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# Invasive Fungal Infections in Patients with Acute Leukemia and Hematopoietic Stem Cell Transplant Recipients

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## 1. Introduction

Patients with acute leukemia (AL) and hematologic stem cell transplant (HCT) recipients are at increased risk for the development of invasive fungal infections (IFI) due to prolonged neutropenia and severe immunosupression. The incidence of IFI in these patients has increased in recent years [1], which likely reflects changes in clinical practice including escalation of treatment intensity; more frequent use of unrelated, mismatched or alternative donors as stem cell source; rising number of multiple transplantations; and more frequent use of T-cell depleted grafts [2-3]. IFI are associated with high morbidity and mortality in the patients with AL and represent a leading cause of infectious mortality in allogeneic HCT (allo-HCT) recipients. Therefore, effective preventive strategies, accurate diagnostic techniques and optimal treatment approaches are required for an optimal management of high risk patients with AL and HCT recipients.

## 2. Clinical syndromes

Clinical symptoms of IFI are often non-specific, therefore a high index of suspicion in high risk patients is necessary for timely diagnosis and prompt treatment. Diagnosis of the IFI could be challenging, particularly in severely immunocompromised patients as a result of blunted inflammatory response due to profound neutropenia and altered T-cell function; hence, in such patients clinical and radiologic findings may be very subtle. In the case of clinical suspicion of IFI aggressive pursuit of a specific microbiologic diagnosis with early recognition and prompt initiation of antifungal therapy are the key components of optimizing treatment outcomes. The most common clinical signs are the symptoms of local or generalized inflammatory response, primarily persistent fever despite the use of broad spectrum antibiotics. Other symptoms significantly depend upon the involved anatomic site and causative pathogen. For example, for mold pathogens, lungs and less commonly sinuses are involved; accordingly, the most common symptomatology include dyspnea, cough, atypical chest pain, hemoptysis, rash, facial pain, nasal congestion and visual symptoms such as periorbital pain, blurred vision and proptosis.
Among numerous potential pathogens *Candida* and *Aspergillus spp.* are the major causative fungi leading to life-threatening infectious complications in AL and HCT recipients [4].

Invasive Candidasis

*Candida* fungemia and invasive Candidasis (IC) often occur during breakdown of mucosal barriers because *Candida spp.* are ubiquitous colonizers of mucosal surfaces [5-6]. Skin was previously considered as major source for dissemination of *Candida spp.,* however as it was recently demonstrated endogenous Candida spp. could frequently disseminate from a patient's gastrointestinal tract [6]. IC occurs more frequently in patients with GVHD and is often associated with the presence of indwelling venous catheters, particularly if they are used for the administration of parental nutritional supplementation.

The most common manifestation of IC is persistent and unexplained fever. In contrast to other fungal pathogens, *Candida spp.* are often identified in the blood by fungal blood cultures, but 50% of patients with autopsy-proven IC had no positive blood cultures isolated prior to death [7]. Hematogenous dissemination may lead to endophthalmitis, which is often associated with visual changes and pain [8]. The lesions of endophthalmitis are due to the inflammatory response to the *Candida* organisms, and thus cannot be seen in neutropenia; it most commonly occurs after neutrophil engraftment and frequently is associated with only subtle retinal lesions, which can be detected by indirect ophthalmoscopy. During hematogenous dissemination *Candida spp.* can invade the skin, joint, muscles and renal tubules, which clinically present as maculopapular skin lesions with a tendency to ulcerate, polyarthralgias, polymyalgias and azotemia [9]. Right upper abdominal discomfort, elevated transaminases, particularly alkaline phosphatase in the persistently febrile patents shortly after neutrophil recovery may indicate the presence of hepatosplenic candidiasis [10]. Disseminated *Candida* can cause central nervous system (CNS) syndromes, including meningitis and encephalitis. In HCT recipients who developed brain abscesses, *Candida spp.* accounted for 33% of all cases [11]. Very rarely *Candida spp.* can cause fungal endocarditis, which is the most serious manifestation of IC with symptoms similar to those of bacterial endocarditis.

Mold infections

Despite being ubiquitous, molds are unable to effectively invade tissues in immunocompetent individuals; thus, invasive mold infections (IMI) such as Aspergillosis, Zygomycosis, and Fusariosis almost exclusively develop in high-risk patients with prolonged neutropenia or in severely immunosuppressed patients [12-13]. Nosocomial IMI among patients with AL or HCT recipients primarily develop as a result of spore transmission via inhalation or direct contact [14]. Opportunistic molds, particularly *Aspergillus and Fusarium spp.* can possibly disseminate by a contaminated water, therefore, water might be a potential source of IMI in the treatment facilities [15]. *Aspergillus spp.* account for the majority of mold fungal infections, primarily mold pneumonias. *Zygomycetes* are indentified in 10% to 20% of mold pneumonias, *Scedosporium spp., Fusarium spp.,* and other molds are responsible for a very small percent of cases [16].

