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Background.

As experience with bone marrow transplantation has improved, larger numbers of patients are undergoing transplantation. As a result, the population of acutely and chronically immunosuppressed patients has increased dramatically, leading to an increased awareness of the role of invasive fungal infections (IFIs) in this population. Most Canadian transplant centers have seen increases in rates of invasive fungal infections, with some institutions reporting rates as high as 20% in high risk allogeneic stem cell transplant recipients. These changes in epidemiology, combined with the availability of new diagnostic tests and antifungal agents have led to the development of a number of management strategies for IFIs. This document will review recent changes in the epidemiology, diagnosis and management of IFIs, focused on the HSCT population.

Epidemiology

Overview: During the 1990s and into the 2000s, transplant centers in the United States and Europe reported an overall decline in the incidence of invasive Candida infections in HSCT and hematological malignancy populations, attributable in large part to the widespread use of fluconazole in prophylaxis. Unfortunately, this trend was accompanied by a significant increase in the number of mold infections, predominately invasive aspergillosis, but also including other more uncommon organisms such as zygomycetes and fusarium. Interestingly, a significant portion of these infections were diagnosed in non-neutropenic patients more than 90 days following transplant. Subsequent epidemiologic studies identified graft versus host disease as an important risk factor for IA, both directly and as a consequence of the immunosuppressive regimens used in treatment.

Rates of IFI: The poor sensitivity and specificity of traditional tests available for diagnosis of IFI has led to underestimation of incidence rates for these infections. Autopsy studies have suggested that even in the modern era, up to 50% of IFI are not diagnosed antemortem, even with the advent of more sensitive diagnostic tests. Despite these limitations, several centres in North America have published incidence data in HSCT patients. A retrospective study of patients undergoing allogeneic bone marrow transplantation in Seattle, Washington found

Timing of IFI	Risk Factor
Early	Prolonged, severe neutropenia Prior IFI
Late	Prior IFI GVHD (particularly intestinal) Alemtuzumab* TNF inhibitor use* Steroid use >* MUD Repeat transplant

an incidence of invasive mold infection of 13.1%, with the majority of these infections attributed to *Aspergillus* (2). A similar retrospective study of patients undergoing non-myeloablative (NMA) bone marrow transplantation in Boston reported a 10.1% incidence of invasive aspergillosis, which was the most common infectious cause of mortality in this population (1). Canadian data is more scarce has mirrored these results. A retrospective review of NMA patients from Maisonneuve-Rosemont transplant program reported an incidence of IA of 15% by 3 years after transplantation, (3) while physicians from Vancouver reported an IA rate of 18.8% post allogeneic transplantation between 2006-2008 (W Ma et al., Poster presentation, ASH 2009) The rate of IA at the McGill University Health Centre was found to approach 26% in a recent one year audit. Further, the mortality of IFI in these cohorts was high, ranging from 60-65%. Collectively, these data suggest a significant burden of disease due to IFI in allogeneic stem cell transplant populations within Canada.

Identification of high risk patients: The availability of larger cohort data in HSCT patients has identified a number of factors that are associated with increased risk of IFI. The classic risk factor of prolonged neutropenia remains the most important consideration for early IFI in the period <30 days post transplantation. However, late IFIs have been associated with a number of other factors as detailed in Table 1.

IFI Diagnosis

Traditional methods: The diagnosis of IFI remains a clinical challenge. Blood cultures are of uncertain sensitivity for the diagnosis of Candidiasis, and of no utility for the diagnosis of mold infections. Respiratory cultures are insensitive for the diagnosis of invasive mold infections, and cannot distinguish contamination or colonization from true infection.

New Diagnostics: The introduction of new diagnostic tests offers some help with this dilemma, but has not resolved this issue. High resolution CT scanning is a very sensitive technique for the identification of pulmonary pathology, but is not specific for IFI. Several non-culture based fungal diagnostics have been developed including the Galactomannan EIA, B-D-glucan assay, and *Aspergillus* PCR. Of these tests, only the Galactomannan test is readily available in Canadian centers.

CT scanning: A number of abnormalities on pulmonary CT have been linked with IFI. The most classic of these is the "halo sign" which refers to a dense nodular consolidation surrounded by an area of ground glass opacification. Later in the course of disease, the "air crescent" sign is seen in which an area of cavity formation occurs inside a nodular density. This is thought to represent necrosis, often develops in association with recovery of leukocytes after chemotherapy. Single and multiple nodules without "halo" changes are also common indicators

of fungal disease. Unfortunately, none of these are specific for IFI and can be seen with other conditions. Further, IFI can present atypically with consolidation, pleural disease or “tree in bud” changes, adding to the challenge of interpreting CT scan findings

