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# Trends in invasive fungal infections, with emphasis on invasive aspergillosis

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## Abstract

Patterns of invasive fungal infections are changing in many ways. Although yeast infections appear to have reached a stable incidence, the number of infections as a result of *Aspergillus* species appears to be increasing. Especially for mould infection, the diagnosis remains difficult and the detection and identification of clinically relevant isolates to the species level requires new validated techniques. Diagnostic tests are becoming more accurate, with biological markers such as PCR, galactomannan and 1,3 $\beta$-D-glucan undergoing clinical validation. This is of importance because an early diagnosis is associated with increased survival. Correct diagnosis and *in vitro* susceptibility testing are becoming imperative for guidance of therapy in the context of changing epidemiology and the emergence of acquired resistance to antifungal drugs, as is insight into host factors that increase susceptibility to invasive mould infection and into the risks associated with new treatment modalities of underlying diseases. Despite improvements in the survival rates of patients with invasive fungal infection in recent years, continued research is required to meet the challenges associated with changes in epidemiology and resistance development.

**Keywords:** Diagnosis resistance, host susceptibility, invasive *Aspergillus*, invasive fungal infection

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## Introduction

Invasive yeast and mould infections in immunocompromised patients (i.e. haematological, solid organ transplant or intensive care unit patients) have consistently shown a lower incidence in comparison with bacterial infections during the past decades [1,2]. Nevertheless, their burden is immense because of the high morbidity and mortality rates in infected patients. Although invasive yeast infections, primarily invasive *Candida albicans* infections, have shown a slight decrease in North American centres during the past decade, the incidence of non-albicans *Candida* infections and those caused by rare yeasts is increasing relatively [1]. Mould infections, especially as a result of *Aspergillus* species, are still increasing [3,4]. Better diagnostic tests and procedures, such as galactomannan detection and high-resolution computed tomography scans, together with the availability of more potent drugs have improved the prognosis for patients with invasive fungal diseases [3], yet the mortality rate has not decreased significantly [1]. However, improvements in the management of patients with invasive fungal infections come with a cost. Opportunistic fungi, other than *Aspergillus* and *Candida*, have emerged, with ensuing difficulties in their diagnosis and treatment. Acquired resistance to azoles and echinocandins has been reported, which also complicates the management of patients with invasive fungal diseases. With highly sensitive diagnostic assays, such as PCR, it may be very difficult to prove the presence of an invasive fungal infection because the detection of circulating fungal DNA is not necessarily associated with clinical manifestations of the fungal disease. Diagnostic tools can be adequately used only if the treating physician is aware of the propensity of patients to acquire a fungal infection. With the changing treatment modalities, new risk groups may emerge, which requires continuous awareness and education. For example, the recognition of patients with increased susceptibility to fungal infections as a result of inherited immunity anomalies, such as impaired NADPH-oxidase activity [5,6],

disturbed interleukin (IL)-10 or tumour necrosis factor (TNF)$\alpha$ production [7–9] and genetic polymorphisms in Toll-like receptors that result in defective production of inflammatory cytokines [10], is of importance. In addition, biological factors such as iron overload and age have also been shown to increase the risk of developing invasive fungal infections [11]. Historically recognized risk factors, such as corticosteroid use and neutropenia, along with myeloablative treatment regimens, further augment the aforementioned risk factors. However, even novel treatment modalities that allow less intensive conditioning, remain associated with invasive fungal infections [12]. In this review, we aim to discuss the changing factors related to the fungus and the host, together with their impact on patient management.