In the case of invasive Aspergillosis (IA), when infection spreads beyond the respiratory tract, patients often develop signs of systemic inflammation and multiorgan failure. In such cases, hematogenous dissemination can lead to involvement of the eye, sinuses and abdominal organs; however *Aspergillus spp.* may involve virtually any organ including CNS. When involvement of the sinuses occurs, IA often clinically resembles Zygomycosis (mucormycosis) of the sinuses. Profoundly immunocompromized patients can occasionally develop gastrointestinal IA from swallowed organisms that subsequently invade the gut

mucosa, which can present with gastrointestinal bleeding or nonspecific signs of an acute abdomen and fever and might be confused with GVHD in allo-HCT recipients or neutropenic eneterocolitis in severely neutropenic patients [17-18]. A very serious complications occur when fungal invasion directly spreads to central nervous system involving various anatomic structures of the brain [19] or to ethmoid sinus with a farther advance to cavernous sinus [20]. Hematogenous dissemination to the brain can present as a clinical presentation of a cerebral infarction. In HCT recipients with brain abscesses *Aspergillus spp.* were causative pathogens in 58% of cases and in the majority of cases brain abscess in these patients were associated with concomitant pulmonary disease [11].
Endocarditits is another serious complication of IA and primarily happens in patients with prosthetic heart valves or prolonged fungemia due to fungal colonolization of the central venous catheters [21]. Symptoms are similar to those of bacterial endocarditis: persistent fever and thromboembolic complication due to septic emboli. Blood cultures are usually negative, in contrast to classical bacterial endocarditis and diagnosis is usually made by demonstration of hyphae in the embolus with a subsequent culture of the organism. Prognosis of these patients is very poor despite an intensive medical and surgical treatment with a mortality approaching 100% [22]. Endophthalmitis and cutaneous septic lesions by *Aspergillus spp.* are similar in appearance to those caused by disseminated Candidasis.
*Zygomycetes* typically enter through the sinuses, lung parenchyma, skin, and gastrointestinal tract with a tendency to disseminate in immunocompromised individuals and clinically resemble IA. A distinctive feature of *Zygomycetes* is their propensity to invade blood vessels and cause thrombosis leading to subsequent necrosis of involved tissues.
Paronychia is often caused by bacteria or yeasts; however, in severely immunocompromised recipients it can be caused by *Fusarium spp.* or other molds and lead to life-threatening IFI [16].

## 2. Epidemiology

Knowledge of the epidemiology of IFI is crucial for development of optimal prophylactic approaches and effective therapeutic strategies for patients with AL and HCT recipients. The epidemiology of IFI has changed significantly over the last two decades. *Candida spp*. were by far the most common causative fungi more than twenty years ago, however the incidence of IMI, particularly IA is increasing. The apparent change may, in part, be due to control of *Candida* by fluconazole prophylaxis in high risk patients.
After its peak in 1980's when *Candida spp*. became the fourth-commonest cause of bloodstream infection in the USA [23], the epidemiology of IC dramatically changed. Before the widespread use of antifungal prophylaxis the incidence of IC in HCT recipients was as high as 25%, but after the introduction of fluconazole prophylaxis in high risk patients the incidence of IC has been steadily decreasing [24]. This phenomenon is mostly caused by a reduction in incidence of *C. albicans* [25], whereas the incidence of azole resistant *Candida spp.* such as *C. glabrata* [26] and *C. krusei* [27] has risen. Analysis of multi-institutional surveys from Europe showed that between September 1997 and December 1999 in patients with hematological malignancies *C. albicans* was responsible for only 35% of cases of candidemia. The majority of cases were caused by non-albicans *Candida spp*, predominantly *C. tropicalis* and *C. krusei* in 24% and 12% of cases respectively [28]. Another retrospective study from a single center in Brazil showed that during a 9-year period (from 1995 to 2003) non-albicans *Candida spp.* were responsible for as high as 79% of all candidemia episodes in

patients with hematological malignancies [29]. Therefore, despite the fact that azole prophylaxis led to a significant reduction in the incidence of IC in high-risk patients, azole-resistant species such as *C. krusei* or *C. glabrata* could be responsible for breakthrough infections [30-31].

The epidemiology of *Aspergillus spp*. in the US is also evolving. Similar to the changing incidence of *Candida* infections, it is likely that the prophylactic use of agents with anti-mold activity is responsible for a change in epidemiology of IA. Over the last 15 years the incidence of IA has tripled in allo-HCT recipients in one large transplant center [32]. If earlier epidemiologic data showed that around 90% of all IA cases were caused by *A. fumigatus* [32], the recent multicenter report demonstrated that in HCT recipients the proportion of IA caused by *A. fumigatus* has decreased to 56% with a relative increase in the incidence of other *Aspergillus spp.* including *A. flavus, A. terreus, A. niger* and *A. versicolor* [33].

In recent years previously rare molds have been emerging as important pathogens in high risk patients. There is a rising incidence of IFI caused by *Fusarium spp., Rhizopus spp., Scedosporium spp.,* although the absolute numbers of these infections are still relatively low [32]. In allo-HCT recipients the incidence of both *Fusarium spp*. and *Rhizopus spp.* infections doubled in the period 1985–1999 and was accounting for 18 % of all IMI [32]. It was noted that in some centers an increase in incidence of Zygomycosis infections occurred after the introduction of voriconazole for prophylaxis and treatment of IFI [34]. Although, data from 25 US transplant centers over a 3-year period showed very low incidence of *Zygomycetes* (less than five cases per 1,000 transplants) with no increased incidence after introduction of voriconazole [35].