Galactomannan (GM) EIA: Galactomannan is a carbohydrate component of the cell wall of *Aspergillus fumigatus*. The GM EIA is predominately directed at detecting this organism, although it may cross react with other *Aspergillus* sp and other fungi, notably *Histoplasma capsulatum*. The test is commercially available and approved for testing of serum samples, but has also been extensively evaluated on bronchoalveolar (BAL) fluid. In serum testing the sensitivity and specificity varies according to the patient population, with a recent meta-analysis finding the overall sensitivity of GM testing in all studies to be 71%. Sensitivity is highest in neutropenic patients, where it approaches 80%. In non-neutropenic patients the sensitivity drops significantly, in the range of 30-50%. Specificity is good, with false positives reported due to dietary GM and other fungi. Notably, false positives related to use of B-lactam antibiotics were commonly reported; this seems to have been principally due to GM contamination of piperacillin-tazobactam. Changes in the production of this product have greatly diminished this issue. Sensitivity and specificity of the serum GM test is maximized through use of serial testing strategies, and demonstrating a reproducible and sustained rise in GM index that exceeds the cutoff level of 0.5. Several studies have examined the use of GM testing of BAL fluid in the diagnosis of IA. The performance characteristics of the GM test are greatly improved on BAL fluid with reported sensitivities as high as 90-95% in some studies, although most authorities recommend the use of a cutoff index of higher than 1.0 for positivity.

B-d-Glucan test (BDG). B-glucan is also a principle carbohydrate component of the fungal cell wall. B-glucan is produced by both *Candida* sp. and *Aspergillus* sp, and as a result the test is not useful for differentiating between different IFIs. The BDG test utilizes an enzymatic detection method, and requires a dedicated technology platform, and specialized collection techniques to avoid contamination with environmental B-glucan. It is not currently approved, nor is it widely available in Canada. Data on the performance characteristics of the BDG test are more limited, in part due to more demanding technical requirements of the tests. Published studies to date suggest that the BDG test is slightly more sensitive than GM for the diagnosis of IA, but at the expense of reduced specificity.

Aspergillus/Candida PCR – these tests remain primarily research tools, as there is no standardized, commercial test format with the exception of the recently licensed *Mycognotica* IA real-time PCR kit which is for use on BAL specimens only. Unfortunately, clinical experience with this test has not found improved performance characteristics as compared with the BAL galactomannan assay.

Diagnostic criteria for IFI: Given the limitations in the diagnostic tools for IFI, several organizations have formulated consensus criteria for the diagnosis of IFI. Although these classifications were developed primarily to allow for uniform recruitment in clinical studies, they have also been used for the development of antifungal algorithms. Using this approach, patients are evaluated based on their risk group, clinical features, and microbiologic results in order to classify any putative IFI into possible, probable or proven disease. These classifications are summarized in Table 2.

Proven	<ul style="list-style-type: none"> • Histopathology demonstrating disease <i>and/or</i> • Growth in culture from tissue biopsy or aspirate from a sterile site
Probable	<ul style="list-style-type: none"> • Presence of 1 host factor criterion <i>and</i> • Presence of 1 clinical feature* <i>and</i> • Microbiological evidence <ul style="list-style-type: none"> • Culture or microscopy from sputum, BAL or sinus • Positive GM from blood, BAL or CSF or B-glucan from blood
Possible	<ul style="list-style-type: none"> • At least 1 host factor criterion <i>and</i> <ul style="list-style-type: none"> ▪ Neutropenia ▪ Persistent fever despite antibiotics in high-risk patients ▪ Signs and symptoms of GVHD • Prolonged corticosteroid use • One clinical feature*

Clinical features*

Lower respiratory tract fungal disease: The presence of 1 of the following 3 signs on CT:

- Dense, well-circumscribed lesions(s) with or without a halo sign
- Air-crescent sign
- Cavity

Tracheobronchitis: One of the following

Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis

Sinonasal infection: Imaging showing sinusitis plus at least 1 of the following 3 signs:

- Acute localized pain (including pain radiating to the eye)
- Nasal ulcer with black eschar
- Extension from the paranasal sinus across bony barriers, including into the orbit

CNS infection: 1 of the following 2 signs:

- Focal lesions on imaging
- Meningeal enhancement on MRI or CT

Disseminated candidiasis: At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks:

- Small, target-like abscesses (bull's-eye lesions) in liver or spleen
- Progressive retinal exudates on ophthalmologic examination