## The Fungus

The taxonomy of several fungi has changed in recent years because of an approach referred to as polyphasic taxonomy, which is based on analysis of macro- and micromorphology, extrolite profiles and $\beta$-tubulin, calmodulin, internally transcribed space (ITS) and actin gene sequences of the isolates [13]. The new taxonomy has a major impact on the number of species, especially within the genus *Aspergillus*. Within the medically important sections *Fumigati*, *Nigri* and *Nidulanti*, numerous new species were identified. For example, the section *Fumigati* (teleomorph *Neosartorya*) now contains 30 species that cannot be readily differentiated by macroscopic and microscopic features alone [13]. Cryptogenic species include *Aspergillus lentulus*, *Aspergillus pseudofischeri* and *Aspergillus udagawae*, and some of these can be differentiated from *Aspergillus fumigatus* by their inability to grow at 48°C. Therefore, sequence-based analysis is required to correctly identify isolates to the species level. Because ITS sequencing, which is commonly performed in clinical microbiology laboratories, does not discriminate among the cryptogenic species, a major change would be required to correctly identify the species. The clinical relevance of molecular identification to the species level remains unclear because the ability of most cryptogenic species to cause invasive fungal disease is presently unknown [14]. It can be expected that, in due course, commercial DNA-based assays will become available for molecular strain identification. However, because the cryptogenic species differ in their susceptibility to antifungal agents compared to *A. fumigatus*, it might be appropriate, in the meantime, to determine the *in vitro* susceptibility of clinically relevant isolates to guide antifungal therapy.

## Diagnostic Tools for Detecting Fungi Causing Infections

For all those involved in the care of patients with invasive mould diseases, the availability of validated, reliable and rapid diagnostic methods as well as effective treatment options comprise important issues [15]. With an adequate diagnostic armamentarium, we can limit the morbidity and mortality as a result of invasive mould infections. The importance of avoiding any delay in the initiation of adequate antifungal therapy on the outcome of patients with invasive fungal diseases has become evident both in invasive *Candida* infections [16] and in invasive aspergillosis [17]. Non-culture-based assays have been increasingly used to diagnose infections earlier, and the detection of galactomannan has become valuable despite the reported heterogeneity in the performance of the test [18]. Overall, galactomannan detection in serum is most useful in neutropenic patients with a haematological malignancy who are not exposed to antifungal prophylaxis active against moulds [19,20]. In this setting, prospective monitoring is sometimes used, which appears to be a feasible strategy, provided that the prevalence of invasive aspergillosis is high [21]. An important issue is whether the use of biological markers, in combination with improved imaging techniques, as a management strategy will help us to better identify those patients who require antifungal therapy. Studies that compare an empirical treatment strategy with such a diagnostic driven strategy indicate that, with a diagnostic driven strategy, the use of antifungal agents can be reduced without compromising the prognosis for the patient [22,23]. However, additional confirmative studies are warranted before such a diagnostic driven approach can become common practise. Indeed, different strategies probably need to be followed in the various treatment phases because the risk of invasive fungal infections differs during each phase.

In non-neutropenic patients, the value of the detection of circulating galactomannan is limited because of low sensitivity. In these patients, galactomannan detection in BAL-fluid might be more appropriate to diagnose invasive pulmonary aspergillosis [24].

Another antigen that can be detected is 1,3-$\beta$-D-glucan (BG), which is a cell wall component of many fungi, including *Candida* and *Aspergillus*. The detection of BG was included as mycological evidence of invasive fungal infection in the recently published revised definitions of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group [25]. Although the clinical

experience with BG detection in patients with invasive fungal infection is still limited, a possible role in the management of patients at risk of invasive *Candida* infection in the intensive care unit is currently being investigated [26].

Although the first PCR for the detection of *Aspergillus* was reported in the early 1990s, this technique is still considered as experimental more than 15 years later. *Aspergillus* PCR appears to have some very promising benefits and may complement properties of other biological tests, such as galactomannan antigen detection. *Aspergillus* DNA can be detected very early in patients with invasive aspergillosis, frequently in a phase devoid of clinical signs and symptoms of invasive fungal infection [27]. Also, exposure to antifungal drugs might improve the sensitivity of the assay as opposed to galactomannan detection, where the reverse is the case [28]. Although a clinically validated commercial format is still lacking, the platforms are becoming more automated and the extraction methods and targets are becoming commercially available. An international initiative that involves many investigators aims to devise a standard for *Aspergillus* PCR, which would subsequently allow clinical validation [29]. The standard for PCR should be available before the next revision of the EORTC/MSG definitions, so that it may be included as mycological evidence.