## 3. Risk factors

Risk factors for IFIs in patients with leukemia and HCT recipients depend on both causative pathogens and host factors. Since the most common fungal pathogens are opportunitistic, they capable of causing life-threatening infections almost exclusively in immunosuppressed host and the IFI risk directly depends on the duration of neutropenia and severity of immunosuppression. Patients with AL are considered high risk for IFI if prolonged (>7 days duration) neutropenia (absolute neutrophil count <100 cells/mm3) following cytotoxic chemotherapy is anticipated and/or significant medical co-morbid conditions such as hypotension, pneumonia, new-onset abdominal pain, or neurologic changes are present. The same criteria apply for HCT recipients alongside with additional risk factors, mainly graft versus host disease (GVHD) and prolonged immunosuppressive therapy. In ASCT recipients significant alterations of humoral and cellular immunity typically resolve within 3 months, therefore, for such patients the risk for IFI beyond that time is minimal. Immune reconstitution happens slower after allo-HCT and usually approaches normality by 1 year if GVHD does not develop. The occurrence of chronic GVHD significantly impairs immune reconstitution by requiring prolonged and often intensive immunosuppressive therapy. These patients remain at a high risk for IFI if chronic GVHD persists and requires continuing immunosuppressive therapy.

Neutropenic patients with AL and HCT recipients are at a risk for IC, particularly in the absence of antifungal prophylaxis. The risk of IC in both AL patients and HCT recipients also depends on the duration of neutropenia, severity of mucosal injury, and the presence of a central venous catheter [36]. Patients with neutropenia are also at risk for IMI,

particularly IA. The underlying disease itself and treatment intensity directly influence the risk of AI; patients with AML have the highest risk for IA with an incidence of approximately 20 times greater than that among patients with lymphoma and multiple myeloma [37]. The precise risk for IA in AML patients varies in different published series, however in most studies it ranges between 5 and 10%, depending on the disease status, duration of neutropenia, and the types of anti-neoplastic agents used for a leukemia treatment [37-42], where patients undergoing chemotherapy for relapsed or refractory leukemia are at greatest risk, whereas patients undergoing an induction chemotherapy for a newly diagnosed leukemia are at a lower risk and those, who are receiving consolidation therapy are at lowest risk. In HCT recipients the risk for IA significantly depends on the type of transplant (ASCT versus allo-HCT); conditioning regimen (myeloablative, non-myeloablative, reduced intensity); stem cell graft source (related, unrelated, haploidentical, umbilical cord, HLA matched or mismatched, T-cell depleted); post-transplant interventions (salvage chemotherapy, prolonged treatment with glucocorticoids and anti-rejection medications); and the development of post-transplant complications, particularly GVHD [43-45]. Overall risk for IA is low in ASCT represents (1–2%) because there is only a brief period of immunosupression and neutropenia in these patients [46]. In allo-HCT recipients, the risk for IA is substantially higher with a trimodal incidence distribution [32, 47-48]. The first peak occurs during the pre-engraftment period where the main risk factor is prolonged neutropenia which is similar to neutropenic patients with leukemia who did not receive transplantation. The second peak occurs between 2 to 3 months after the allo-HCT in patients with acute GVHD being treated with corticosteroids. The third peak occurs after one year post-transplant in patients who continue to require systemic immunosupression for extensive chronic GVHD. Late onset (41–180 days) occurrences of IA were more common in recipient of mismatched or unrelated donor HCT; in patients who received T-cell depleted or CD34-selected stem cell products; in patients receiving corticosteroids; in patients with neutropenia, lymphopenia, GVHD, CMV disease; and in patients with respiratory virus infections. Other factors such as iron overload and a toll-like receptor 4 polymorphism are recognized as independent risk factors for IA in HCT recipients [49-50].
Among less frequent IMI, Zygomycosis tends to develop relatively late, usually after 90 days post HSCT and is more commonly seen in patients with underlying MDS, chronic GVHD and patients receiving treatment of GVHD [32]. Alternatively, severe, but rare infections with *Scedosporium spp,* occurring early, usually in neutropenic AL patients and during the pre-engraftment period in HCT recipients [51].

## 4. Diagnostic approaches

To improve outcomes of high risk patients with AL and HCT transplant recipients it is critical to establish the diagnosis of IFI early, but currently there is no single diagnostic method that has a sufficient sensitivity and specificity to determine IFI. Therefore, timely diagnosis of IFI should be made on the basis of a constellation of clinical signs, confirmatory imaging studies and laboratory findings.

Imaging studies.

Even minor abnormalities on chest radiographs (CXR) in high risk patients should prompt further investigation, which often includes computed tomography (CT) of the chest. When suspicion of invasive pulmonary Aspergillosis is high, it is very important to pursue CT

scanning of the chest, because CXR may be negative in up to 10% cases of invasive pulmonary Aspergillosis, whereas only 3% CT scans are falsely negative [52]. If possible, images need to be compared with prior ones to exclude over interpreting persistent abnormalities, which are not related to acute infection, such as scarring or other tissue changes caused by previous radiation or administration of chemotherapy. The pulmonary infiltrates due to IMI are generally nodular. CT imaging findings that are more specific radiologic signs of IA are early on a "halo sign" when the central nodular area of fungal invasion is surrounded by a ground-glass appearing hemorrhage and a "crescent sign" may occur later as a result of necrosis and cavitation of lung tissue. In a large multicenter study, most patients with documented IA had one or more dense nodules and nearly two thirds had a halo sign [53]. The chest CT defines the extent of the disease process with a greater accuracy than CXR and can also be used repeatedly to monitor the response to therapy. Better visualization of lung tissue on CT scan also helps to define optimal sampling sites and to select the most appropriate invasive diagnostic procedure, such as with bronchoalveolar lavage with or without transbronchial biopsy, image guided needle biopsy, video-assisted thoracoscopic or open lung biopsy. Importantly, during therapy lung infiltrates and clinical symptoms may appear or get worse with immune reconstitution and resolution of severe neutropenia when an inflammatory response develops at the site of tissue damage by an invasive fungal pathogen. It was demonstrated that initiation of therapy directed against Aspergillus when a halo sign has been identified resulted in significantly improved survival [54-55].