Licensed Antifungal Agents

Azoles – The azole class of antifungals act through the inhibition of ergosterol biosynthesis, an essential sterol component of fungal cell membranes. The first generation triazoles include fluconazole, and itraconazole. Fluconazole is a water soluble molecule with excellent bioavailability and can be given IV and po. It is active against yeast but has no significant activity against molds. In contrast, itraconazole is a highly lipophilic molecule with erratic absorption, which distributes extensively into most tissues and has intrinsic anti-*Candida* and anti-*Aspergillus* activity. The cyclodextrin solution of itraconazole is preferred for improved absorption to be taken without food. Two second generation triazoles, voriconazole and posaconazole are now available, and have a broader activity against molds. Voriconazole, a moderately lipophilic molecule is available both IV and po, and is the treatment of choice for invasive aspergillosis. Posaconazole, like itraconazole, is highly lipophilic and is currently only available in an oral suspension. Posaconazole has the broadest spectrum of activity of the azoles. Azoles are subject to a number of drug interactions through inhibition of hepatic cytochrome P450 enzymes, often necessitating alterations in dose of concomitant medications. Therapeutic failures with azoles are also observed when given concomitantly with metabolic inducers such as phenytoin or rifampin.

Polyenes. Amphotericin B deoxycholate (AMB) and the lipid-associated formulations of amphotericin B (L-AMB) bind directly to fungal ergosterol where they form pores in the cell membrane. When administered IV, amphotericin compounds produce significant infusion and renal toxicity which is attributed to AmB binding to cholesterol in human cell membranes. These side effects are somewhat alleviated with the lipid formulations via the use of the lipid carrier which is intermediate in affinity for AmB, between cholesterol and ergosterol. Amphotericin B compounds remain the broadest spectrum agents available.

Echinocandins. The echinocandins inhibit the production of B-1,3-glucan, an important component of the fungal wall. There are three currently available members of this class: caspofungin, micafungin and anidulafungin. All are available in IV formulation only. These drugs are extremely well tolerated with minimal drug-drug interactions, and have both anti-*Candida* and anti-*Aspergillus* activity. Clinical experience is greatest with caspofungin, although there is no evidence to date suggesting meaningful differences in their activity.

Management strategies.

A variety of strategies have been explored in the management and prevention of invasive fungal infection in the transplant patient. These include:

Directed therapy. The limits to diagnosis of invasive fungal infection combined with the high rates of mortality in established infection have argued against the

use of antifungal therapy only for patients with confirmed infection. However, once a diagnosis of a given IFI is confirmed, empiric or prophylactic antifungal therapy may need to be modified. Specific therapy according to fungal etiology is outlined below:

The choice of first line therapy for invasive aspergillosis is voriconazole, with L-AMB as an alternative agent. For the treatment of refractory disease there is no universally accepted standard approach, with some authorities recommending a switch to another class of antifungal (i.e. a L-AMB or an echinocandin). Others favour the use of combination therapy with two new agents (ie a L-AMB plus echinocandin or second generation azole plus echinocandin). In the absence of new evidence, switching from voriconazole to another azole (ie posaconazole or itraconazole) should probably only be used in cases of voriconazole intolerance, and not refractory disease. Similarly, L-AMB is recommended for the therapy of IA breakthroughs of broad spectrum azole prophylaxis, rather than another azole. The duration of therapy is not well defined, though a minimum of six weeks is likely necessary. Serial galactomannan determinations are useful in monitoring response to therapy, with most patients s

For invasive candidiasis, most experts would favor initial therapy with an echinocandin, although amphotericin B remains an acceptable option in neutropenic patients. Most authorities would avoid the use of first-line therapy with fluconazole in the neutropenic patient. Stepdown therapy to oral fluconazole or voriconazole should be considered in the stable patient responding to therapy when culture results are available. Therapy for candidemia should be continued for two weeks following the sterilization of blood cultures.

The treatment of zygomycosis is less well defined, though the highest success rates have been reported with a combination of lipid-associated amphotericin B and surgical debridement. Posaconazole, either in combination with L-AMB or as step-down therapy is gaining widespread use, and has been reported to yield high success rates in case series and anecdotally.

Pre-emptive (Early directed) Therapy. Pre-emptive therapy algorithms use a combination of routine screening with new diagnostic tests (ie serum GM) with CT scanning and aggressive BAL to attempt to identify patients with IFI early in the course of disease, while obviating the need for empiric antifungal therapy in high risk patients. Importantly, these approaches are aimed at the detection of invasive aspergillosis only, and are usually combined with fluconazole prophylaxis for the prevention of invasive candidiasis. Two published studies have suggested that this approach is associated with reduced antifungal use and no increase in mortality when used for neutropenic patients at risk for IA. Limitations in the availability of GM testing and rapid CT scans have prevented widespread implementation of this strategy. Pre-emptive therapy is an attractive approach for the management of patients at intermediate risk for IA (i.e. AML

consolidation, autologous transplantation, neutropenia during non-myeloablative allogeneic transplantation), or patients at high risk for IA in centers with a documented low incidence of disease.