## Acquired Resistance

Although intrinsic resistance is known in the case of certain drug–*Aspergillus* species combinations, acquired resistance remains uncommon. Resistance has been reported to emerge in patients with an aspergilloma or with chronic aspergillosis who have been treated with itraconazole [30]. Resistance is most commonly associated with point mutations in the *Cyp51A* gene and most isolates exhibit a phenotype of cross-resistance [31]. In addition, resistance has been observed to have emerged in patients with acute invasive aspergillosis in the Netherlands, with 6–12.8% of patients harbouring a resistant isolate [32]. *A. fumigatus* isolates resistant to itraconazole and with reduced susceptibility to voriconazole and posaconazole were found and, in patients infected with resistant isolates, azole therapy was unsuccessful [33]. The azole-resistant isolates appear to be as virulent as wild-type isolates [32], and even cases of azole-resistant central nervous system aspergillosis were reported that were very difficult to manage [34]. Because a single highly dominant resistance mechanism was found in the Dutch clinical isolates, it was suggested that azole resistance might have developed through exposure to azole fungicides in the environment rather than in azole-treated patients [35]. Indeed, *A. fumigatus* isolates resistant to medical triazoles were cultured from the hospital indoor environment and from soil samples and compost. These isolates were found to have resistance mechanisms identical to those found in the majority of clinical isolates, and genotyping showed clustering of resistant isolates of clinical and environmental origin [35]. This suggests that patients with azole-resistant aspergillosis might acquire the isolate from the environment, which is also the primary route of transmission for azole-susceptible *A. fumigatus*. Furthermore, azole-resistant *A. fumigatus* may be spreading in the environment,

All of the above complicates the implementation of effective measures that prevent further dissemination. Although the clinical efficacy of voriconazole and posaconazole against azole-resistant aspergillosis remains unclear, the use of these azoles should be avoided in acute infection until more experience has been gained.

Elevated MIC values of echinocandins with occasional treatment failure have been reported for strains of *Candida*, but the relation between MIC and clinical response remains unclear [36]. However, resistance to echinocandin drugs among clinical isolates was associated with amino acid substitutions in two 'hot-spot' regions of Fks1, the major subunit of glucan synthase [36,37]. The Fks1-mediated resistance mechanism is conserved in a wide variety of *Candida* species and can account for intrinsic reduced susceptibility of certain species. Fks1 mutations confer resistance in both yeasts and moulds, and sporadic *A. fumigatus* isolates have been found that are resistant to echinocandins. However, in some phenotypic caspofungin-resistant isolates, no mutations were found in the Fks1gene, indicating that other, yet unknown, mechanisms are present [38,39]. Of note is that caspofungin (i.e. echinocandins) may play a pivotal role in a possible synergy between the antifungal drug and the host immune system [40]. Echinocandins can induce (even at low concetrations) morphological changes in hyphae of *A. fumigatus*, which, as a concequence, increase glycan $\beta$ exposure, resulting in an increased Dectin-1-mediated inflammatory response by macrophages, as well as in enhancement of the activity of polymorphonuclear neutrophils (PMNs) [41,42].

Given the limited number of evidence-based treatment options in invasive candidiasis and invasive aspergillosis (Table 1), the loss of a class of antifungals, as a result of acquired resistance, significantly complicates patient management. In the coming years, priority should be given to monitoring of the extent and spread of (azole) resistance in opportunistic fungi, to the determination of interpretative breakpoints for all clinically used antifungal agents, and to the development of antifungal compounds that are active against new targets.