Laboratory approaches.

*Candida spp.* are often detected in peripheral blood of patients with IC, however, blood cultures have a low sensitivity for the diagnosis, being negative in up to 50% of cases with autopsy-proven disseminated Candidiasis [56]. Blood cultures can also be positive for a variety of other fungal pathogens, but blood cultures are practically never positive in the case of disseminated Aspergillosis or Zygomycosis [7].

*Aspergillus* was isolated from sputum in only 8% to 34%, and from BAL in 45% to 62% of patients with invasive Aspergillosis [57]. In case of pulmonary Zygomycosis, it is poorly isolated from sputum, with positive cultures <25% and the yield of BAL is not higher [58]. Fungal element identification in tissue of the presumed site of infection can be considered as a "gold standard" however, possibility of colonization of the respiratory secretions by environmental fungi may lead to over diagnosis. Tissue biopsies in the case of visceral fungal infections are frequently difficult to obtain due to critical condition of the patient and concomitant severe thrombocytopenia. If there is cutaneous involvement, punch biopsy of skin lesions are often required to establish the diagnosis. For a suspected fungal infection special stains for fungal organisms, such as the Gomori methenamine silver stain, are necessary because routine histologic stains may often not be sufficient to visualize the tissue invasion by fungi.

Detection of yeasts or fungal elements (hyphae) in the tissue by microscopic examination should be followed by a subsequent culture to identify a specific organism, since morphology is not sufficiently specific. *Aspergillus* is characterized by narrow, septated hyphae with acute angle branching and, in comparison with other fungi, it grows relatively rapidly with a visible growth within 3 days if inoculum size is adequate. The hyphae of *Fusarium* in tissue resemble those of *Aspergillus*. *Zygomycetes* characteristically are pauciseptate, wider, ribbon like in contrast to *Aspergillus* in tissue. Positive cultures of tissue

of most molds, but especially Zygomycosis is challenging given the difficulty of extracting fungal elements from infected tissues [59]

Because of numerous limitations related to culture techniques, adjunctive non-cultural methods such as galactomannan (GM) antigen, b-(1-3)-D glucan (BG) and PCR-based methods are now being implemented for a timely diagnosis of IFI, primarily IA, particularly when tissue sample is not obtained [60-63]. If PCR-based techniques are still considered investigational [63], the clinical value of serum GM and BG assays was confirmed in prospective trials. Both tests now are accepted as supplementary diagnostic tests for an early detection of common IFI in high risk patients. A positive GM test preceded the development of clinical symptoms of IFI in the majority of patients in studies [60]. The BG has high sensitivity and specificity with a capability to detect many clinically relevant fungal pathogens including *Candida spp, Aspergillus spp, Pneumocystis spp,* and *Fusarium spp,* but not *Zygomycetes spp* or *Cryptococcus spp.* [62, 64]. Its strength is the ability to detect a variety of the most common relevant fungal pathogens in AL and HCT patients; its weakness is its inability to determine which pathogen is present. In patients with AML and MDS receiving chemotherapy the negative predictive value of BG approached 100%; a single positive test and two or more sequentially positive results had the specificity of 90% and > 95% respectively [64]. The role of BG assay in HCT recipients has not been well studied [65] and requires further investigation.

The serum GM assay detects *Aspergillus* and *Penicillium spp.* (a rare pathogen in AL and HCT patients). GM is a major component of *Aspergillus* cell walls and released during a rapid growth of hyphae. The serum GM assay can identify the presence of fungal growth in tissues during invasive infection and often quite early even before the development of any clinical signs, radiologic abnormalities or fungal growth in culture. Detection of GM antigen by ELISA in the serum has been widely retrospectively and prospectively studied with a wide range of reported sensitivities depending on the study population and definitions of the test positivity [60, 66-74] . The GM assay had only moderate accuracy in the patients with hematologic malignancies with a pooled specificity of 58-70% and sensitivity of 92-95%, and in HCT recipients a pooled specificity of 65-86%- and sensitivity 65-82% [75]. The predictive accuracy of GM is negatively affected by a concomitant use of piperacillin-tazobactam [76] and amoxicillin-clavulanate [77] due to the presence of GM in the antibiotic formulations, and a false positive GM signal may be detected up to 5 days after discontinuation of these medications. The use of antifungal prophylaxis with agents having anti-mold activity could be associated with falsely negative GM tests [67]. False positivity of GM assay was also described in case of infection with *Penicillium spp.* [78] and *Histoplasma capsulatum* [79] because these microorganisms share cross reactive antigens.

GM can also be detected in other body fluids during IA infection involving those fluids or adjacent tissues, including urine, CSF and bronchoalveolar lavage (BAL) [80-82]; however, clinical utility of the assay obtained from other sources than serum is under clinical investigation. Preliminary data has suggested that detection of GM in BAL fluid [83] could be an reliable adjunct test with a specificity and sensitivity exceeding of those in BAL fungal culture [84-85] and some studies suggest BAL GM to be more sensitive than serum GM [86]

PCR assays to detect and identify fungal pathogens are being developed and ongoing clinical testing, but not yet available for a commercial use [74]. Although development and clinical implementation of PCR assays for fungal species such as *Aspergillus spp.* holds an enormous promise, multiple obstacles, particularly widespread environmental fungal contamination and difficulties in DNA extraction, must be overcome before PCR-based techniques will be clinically useful [87-89].

