An overview of invasive fungal infections in immuno-compromised hosts

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ABSTRACT

Background: With the advances in medical care, invasive fungal infections possess a significant health problem especially in immunocompromised patients. These infections have varied aetiological agents which are commonly found in soil, water, plant debris and organic substrates. Aim: The overview of different fungal aetiological agents, newer and rapid diagnostic modalities and overall treatment and prevention options available is presented in this article. Methods: Literature search was performed in PubMed by using MeSH terms ‘mycoses’ and ‘immunocompromised host’. Only relevant review articles published within the last five years were considered. Google Scholar search engine was also used. Results: Common invasive fungi include Candida spp., Cryptococcus spp., Aspergillus spp., Rhodotorula spp., Fusarium spp., Mucormycotina, Pheohyphomycosis spp., Pneumocystis jirovecii, Scedosporium spp., and endemic mycoses such as Penicillium, Histoplasma and Blastomyces. A high degree of suspicion is required for early diagnosis and optimal management of these infections. Conclusion: Early and rapid diagnosis of causative fungal agents is required so that appropriate treatment can be initiated. Adequate preventive measures must be applied in an immunocompromised host that can prevent development of drug resistant super-infections. Key words: Invasive fungal infections, immunocompromised patients, rapid diagnosis, treatment, prevention

INTRODUCTION

During last three decades, the frequency of invasive fungal infections (IFIs) in immunocompromised (IC) hosts has increased remarkably and associated with excessive morbidity and mortality. This is mainly related to advances in the medical care, thereby increasing the number of at-risk immunocompromised populations. Majority of IFIs are due to opportunistic fungal pathogens that are commonly found in soil, water, plant debris, and other organic substrates.

Major risk factors for IFIs include neutropenia < 500 neutrophils/ml for more than 10 days, solid organ transplantation, blood and bone marrow transplantation, neoplastic diseases including haematological malignancies, chemotherapy, HIV infection, prolonged systemic therapy with corticosteroids and
newer immunosuppressive agents. Other risk factors are malnutrition, severe burns, prolonged stays in intensive care units (ICUs), invasive medical procedures, major surgery, advanced age, and premature birth.\[1,2\]

Well known opportunists like \textit{Candida albicans}, \textit{Cryptococcus neoformans}, and \textit{Aspergillus fumigatus} are being reported for causing life threatening systemic infections in IC hosts. New and emerging fungal pathogens that include species of non-albicans \textit{Candida}, non-neoformans \textit{Cryptococcus}, \textit{Aspergillus} other than \textit{A. fumigatus}, opportunistic yeast-like fungi such as \textit{Trichosporon Spp.}, \textit{Rhodotorula Spp.}, \textit{Geotrichum capitatum} (\textit{Blastoschizomyces capitatus}), the zygomycetes such as \textit{Fusarium Spp.}, \textit{Acremonium Spp.}, \textit{Scedosporium Spp.}, \textit{Paecilomyces Spp.}, \textit{Trichoderma Spp.}, and a wide variety of dematiaceous fungi.[3,4,5]

Due to complexity of the patients at-risk for infections and the diverse and ever increasing array of fungal pathogens, opportunistic mycoses pose considerable diagnostic and therapeutic challenges.[6] Diagnosis depends upon clinical suspicion and the collection of appropriate material for microscopy, culture, histopathology, antigen detection, molecular tests and imaging. Detection of the infecting fungi is extremely important for proper management of infection due to the less common opportunistic fungi. Some of these fungi are inherently non-susceptible to standard azole or polyene therapy and may require the use of alternative anti-fungal agents. In addition, it may also require surgical management and reversal of underlying impairment of host defences.[7] The overview of different fungal etiological agents, newer and rapid diagnostic modalities and overall treatment and prevention options available is presented in this article.

**METHODOLOGY**

Literature search was performed in PubMed by using MeSH terms ‘mycoses’ and ‘immunocompromised host’. Only the relevant review articles published within last five years were considered. Also the Google Scholar search engine was used for the collection of articles.