**TABLE 1. Licenced therapeutical indications of systemic antifungals and invasive infections**

| | Conventional amphotericin B | Liposomal amphotericin B | Amphotericin B lipid complex | Caspofungin | Anidulafungin | Micafungin | Fluconazole | Voriconazole | Posaconazole |
|---|---|---|---|---|---|---|---|---|---|
| Class | Polyene | Polyene | Polyene | Echinocandin | Echinocandin | Echinocandin | Azole | Azole | Azole |
| Prophylaxis | | | | | | *Candida* infection in patients undergoing allogeneic HSCT or patients expected to have neutropenia for >10 days | *Candida* infection in patients undergoing HSCT | | Invasive *Candida* and *Aspergillus* infections in patients receiving chemotherapy for acute myeloid leukaemia or myelodysplastic syndrome, and HSCT patients with GVHD undergoing high-dose immunosuppression. |
| Empiric therapy | | In febrile neutropenia | | In febrile neutropenia | | | | | |
| Primary therapy | Invasive fungal infections including aspergillosis, cryptococcosis, North American blastomycosis, systemic candidiasis, coccidioidomycosis, histoplasmosis, zygomycosis, and infections as a result of related susceptible species of *Conidiobolus* and *Basidiobolus*, and sporotrichosis | Cryptococcal meningitis | | Candidaemia in neutropenic and non-neutropenic patients and other *Candida* infections | Candidaemia and other *Candida* infections in non-neutropenic patients | Invasive candidiasis | Invasive candidiasis and other invasive-*Candida* infections in non-neutropenic patients<br>Cryptococcal meningitis | Invasive aspergillosis<br>Fluconazole-resistant *Candida* infections<br>Candidaemia in non-neutropenic patients<br>Infections caused by *Scedosporium apiospermum* and *Fusarium* spp. | |
| Salvage | | *Aspergillus*, *Candida* or cryptococcal infection refractory to conventional amphotericin B therapy | Treatment of invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B therapy | Treatment of invasive aspergillosis refractory to/intolerant of other therapies | | | | | Invasive aspergillosis, fusariosis, chromoblastomycosis, coccidioidomycosis in patients refractory to or intolerant of first-line therapy with other antifungal agents. |

HSCT, haematopoietic stem cell transplantation.
The decision to use Mycamine should take into account a potential risk of the development of liver tumours. Mycamine should therefore only be used if other antifungals are not appropriate. EMEA: http://www.emea.europa.eu/humandocs/Humans/EPAR/mycamine/mycamine.htm.

## Host-Related Factors Predisposing to Mould Infections

Several well described and established factors have been defined that predispose patients for invasive mould infections, such as invasive aspergillosis. Some of them, such as neutropenia as a result of meyloablative therapy in acute leukaemia or allogeneic haematopoietic stem cell transplantation (HSCT), are ubiquitous in their consequence of increasing the risk of mould infections. Although yeast infections are often acquired through disruption of the integrity of the mucosal barrier, mould infections are primarily caused by inhalation of conidia and, consequently, the lungs are frequently the primary site of infection. Yet, the intestine also has been reported to be a possible site of invasive *Aspergillus* infection [43], especially in chronic graft-versus-host disease (GvHD), or in patients with mucosal barrier injury related to the conditioning regimen.

## Genetic Factors Predisposing to Invasive Aspergillosis

Germination of conidia is the first step in the pathogenesis of invasive aspergillosis. One of the backbones of the host defence that may prevent *Aspergillus* invasion is formed by early recruitment of PMNs to the site of infection [6]. Together with alveolar macrophages, their role is essential in exertion of the NADPH-oxidase activity within PMN aggregates to prevent hyphal proliferation and tissue invasion. Defective oxidant production within PMN aggregates largely contributes to host susceptibility to invasive aspergillosis. This can explain why neutropenia, defective NADPH-oxidase and corticosteroids, which delay the recruitment of PMNs, are important predisposing factors for invasive mould infection [5,6].