## 5. Treatment and prevention

There are four common antifungal strategies used in current clinical practice to combat IFI. These include antifungal prophylaxis, empirical therapy, pre-emptive (or diagnostics-driven) therapy and treatment of proven and probable IFI.

Antifungal prophylaxis

The goal of antifungal prophylaxis is to prevent IFI with an administration of the antifungal agent(s) in high risk patients. Antifungal prophylaxis is a rapidly evolving field. Prophylaxis is considered clinically beneficial when the risk of a life-threatening IFI outweighs the risks of toxic effects and drug interactions, and the risk for emergence of drug resistance associated with the antifungal agent used. Optimally, antifungal prophylaxis should also be cost-effective. Therefore, the choice of empirical antifungal treatment is considered based on the prevalence of the most likely fungal pathogens, along with considerations about toxicities, resistance, and cost.

The most widely used agent for IFI prophylaxis in patients with AL and HCT recipients is fluconazole, an agent with activity against most (but not all) *Candida spp*.. Prospective studies demonstrated reductions in IFI caused by yeast organisms when fluconazole was given prophylactically at the dose of 400 mg daily in allo- HCT and ASCT recipients [90-91]. In one trial prophylaxis with fluconazole resulted in a statistically significant reduction of IFI to 3% as compared to 16% in the placebo group [90]. Administration of fluconazole up to engraftment was able to effectively prevent IFI caused by *Candida spp.* with the exception of *C. krusei.* Although there was no difference in overall mortality in patients who received prophylaxis with fluconazole as compared to those who received placebo, infection related mortality was significantly reduced [90]. In another trial [91], patients who received fluconazole prophylaxis during the first 75 post-transplant demonstrated a statistically significant clinical benefit with a reduction of IFI from 18% to 7%, a decrease in infection-related mortality from 22% to 12%, and improvement of overall mortality from 35% to 20% as compared to placebo group [91]. The enduring benefit of fluconazole prophylaxis in HCT recipients even beyond the time the drug was given was noted in a follow-up analysis of the Slavin study [92]. Based on such benefits fluconazole at a daily dose of 400 mg was widely accepted as a standard of care for IFI prophylaxis in high risk HCT recipients, endorsed by consensus guidelines [93]; however controversy remains regarding the optimal duration of prophylaxis and whether to stop it at the time of engraftment or continue until GVHD resolves and its treatment has ceased. It is also not completely clear if patient groups who at a lesser risk for a development of IC than recipients of myeloablative HCT, such as patients with AL, ASCT and non-ablative transplant recipients derive similar clinical benefit from fluconazole prophylaxis.

Another widely studied agent for antifungal prophylaxis in high risk patients is itraconazole, which has activity against *Aspergillus spp.* in addition to *Candida spp.* [94]. Comparison of itraconazole to fluconazole as a long term prophylactic agent (until day 100 or 180 post-transplant) in HCT recipients showed no difference in overall survival [95-96]; in one of the trials a reduction in the incidence of IFI in itraconazole arm was noted [95] but not in the other [96]. Despite its attractive anti-mold activity, the lack of proven clinical advantage over fluconazole [96-98], suboptimal bioavailability [95], higher mortality rate [95-96] and a higher incidence of adverse events, particularly more renal and hepatic toxicities [96] associated with itraconazole administration make this agent suboptimal for

routine prophylaxis of IFI in high risk patients. In a meta-analysis of itraconazole trials, an *Aspergillus* protective effect was noted in patients given a bioavailable daily dose of at least 200 mg twice daily (typically seen when itraconazole is given as oral solution at a twice daily dose of 200mg per dose [94] .

Voriconazole is a well-tolerated triazole with an extended-spectrum of activity including activity against *Aspergillus spp.*. A large multicenter, randomized trial compared voriconazole with fluconazole for prevention of IFI after myeloablative allo-HCT [99]. In that trial patients were randomized to receive antifungal prophylaxis with study drugs for 100 days or for 180 days if patients were considered high risk. All patients were intensively monitored with twice weekly serum GM assays for initial 60 days, then at least weekly until day 100. In the case of positive GM or clinical signs suggestive of IFI patients were followed with radiographic studies and invasive diagnostic procedures for confirmation of IFI. Despite statistically non-significant trends to fewer IFI, particularly IA, and less frequent use of empiric antifungal therapy in voriconazole arm, the primary endpoint- fungal-free survival (alive and free from proven, probable, or presumptive IFI patients) at 6 months was similar in both groups. In addition, there was no difference in incidence of severe adverse events, relapse-free and overall survival at 6 months. Thus, prophylaxis with voriconazole did not improve 6-month fungal-free and overall survival as compared to fluconazole if intensive monitoring and a structured approach for empiric antifungal therapy were used [99]. Voriconazole is available in both intravenous and oral formulations, which allows its extended use to prolonged periods of high risks for the development of IFI. One of the major drawbacks of voriconazole is the lack of activity against *Zygomycetes*. Although multicenter studies with voriconazole [35, 99-100] showed no increased incidence of IFI caused by *Zygomycetes* in patients received voriconazole prophylaxis, there are single center retrospective case series suggesting an association of voriconazole use with more Zygomycoses. Thus, this issue bears further scrutiny over time. This is quite difficult because of the difficulty in culturing *Zygomycetes* in tissue. Echinocandins have a potential advantage over fluconazole given the broader spectrum of activity, in particular, against fluconazole resistant *C. krusei* or *C. glabrata*, and the anti-*Aspergillus* activity, although the latter has not yet been proved in clinical trials. A prospective comparison of micafungin to fluconazole for antifungal prophylaxis in neutropenic patients undergoing HCT showed compatible efficacy, safety profile, overall survival and reduced the need for empiric antifungal therapy in patients receiving micafungin [101]. The major disadvantage of micafungin use for a fungal prophylaxis is the need for intravenous administration and substantially greater cost; however this agent might be preferred over fluconazole in the centers with high prevalence of infections caused by non-albicans *Candida spp.*

