered to specifically compare these two agents in terms of efficacy. Liposomal AMB, which is the most expensive agent, also appeared to be the less toxic of the two in that study, even though some of the toxic reactions that occurred may have been either insignificant or preventable. Hence, the overall cost-effectiveness of these formulations remains undefined.

The optimal dose of lipid formulations of AMB in the treatment of IA is also unclear. Clinical evidence indicates that AMB (both AMB deoxycholate and the lipid formulation) may produce a dose response in IA cases [87, 88]. This concept was challenged in the only randomized trial of IA ever published, which compared liposomal AMB given at 1 mg/kg/day with liposomal AMB given at 4 mg/kg/day; this study found no difference in the overall response rate [89]. However, in the subset of patients with documented IA, the response rate was 58% and 37% in the 4 and 1 mg/kg/day groups, respectively.

Additionally, the optimal duration of therapy for IA is uncertain. Such therapy should be highly individualized with respect to the resolution of all symptoms and signs of the infection, radiologic near-normalization, negative

cultures, and ideally, restoration of the impaired immune defenses.

Among specific agents, the role of itraconazole in initial therapy for IA remains to be clarified. Most of the available literature describes the efficacy of oral itraconazole only in less heavily immunosuppressed patients having IA [76, 90], as intravenous (i.v.) itraconazole was only recently approved for use. The i.v. preparation of itraconazole offers the possibility of achieving rapidly and reliably therapeutic serum levels of the drug. In addition, there are recent encouraging data from an open-labeled multicentered European study of 31 cases of IA indicating that i.v. itraconazole followed by oral itraconazole is safe and results reliably in therapeutic levels and good response rates [91]. Intravenous itraconazole could be a viable option for primary therapy of IA, especially in clinically stable, less immunosuppressed patients. However, if there is concern about the bioavailability of itraconazole because of interactions with other medications given concomitantly, then another agent should be used, even in stable patients, unless the itraconazole level can be routinely monitored in a timely fashion.

The role of adjunctive surgery in the management of IA also has not been addressed in a conclusive way. Infarcts and tissue sequestration are common causes of antifungal therapy failure [92]. *Aspergillus* endocarditis, endophthalmitis, osteomyelitis, and arthritis are indications, even though the optimal timing of surgery is not clearly defined. Resection of infected pulmonary tissue is beneficial for some patients [93, 94]. Residual cavitary lesions, especially when they contain fungus balls, after successful antifungal therapy may cause late exsanguinating hemorrhage or reactivation of infection during subsequent myelosuppressive chemotherapy. Removal of these lesions, if surgically feasible, should be considered. Surgical intervention may be life-saving for acute pulmonary hemorrhage, even when performed early in the disease process [95]. It is less evident that early removal of well-circumscribed lesions close to pulmonary arteries is beneficial. Likewise, the value of late “debulking” of a pulmonary mass if the patient has multiple fungal lesions that cannot be completely resected is uncertain. Recent uncontrolled data suggest that resection of the lobe most adversely affected by IA could be beneficial in treating pulmonary lesions that worsened despite the use of intense antifungal therapy and stable or improving lesions if the patient is a candidate for high-risk BMT [59]. Surgery also plays a role in the management of *Aspergillus* sinusitis, but the extent of the procedure is poorly defined.

In addition, the availability of i.v. triazoles that are active against *Aspergillus* spp. (i.v. itraconazole; investigational triazoles, such as voriconazole, ravuconazole, and SCH59884) and a new class of antifungal agents that inhibit fungal cell wall synthesis, the echinocandins, caspofungin, FK463, and V-002 offers new therapeutic alternatives (Table 1) [96]. New investigational triazoles show impressive activity in vitro and in animal models of aspergillosis; early clinical experience suggests that they are also active in humans as both salvage [97, 98] and primary therapy for IA [99]. Their availability in oral form also allows their use in long-term therapy. However, in view of the increased and prolonged use of itraconazole, either prophylactically or therapeutically for IA, it is unclear whether cross-resistance would occur, thereby devitalizing this drug [80]. Echinocandins are static drugs in vitro against *Aspergillus* spp., but their activity in animal models and selected groups of patients with IA appears promising [96, 100, 101]. Finally, terbinafine, a squalene epoxidase inhibitor, has been shown to be efficacious against *Aspergillus* spp., and its use in combination with azoles has been shown to result in synergy in vitro. More of such studies are needed. Finally, emerging studies suggesting comparability of newer triazoles (e.g., itraconazole, voriconazole) with AMB-based regimens for refractory febrile neutropenia [102] as well as the ongoing comparison of echinocandins with liposomal AMB for refractory febrile neutropenia will evaluate the rate of breakthrough IA.