In addition, other host-related factors (e.g. the production of IL-10 and TNF$\alpha$) have a role in the development of invasive mould infections such as invasive aspergillosis. IL-10 acts as a major regulatory cytokine of inflammatory responses by controlling the balance between inflammatory and humoral responses [44]. It operates by impairing the antifungal effector function in phagocytes and the secretion of proinflammatory cytokines in macrophages, T-cells, neutrophils and dendritic cells [44,45]. Single nucleotide polymorphisms in the IL-10 gene promoter were found to be an independent predictive factor for the development of invasive aspergillosis in a study with patients treated by allogeneiec HSCT [8]. In that study, patients with the ACC haplotype (associated with decreased IL-10 production) had a nine-fold lower risk of developing invasive aspergillosis compared to control patients with unaffected IL-10 production. Among those with the ATA haplotype (associated with increased IL-10 production), a significantly higher incidence of invasive aspergillosis was observed. Because the precise role of IL-10 is currently not yet fully understood, further studies are required to determine the definite value of these observations.

High levels of proinflammatory cytokines, such as TNF$\alpha$ and lymphotoxin $\alpha$), are required for adequate control of an invasive fungal infection. Because 60% of the variation in TNF$\alpha$ production is considered to be genetically determined, this factor may explain in part the inter-individual differences in the risk of patients undergoing similar immunosuppressive treatment regimens to develop invasive fungal infection (i.e. in addition to factors related to exposure to the fungus). In HSCT recipients, genetic differences in the TNF$\alpha$ receptor type 2 promoter have also been shown to have a pivotal role in host susceptibility to invasive aspergillosis [9]. Another defect in the innate immunity mechanism can also increase host susceptibility to invasive mould infection. Toll-like receptors are transmembrane proteins on the surface of immune cells that interact with several adapter proteins to activate transcription factors, resulting in the production of inflammatory cytokines and activation of the adaptive immunity [10]. Because *Aspergillus*, once present in the human body, activates innate immune cells through Toll-like receptors 2 and 4, the absence or weakness of this signal by epithelial cells of recipients or phagocytic cells from the stem cell donor can lead to an increased risk of acquiring invasive aspergillosis. Single nucleotide polymorphisms in Toll-like receptors 4 haplotype S4 in unrelated donors of HSCT recipients were associated with increased host susceptibility to invasive aspergillosis. Similar observations were reported in a single study investigating the presence of polymorphisms in Toll-like receptors 1 and 6 [46]. Polymorphisms in this area of the innate human immunity system are also considered to be associated with an increased risk of invasive yeast infections such as invasive candidiasis [47]. Another factor that was shown to be associated with increased susceptibility to invasive aspergillosis is polymorphism in the plasminogen gene [48].

The number of different host-related genetic factors predisposing to an invasive fungal infection is high and increasing (Table 2), although their specific and additive roles are yet to be fully understood.

## Biological Factors

Along with the aforementioned direct host-related factors, iron overload, which can be acquired during the course of

**TABLE 2. Host-related factors that influence the susceptibility to invasive fungal infection, and possible underlying mechanisms**

| Genetic factors | Mechanism |
|---|---|
| Defective oxidant production within PMN Aggregates | Impaired neutrophil recruitment |
| SNPs in IL-10 promoter gene | High IL-10 levels with consequently impaired cytokine production |
| TNFα receptor 2 gene promoter disturbance | Impaired (mainly too low) TNFα levels with consequently impaired control of infection |
| SNPs in Toll-like receptors 4 haplotype S4. Polymorphism in Toll-like receptors 1 and 6 | Impaired immune signal at time of infection. Defect in production of inflammatory cytokines |
| Polymorphism in the plasminogen gene | Impaired immune response at time of infection |

IL, interleukin; PMN, polymorphonuclear neutrophil; SNP, single nucleotide polymorphism; TNF, tumour necrosis factor.