Posaconazole is a triazole with a broad spectrum of activity against both yeasts and molds. It is active against pathogens such as the *Zygomycetes* which are resistant to many widely used antifungal agents. Such a broad spectrum of activity makes this agent an attractive alternative to fluconazole for prophylaxis of IFI. Posaconazole is approved for prophylaxis in neutropenic patients with AML or MDS based on the results of randomized multicenter studies where it was shown to be superior to fluconazole or itraconazole in the prevention of IFI [102]. In that study prophylactic administration of posaconazole resulted in improved overall survival, although at the cost of higher incidence of serious adverse events attributable to study drug [102]. Since significant numbers of the patients in the posaconazole group were receiving chemotherapy there was a possibility that greater toxicity of the posaconazole may have resulted from a harmful interactions with

chemotherapy agents. This has not been adequately studied. In patients with GVHD posaconazole was similar to fluconazole in prevention of all types of IFI and, not surprisingly given its anti-mold activity, superior in reducing the incidence of IA and mortality related to fungal infections as compared to fluconazole [30]. Based on these results posaconazole was approved in the US for antifungal prophylaxis in high risks patients, however this agent has not been studied in the pre-engraftment phase of HCT.

Development of extensive chronic GVHD significantly increases the risk of late IFI (more than 100 days post-transplant), predominantly IA, with a reported incidence as high as almost 40% in one series [103]. Therefore, anti-mold prophylaxis with posaconazole in patients with extensive chronic GVHD could be beneficial in these patients.

Amphotericin B (AmpB) is not currently used for prophylaxis of IFI due to its excessive toxicity and infusion-related events. However, recently aerosolized AmpB was prospectively tested in patients with AL and allo-HCT recipients and led to reduction in the incidence of invasive pulmonary Aspergillosis; however administration of the drug was interrupted in a substantial proportion of patients (45%) due to cough during inhalation, weakness preventing the use the aerosol delivery system and technical problem with the aerosol delivery system. Moreover, aerosol dose and delivery device have not yet been determined, therefore further study is necessary before the clinical utility of this agent can be determined [104].

The risk for IFI is substantially lower in the majority of ASCT recipients as compared to allo-HCT recipients. These patients usually do not require routine anti-yeast prophylaxis. However, for high risk sub-populations of ASCT recipients such as patients with underlying hematological malignancies, history of prolonged neutropenia, significant mucosal damage, and treatment within fludarabine or 2-CDA within 6 months prior ASCT ant-yeast prophylaxis is recommended [15].

Empirical treatment

Empirical treatment is an initiation or modification of an existing antifungal treatment in high risk patients with persistent fever of unknown source unresponsive to antibacterial agents. Although, approximately one-third of neutropenic patients with cancer receive an antifungal drug due to persistent fever, only less than 5% of these patients subsequently demonstrate the presence of IFI [102, 105-107]. Therefore, initiation of empirical antifungal therapy solely on the basis of persistent fever can be legitimately questioned.

The most common fungal pathogen in neutropenic patients is *Candida*. Anti-yeast prophylaxis dramatically reduces the risk for IC. However, in persistently febrile neutropenic patients not given antifungal prophylaxis IMI or fluconazole-resistant *Candida* infections represent the most common threats. Oral, non-absorbable antifungal drugs, such as oral AmpB, nystatin and clotrimazole troches are capable of reducing superficial colonization and control local mucosal candidiasis, but, surprisingly, do not reduce the risk for IC.

There is not enough data for recommendations regarding a particular empirical antifungal agent for patients who are already receiving anti-mold prophylactic coverage, however changing treatment to a different class of intravenous anti-mold agent should be considered, given the possibility that breakthroughs of fungal infection could be caused by resistant organisms. Another concern is that breakthrough IFI may be due to inadequately low serum levels of voriconazole or posaconazole when these agents administered orally because of the variability of blood levels after HCT [108-109].

For more than 30 years AmpB has been used for an empirical antifungal treatment, but with development of newer agents, its therapeutic role in the current management of fungal infections is minimal, due to excessive toxicity, particularly nephrotoxicity and infusion-related events. Alternative formulations of AmpB such as liposomal AmpB, AmpB colloidal dispersion, AmpB lipid complex; azoles with anti-mold activity such as itraconazole or voriconazole, and caspofungin are widely used because of better tolerability and reduced systemic toxicity, however there are no data that have demonstrated their superior efficacy to AmpB [105, 107, 110-112].

Voriconazole is recognized by many experts as a suitable alternative to a liposomal AmpB as an empirical antifungal therapy in patients with neutropenia and persistent fever [106] and currently widely used for an empirical therapy for high risk patients, particularly for an empirical treatment of probable IMI.