Empiric Therapy. Empiric therapy remains the most common strategy for the management of IFI in high risk patients. Addition of empiric antifungal therapy after 48-72 hours of persistent fever unresponsive to broad spectrum antibacterials has been studied extensively in the neutropenic host. Initial studies from the 1980's found that empiric antifungal therapy reduced the incidence of IFI, and were associated with a trend towards increased survival. These effects were predominately mediated through a reduction in the number of invasive Candida infections. Recent non-inferiority trials comparing Amb to L-AMB, caspofungin to L-AMB, and voriconazole to L-AMB suggested that caspofungin but not voriconazole were non-inferior to L-AMB. Although these studies have guided our choice of empiric antifungal therapy, they have been criticized for incorporation of large numbers of low risk patients combined with the use of composite endpoints that incorporated drug tolerance as part of the indicator of success. This approach resulted in a significant bias of the results towards tolerability of the agent rather than antifungal efficacy. Collectively, these factors have led to a diminished enthusiasm for empiric antifungal therapy, and the suggestion that pre-emptive and prophylactic approaches may be more appropriate for some at-risk populations to reduce overtreatment of low-risk groups, and reduce the incidence of disease in the highest risk groups.

Prophylaxis: A variety of antifungal agents have been evaluated in the management of fungal infections in allogeneic bone marrow transplant patients. The first agent to be systematically evaluated was fluconazole. Fluconazole use in the first 75 days after allogeneic transplantation was found to reduce invasive fungal infections, as well as all cause mortality. This survival advantage was maintained as long as 8 years after transplantation in follow up studies. As a result, most centers recommend the use of fluconazole in the first 75 days after allogeneic transplantation. The use of mold-active antifungals post allogeneic transplantation has also been evaluated in several studies. Itraconazole was evaluated in a randomized trial compared with fluconazole after allogeneic transplantation. Although itraconazole reduced the number of mold infections (5% vs 12%), this was not associated with an improvement in overall or fungal free survival. In addition, itraconazole was less well tolerated and associated with more hepatotoxicity than fluconazole. Of the echinocandins, micafungin was also compared with fluconazole in a mixed group of neutropenic patients that included some allogeneic and autologous transplant patients. Micafungin was associated with a reduction in proven, probable and possible cases of IFI as compared with fluconazole, but did not reduce overall mortality. Voriconazole has also been studied in the allogeneic transplantation population in two independent trials. In both trials, voriconazole was non-inferior to comparator,

and not associated with a reduction in overall mortality. Finally, posaconazole has been evaluated in the prophylaxis of allogeneic bone marrow transplant patients with graft vs host disease (GVHD). Posaconazole was more effective than fluconazole at preventing IFIs, but did not improve overall survival. Collectively, the available clinical studies support the use of fluconazole post allogeneic transplantation, but are insufficient to suggest that the use of mold-active agents during post-transplantation neutropenia, or GVHD is an effective strategy. Some centers have attempted to extrapolate from the current data to identify sub-populations at highest risk for IFI (i.e. matched, unrelated donor transplants, alemtuzumab use, Grade III-IV GVHD) for prophylaxis with mold-active agents. These approaches should be evaluated in the context of a clinical trial at the present time.

Environmental control. The ubiquitous nature of fungi precludes a complete avoidance of exposure after transplantation. However, environmental containment at periods of high risk has been found to be effective at a number of centers in the reduction of IFI. In particular, the use of laminar flow rooms, and careful containment of hospital renovations and construction is strongly recommended for neutropenic patients post transplantation, as is counseling about environmental exposures post hospitalization.

Table: Antifungal agents' spectrum of activity and dosing range for prevention and treatment of IFIs in HSCT

Agent	Dose	<i>Candida</i> species				<i>Aspergillus</i>	<i>Zygomycetes</i>	<i>Fusarium</i>
		<i>albicans</i>	<i>glabrata</i>	<i>krusei</i>	<i>parapsilosis</i>			
Fluconazole	400mgd po/IV q24h	+	+/-	-	+	-	-	-
Itraconazole	200-400mg q12h po/IV*	+	+/-	-	+	+	-	-
Voriconazole	6mg/kg IV X 2 then 4mg/kg IV q12h 300mg bid po	+	+	+	+	+	-	-
Posaconazole	600-800mg/d po in divided doses	+	+	+	+	+	+	+/-
L-AmB	3mg/kg/d	+	+	+	+	+	+	+/-
AmB	1mg/kg/d	+	+	+	+	+	+	+/-
Caspofungin	70mg IV X1 then 50mg IV/24h	+	+	+	+/-	+	+/-	-
Micafungin	50-150mg IV q24h	+	+	+	+/-	+	+/-	-
Anidulafungin	100-200mg IV then 50-100mg IV q24h	+	+	+	+/-	+	+/-	-

* Special access only in Canada

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