The lack of effective treatment of IA has made the concept of combination therapy for it theoretically appealing. Combinations of AMB with either flucytosine or rifampicin have been used despite conflicting in vitro and animal data. To date, no clinical studies have convincingly determined whether these combinations are more beneficial than therapy using AMB alone [103]. For instance, the sequence of administration of itraconazole in combination with AMB has produced a spectrum of responses ranging from synergy to antagonism. Furthermore, evidence from preclinical studies suggests that prior or concomitant administration of itraconazole produces antagonism [104]. With the recent introduction of echinocandins, which have a different mechanism of action (inhibition of cell wall synthesis), it is important to determine whether new combinations (e.g., azoles plus echinocandins, AMB plus echinocandins, terbinafine plus azoles, AMB plus azoles and echinocandins), given either concomitantly or sequentially, would result in additive or synergistic effects. For example, there is some preclinical evidence that echinocandins may augment the efficacy of AMB [105]. The sequence and timing of these combinations are important areas of future investigation. These new options, along with the expected routine implementation of CT and PCR/GM detection in the management of IA, will transform the management of this disease, modeling that of cytomegalovirus or other chronic diseases such as cancer (Table 2).

The difficulties in standardization of the in vitro microdilution-based susceptibility testing methods in filamentous fungi are well known [106]. This is a field still in its infancy, and the correlation between in vitro susceptibility testing and outcome in IA is still controversial [107, 108]. Both preclinical and clinical evidence suggest that some non-*fumigatus Aspergillus* spp., such as *Aspergillus terreus* [109] and possibly *Aspergillus flavus*, are less susceptible to AMB.

Table 1 New antifungal agents under clinical testing for the treatment of invasive aspergillosis

Antifungal	Trade name	Usual adult dosage	Mechanism(s) of action	Spectrum/comments
Polyenes				
Liposomal nystatin	Nyotran	0.25–4.0 mg/kg/day	Interaction with ergosterol, intercalation of fungal membrane, increased membrane permeability to univalent and divalent cations, cell death	Similar to lipid formulations of amphotericin B; frequency of nephrotoxicity is lower with lipid formulations
Itraconazole	Sporanox	200–400 mg i.v. q12 h×2 days, then q24 h	Selective inhibition of cytochrome P-450-14-α-demethylase, accumulation of lanosterol leading to perturbation of fungal cell membrane	Like with ketoconazole, drug interactions and poor absorption (capsules) are common causes of clinical resistance. Marked interpatient variability in serum levels secondary to variation in P450 genotype, which affects drug metabolism
Voriconazole		200 mg i.v. or p.o. q12 h	Similar to itraconazole	Active against invasive moulds, including *Fusarium* spp. Not active against *Zygomycetes*. Cross-resistance with fluconazole?
Posaconazole		200 mg p.o. q.i.d. ×7 days, then 400 mg b.i.d.	Similar to itraconazole	More active than itraconazole against invasive moulds, including *Aspergillus* and *Fusarium* spp., and possibly *Zygomycetes*
Echinocandins				
Caspofungin	Cancidas	70 mg i.v. day 1, then 50 mg q24 h	Inhibition of cell-wall glucan synthesis, leading to susceptibility of the fungal cell to osmotic lysis	Spectrum essentially limited to *Candida* and *Aspergillus* spp.
Micafungin (FK463)			Similar to caspofungin	Same as caspofungin
Anidulafungin (VER003)			Similar to caspofungin	Same as caspofungin
Allylamines				
Terbinafine	Lamisil	250 mg p.o. q.d.	Inhibition of squalene epoxidase, resulting in ergosterol depletion and accumulation of toxic sterols, fungistasis	Poor intrinsic activity against common moulds precludes use as monotherapy Exhibits activity in combination with azoles in the treatment of azole-resistant aspergillosis

Table 2 Future approaches in the management of invasive aspergillosis: a hypothetical scenario showing the increasing complexity that reflects increasing options for diagnosis and therapy

AD, antifungal drug; SM, biochemical surrogate marker; CT, computed tomography
[a] Alone or in combination

Setting	Interaction
Primary prophylaxis	AD-1, surveillance with SM
Empiric therapy	switch[a] to AD-2, combination of SM±CT
Pre-emptive therapy	increase dose of AD-2 vs. switch[a] to AD-3, SM+CT to assess response
Targeted therapy	AD-2 vs. AD-3 or AD-4, SM+CT to assess response
Induction	
Consolidation	
Maintenance	
Secondary therapy	oral AD, SM surveillance with SM

Finally, the role of immunomodulators in the management of IA remains unresolved [31]. As is the case with the other refractory opportunistic mycoses, in anecdotal clinical reports, the beneficial adjunctive role of immunomodulation using cytokines or the infusion of immune effector cells in various combinations (e.g., granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, γ-INF, granulocyte-macrophage colony-stimulating factor-primed white cell transfusions) in refractory or recurrent IA cases is only suggestive [31]. However, there is substantial preclinical evidence supporting the role of immunomodulation in the control of *Aspergillus* spp. Further studies are needed in this important area of clinical investigation.

Conclusions

Significant progress in the control of IA has been made, yet formidable challenges remain. Priorities for the future include further development of sensitive, specific diagnostic tests that detect infection early and reliably in immunocompromised hosts, additional investigation of immunorestoration, and continuous introduction of new antifungal agents for therapy and prophylaxis that provide broad-spectrum activity and efficacy despite deficient host defenses.

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