Pre-emptive therapy

The need for empirical antifungal therapy in febrile high risk patients have been questioned because of the very low percentage of such fevers that are actually due to IFI [42]. The advances in our ability for early detection of fungal infections have ushered in a new strategy, pre-emptive therapy. Pre-emptive treatment refers to therapy of highly suspected IFI supported by the presence of clinical symptoms, radiological findings and/or adjunctive laboratory tests [54-55, 113].

The feasibility of preemptive antifungal therapy in high risk patients with the use of serial GM testing and early CT scans was prospectively evaluated in 131 neutropenic episodes. All patients were given fluconazole prophylaxis to eliminate *Candida* as a possible cause of persistent fever. Forty one of 117 episodes (35%) met the criteria for empirical therapy persistent or recurrent fevers [55]. Use of preemptive approach in such patients was associated with a 78% reduction (from 35% to 8%) of antifungal therapy without a compromise in identifying (and treating) IFI. Indeed, early detection allowed for initiation of antifungal therapy earlier with the pre-emptive strategy. The clinical utility of preemptive antifungal therapy was also evaluated in a prospective study, which included patients with AML receiving induction or consolidation therapy, ASCT recipients and in other patients with prolonged neutropenia, however, allo-HCT recipients were excluded [114]. In that study, clinical symptoms, chest CT findings indicative of IFI, documented *Aspergillus* colonization or a positive GM were used to initiate a preemptive therapy with either AmpB or liposomal AmpB, depending on renal function. Although there was no difference in overall mortality, probable or proven IFI were more commonly detected in preemptive treatment arm than in the empirical treatment one (13/143 vs. 4/150). An increase in IFI was seen only in the subset of patients who did not receive antifungal prophylaxis and the increase was due to an increased incidence of IC [114]. This emphasizes the need for anti-*Candida* prophylaxis, if the screening testing is designed primarily for detecting *Aspergillus*.

In another study PCR-based preemptive antifungal therapy was compared with an empirical treatment with liposomal amphotericin B in patients after allo-HCT [113]. PCR-based approach led to an increased use of anti-fungal therapy and reduced 30-day mortality, but there was no difference in the incidence of IFI or 100-day survival.

These observations suggest that some of high risk neutropenic patients with persistent fever may not need an automatic empirical antifungal therapy if they receiving anti-yeast prophylaxis and closely monitored under certain specific conditions [114-117]. However, if

laboratory, clinical or radiologic abnormality suggestive of probable fungal infection is identified, prompt initiation with of antifungal therapy with an agent that includes anti-mold activity is needed.
Despite being an attractive alternative, the preemptive approach is currently regarded as investigational by many and not widely accepted although its use is embraced and its promise is discussed in consensus guidelines [118]

Treatment of established IFI

Early recognition and treatment of invasive fungal infections in AL and HCT recipients are important clinical challenges. Early initiation is a key to optimizing treatment outcomes for both *Candida* and *Aspergillus* [119-121]. The European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC-MSG) has proposed criteria to define proven or probable IFI [122]. New antifungal agents offer choices that in some cases are less toxic than older drugs and in other cases are more efficacious. Novel strategies that combine the new diagnostic tools with new drugs, are being evaluated to change our approaches to these deadly infections [123]

Treatment of IC.

Candidemia is associated with a high mortality, especially in patients with delayed treatment, therefore antifungal therapy in patients with IC should be started as soon as possible [119]. If a central venous catheter(s) is in place and suspected to be causative for IC, it should be removed. Although AmpB was historically the preferred therapy with excellent activity against most *Candida spp.,* lipid amphotericin formulations were just as effective with less toxicity. Other agents are also effective treatment options. Multiple studies, conducted mostly in non-neutropenic patients and patients with solid malignances, demonstrated a high efficacy of fluconazole in patients with candidemia [119]. In patients with IC fluconazole was found to be non-inferior and significantly better tolerated then AmpB [119] IC, but less effective than anidulafungin IC [124]. Although well tolerated, there are gaps in its spectrum of activity with *C. krusei* being intrinsically resistant and many strains of *C. glabrata* requiring higher fluconazaole concentrations for inhibition and some strains being outright resistant. Despite the fact that anidulafungin was mainly studied in non-neutropenic patients, better efficacy [124] and emergence of fluconazole-resistant *Candida spp.* such as *C. krusei* or *C. glabrata* [125-126] make the echinocandins preferable choices for the initial treatment of IC in patients with AL and HCT recipients. After the confirmation of susceptibility of *Candida spp.* to fluconazole clinically stable patients without prior azole exposure can be stitched to fluconazole. Initial preferred dose of fluconazole is 800 mg a day with a subsequent reduction to 400 mg a day in clinically stable patients with resolved neutropenia [127]. In a large randomized study caspofungin showed compatible efficacy and significantly better side effects profile in comparison to AmpB in neutropenic patients with candidemia [128]. Given its good safety profile and efficacy against non-albicans *Candida spp*. caspofungin is indicated in severely ill, clinically unstable neutropenic patients or patients with hepatosplenic Candidasis [129]

Treatment of IMI

Voriconazole is the preferred initial agent for the treatment of IA [130] . It is effective and well tolerated both as primary and salvage treatment [131]. A large randomized study showed a higher response and survival rate with fewer *Aspergillus*-related deaths and side effects with voriconazole compared to AmpB for primary therapy of IA [115].

Although there is a very limited data regarding posaconazole activity as a first line agent in the treatment of IA as an initial agent of choice, substantial evidence supports its activity in the salvage setting after a failure of or intolerance to other antifungal treatments [132]. There are few data regarding clinical role of caspofungin as a first-line treatment for IA, but it is effective with a response up to 50% as a salvage agent in patients with IA who failed or intolerant to standard antifungal therapy [133]. The role of micafungin or anidulafungin for the treatment of IA has not been well studied but are active as salvage therapy.