treatment of the underlying disease, can lead to increased host susceptibility to fungal infections. This has been shown for myeloma patients [11] and for HSCT recipients [49]. The mechanism is considered to act through the increased availability of iron for fungal proliferation and the negative effects of iron overload on the antimicrobial functions of neutrophils, monocytes and natural killer cells. Among other biological host factors, older age is also increasingly recognized as a risk factor for several serious infections because this group of elderly patients is demographically becoming larger. In a multivariable analysis, age was found to be an independent risk factor for invasive mould infections in HSCT patients, in contrast to other biological factors such as acidosis and hyperglycaemia that did not increase the risk [50].

The trend to manage high-risk patients in the outpatient setting presents new challenges for the early diagnosis and treatment of invasive fungal infections. Because intensive diagnostic monitoring is commonly precluded in the outpatient setting, antifungal prophylaxis, with, for example, posaconazole, may be a feasible approach for high-risk patients. Exposing large patient groups to antifungal drugs such as the azoles may have negative consequences with regard to the development of resistance, toxicity or drug interactions. However, increased insight into the role of genetic and biological host factors concerning the risk of developing invasive fungal infection may help us to target only those patients who are at greatest risk. Recognition of genetic or biological factors is therefore needed in the pre-selection period in the case of transplant recipients or early in the course of therapy because the impact of these factors has been shown to be significant [51]. Efforts directed towards discovery and validation of new strategies that incorporate these factors should be undertaken.

## Treatment-related Factors Predisposing to Invasive Fungal Infections

Among immunocompromised patients, invasive mould infections occur most often in the setting of haematological diseases. This is especially related to aggressive treatment regimens, resulting in prolonged periods of cytopenia and the use of high doses of corticosteroids. Also in patients who receive less myeloablative regimens, such as the novel purine antagonists and antibody therapies, the risk of developing invasive fungal infection remains significant [52,53].

Patients who have survived invasive aspergillosis have an increased risk of the fungal disease relapsing during subsequent immunosuppressive treatment episodes. Martino *et al.* [54] performed a study in which a comparison was made between reduced, as opposed to conventional (i.e. intensive), conditioning regimens in patients with proven previous invasive mould infection. Despite the retrospective design of this study, significant differences in relapse rates were observed. The augmentation of the risk of a relapse of invasive aspergillosis was noted, especially during the first 30 days after a transplantation [54]. In addition, cytomegalovirus (CMV) disease, cord blood or bone marrow stem cells as a source of the transplant, and severity of GvHD were found to be important factors in relation to the risk of relapse of invasive aspergillosis. Throughout the entire treatment period, the status of the underlying disease, the duration of neutropenia, as well as duration of previous antifungal therapy (i.e. from start of therapy until the day of transplantation), determined the likelihood of the reactivation of invasive aspergillosis. The latter is of importance because it suggests that a certain total dose and duration of antifungal therapy is needed for an invasive fungal infection to be under control. In this study, voriconazole was found to show a trend towards an effective prophylaxis against relapse of invasive fungal infection, especially as a result of moulds. When the results of this retrospective study were set in a risk assessment model (except for the intensity of conditioning), this model proved to accurately predict the probability of invasive aspergillosis relapse. Together with the aforementioned validated diagnostic techniques, such as galactomannan antigen testing, this model should be investigated further.

Other, time-dependent, biological host factors were evaluated for their role in the risk of acquisition of invasive mould infection in HSCT-recipients by Garcia-Vidal *et al.* [51]. Notably, the majority of invasive mould infections (of which 87% were invasive aspergillosis) occurred late after HSCT (i.e. more than 40 days after transplantation). Factors predis-

posing to early occurrence of the infection were the existence of underlying disease and were related to the type of transplant, such as unrelated or mismatched HSCT along with conditioning with ATG. An increased incidence of invasive mould infection during the early phase of immune reconstitution was associated with hyperglycaemia, lymphopenia and the number of blood transfusions as marker for iron overload. Interestingly, the number of blood transfusions was associated with a higher incidence of mould infection during the early as well as late phase, but ferritin, a more accurate marker of iron overload, was not found to be associated with increased proneness to invasive mould infection. For the period beyond 40 days after transplantation, as in other studies, the severity of GvHD, the presence of CMV disease, the high number of transfusions, and receipt of high doses of corticosteroids determined the risk of an invasive mould infection [52,55,56].