Efficacy and safety of AmpB lipid complex (ABLC) as first-line or second-line therapy was demonstrated in large numbers of patients with hematologic malignancy or HCT recipients [134]. Another study showed that one third of allo-HCT recipients with IA responded to ABLC, with a response of 41% and 21% when ABCL used as a first line treatment and in patients with GVHD respectively [135]. In both studies administration of ABLC had only minimal effects on renal function in the majority of patients. Liposomal AmpB also has been shown to be effective as salvage therapy with fewer infusion related and renal toxicities. Two doses of liposomal AmpB were evaluated (10mg/day versus 3 mg/day) to determine the optimal dose for initial therapy. Both doses had comparable response rates but the 10 mg/day dose was associated with more renal toxicity [136]

The treatment options for Zygomycosis are more limited and less well studied. Patients with Zygomycosis showed a good response to high doses of liposomal AmpB (dose at least 5 mg/kg) [59] or ABLC [137]. In the case of CNS or sinus involvement additional surgical resection of necrotic tissue in patients with Zygomycosis significantly appears to be associated with improved survival as compared to antifungal therapy alone [137-138]. Posaconazole is an effective agent with response rates of about 50–80% when uses as a salvage treatment in patients with Zygomycosis [139-140].

Therapeutic Drug Monitoring

Therapeutic drug monitoring has not been found to be useful for the polyene and echinocandin classes of antifungal drugs. However, the oral formulations of the extended spectrum triazoles have been found to have variability in blood concentrations and multiple drug interactions. Multiple studies have explored whether these variations have clinical consequences in terms of efficacy. Itraconazole oral formulations have been seen to have variable bioavailability in multiple studies [141-145]. Higher doses appear to have greater antrifungal effects in both animals and humans [141, 145-148] . Voriconazole , when taken orally also has been shown to havevariable bioavailability, especially in HCT recipients [149-151].Voriconazole is metabolized in the liver via the cytochrome P450 dependent mechanism, predominantly by CYP2C19 isoenzyme which exhibits genetic polymorphism resulting in reduced drug metabolism in 15–20% patients of Asian descent, and 3–5% of Caucasians and Blacks [150]. Low voriconazole levels have been have been reported in patients with documented breakthrough infections, whereas super therapeutic levels may lead to hepatotoxicity and encephalopathy [150-151]. Less information is known about posaconazole, but it too has been shown to have variable bioavailability [152-156]. There is some suggestion that low levels may be associated with lower effectiveness [156-157].

Controversy remains regarding the use of routine monitoring of azole drug levels, however most experts agree that measurement of levels should be performed in patients with documented breakthrough infection or infections not responding to oral triazole therapy. A change to, or addition of, different class antifungal agent is recommended until the determination of the drug blood level. If drug level is found to be sub-therapeutic, resuming the drug at a higher dose should be considered.

Combination antifungal therapy

Combination antifungal therapy may theoretically lead to better efficacy with shorter courses of therapy, reduced toxicity and emergence of resistance. However potential drawbacks of combination therapy might include potential antagonistic effects of the antifungal drugs used in combination, potential increase in toxicity, and greater cost may offset the potential value of this approach [158]. A randomized trial demonstrated that in non-neutropenic patients with candidemia due to species other than *C. krusei* combination therapy with fluconazole and AmpB resulted in a faster clearance of fungi from the bloodstream as compared to fluconazole alone [159]. Based on this result combination of fluconazole and AmpB could be recommended as an option for the treatment of IC [160], however the data regarding combination antifungal therapy for candidemia in neutropenic patients with leukemia and HCT recipients is lacking. Small retrospective studies showed better responses to the combination of caspofungin plus liposomal AmpB for primary IA infection [161] or for refractory pneumonia [162]. Another small retrospective study compared the voriconazole plus caspofungin combination to a single-agent voriconazole. In this study an improved 3-month survival in HCT recipients or patients receiving chemotherapy with IA who had failed primary therapy with the combination, but there was no difference in survival at 1 year [163]. Thus currently there is no data supporting a benefit of combination therapy in patients with IA.

## 6. Conclusion

Over the past two decades, significant progress has been made in the prophylaxis, diagnosis and treatment of IFI in patients with AL and HCT recipients. Introduction of newer antifungal agents, better supportive care and more effective diagnostic tools resulted to a considerable improvement of clinical outcomes for such high risk patients. However, despite advances in prevention and management of systemic fungal infections, IFI remain a significant clinical problem for AL patients and HCT recipients. Further progress should be made towards improvements in diagnostic techniques, development of novel antifungal drugs and introduction of immunotherapy approaches for high risk patients. Future efforts should also be focused on better understanding of fungal immunobiology and implementation of personalized therapy based on immunologic, metabolic and genetic profiles of high risk patients.

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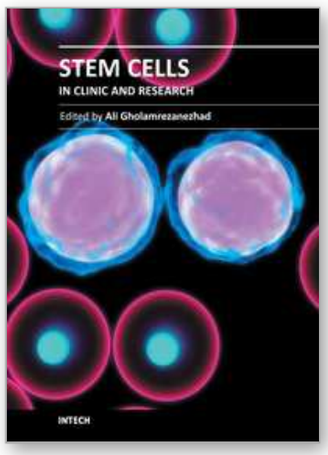


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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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