## The Impact of Novel Treatment Strategies Used for Haematological Diseases

Concerning the novel therapies, alemtuzumab, an antibody directed against T-cells, was associated with an increased incidence of opportunistic infections. In the first cohorts of alemtuzumab-treated patients, invasive aspergillosis appeared to be the most frequent opportunistic infection [53]. This was seen both in patients treated solely with alemtuzumab as well as in those given alemtuzumab as part of the conditioning regimen of an allo-HSCT. Subsequent reports suggested that especially patients treated with alemtuzumab, for rejection of the transplant or as salvage therapy, were at highest risk of an opportunistic fungal infection [52,57]. Of note, in most HSCT-recipients treated with alemtuzumab, invasive fungal infection tended to occur relatively late (i.e. more than 1 year after transplantation). Similar results were reported with fludarabine, a purine antagonist, which allows for a less myeloablative conditioning regimen. Despite the possible advantage of a less intensive conditioning, fungal infections, together with CMV disease, remained significant clinical problems [12,58].

Although less myeloablative conditioning in HSCT is preferred because it may diminish the risk of early infectious complications, including invasive fungal diseases, one should be aware that, even long after these procedures, the cellular immunity remains impaired, with a consequent continued risk of invasive fungal infection [12].

## Conclusions

Basic research and clinical trials have increased our understanding of the interplay between the fungus and the host (Fig. 1). More accurate diagnostic tools and effective antifungal drugs have improved the prognosis for patients at high risk of invasive fungal diseases. However, our efforts are threatened by resistance development and shifts in the epidemiology of fungal pathogens towards less common moulds. It would be unrealistic to expect that, in the near future, we will overcome the morbidity and mortality associated with

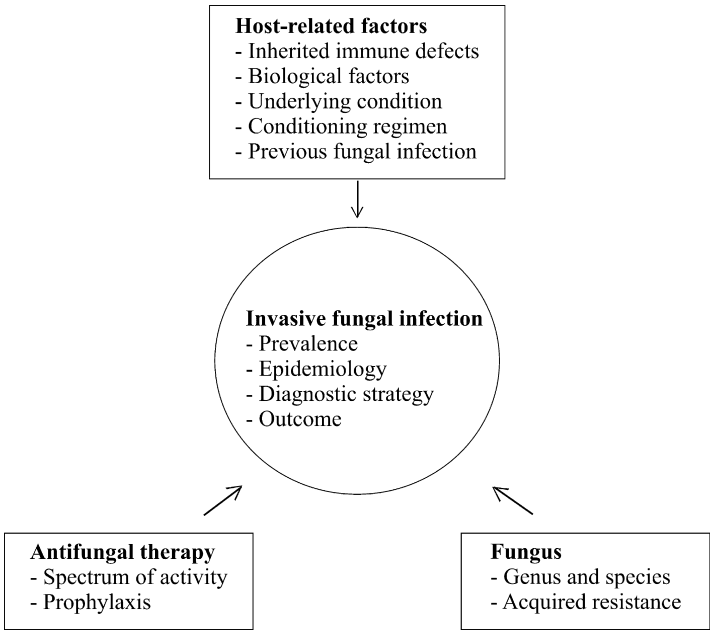


**FIG. 1.** Interplay among factors related to the host, the fungus and antifungal drugs.

invasive fungal infections. By contrast, every effort needs to be made to continue improving the survival of patients who suffer from fungal complications of immunosuppressive therapies.

## Transparency Declaration

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