**REVIEW**

### Organisms

**\textit{Candida} spp.**

\textit{Candida} spp. is currently one of the five principal causes of nosocomial blood stream infections, especially among patients taking broad spectrum antibiotics for a prolonged period, under immunosuppressive therapy, total parenteral nutrition, patients exposed to multiple invasive procedures, and prolonged hospitalization (> 30 days).[8,9] Although \textit{C. albicans} is the most common cause of invasive fungal infections, the growing number of new and emerging yeast infections from non-albicans \textit{Candida} species is increasingly recognised.[5,10] These are \textit{C. tropicalis}, \textit{C. glabrata}, \textit{C. parapsilosis}, and rarer isolates such as \textit{C. guilliermondii}, \textit{C. pelliculosa}, \textit{C. krusei}, \textit{C. kefyr}, \textit{C. rugosa}, \textit{C. lusitaniae}, \textit{C. famata}, \textit{C. norvegensis} and \textit{C. dubliniensis}.[11] \textit{Candida} is isolated from 84 to 88 percent of mucocutaneous surfaces in hospitalized patients. Colonization and adhesion leading to biofilm production to the mucosal surface is the most important step in the process of systemic candidiasis. From colonizing \textit{Candida} spp. extracellular enzymes such as phospholipases, proteinases and hydrolytic enzymes contribute to host tissue invasion.[12] \textit{Candida} hyphal forms are also able to release hydrolytic enzymes and can specially invade epithelial and endothelial cells.[13] Clinical diagnosis of invasive \textit{Candida} infection is difficult, positive blood and sterile fluid can be delayed and yield positive results only in 50 to per cent cases of disseminated infection.

**\textit{Cryptococcus} spp.**

Genus \textit{Cryptococcus} belongs to basidiomycetes, which contain more than 500 species; only \textit{C. neoformans} and \textit{C. gattii} are considered as principal pathogens in IC patients. Primary infection due to \textit{C. neoformans} that usually acquired by inhalation is common and most of these cases are asymptomatic in the lung. \textit{C. neoformans} can cause severe form of meningitis and meningo-encephalitis in patients with AIDS and corticosteroid use.[14] Disseminated Cryptococcal infection can cause clinical manifestations in the skin, ocular, soft tissue and bone and joints.
Saprophytic non-neoformans Cryptococci are occasionally reported especially those with advanced stage of HIV infection patients with cancer who are undergoing transplant surgery.\cite{15} These are C. laurentii, C. albidus, C. curvadus, C. humiculus, and C. uniguttulatus.\cite{15} In Cryptococcus Spp. the polysaccharide capsule protects the organism against the host immune system. Other pathogenic factors include melanin production, mannitol secretion, superoxide dismutase, proteases and phospholipases.\cite{16}

**Aspergillus ssp.**

Aspergillus is ubiquitous, thermophilic filamentous fungi that are transmitted by inhalation of air borne conidia. A. fumigatus, A. flavus, A. niger and A. terreus are commonly associated with human infection. Risk factors to invasive aspergillosis are neutropenia, advanced haematological disease, severe burns, and autoimmunne diseases are at-risk.\cite{22}

**Trichosporon spp.**

Trichosporon genus is one of the basidiomycetous yeast producing septate hyphae, arthroconidia, yeasts and pseudo-hyphae. Trichosporon Spp. can be found in soil, fresh water, and are part of normal flora of the human skin, and gastrointestinal tract.\cite{5} The risk-factors associated with infection are malignant haematological disease, severe burns, AIDS, chronic corticosteroid use, and heart valve surgery.\cite{24} It is second most common cause of yeast fungemia (after Candida Spp.) in patients with malignant haematological disease.\cite{5} The important species causing invasive fungal infections are T. asahii, T. asteroids, T. cutaneum, T. inkin, T. mucoides, and T. ovoides.\cite{21}

**Rhodotorula spp.**

Genus Rhodotorula, a basidiomycetes yeast which produces carotenoid pigments (yellow to red), multilateral budding cells, rudimentary pseudohyphae and occasionally a faint capsule.\cite{5} Rhodotorula species are environmental fungi that can be found in soil, fresh water, fruit juice, milk, shower curtains and tooth brushes. R. mucilaginosa is the most common cause of fungemia, followed by R. glutinis, and R. minuta.\cite{21} Patient who is undergoing bone marrow transplant, AIDS, previous abdominal surgery, cirrhosis, burns, and autoimmune diseases are at-risk.\cite{22}

**Other uncommon yeasts**

Geotrichum is very similar to Trichosporon yeast.\cite{5} It is rarely present as bloodstream infection or disseminated infection in immunocompromised hosts. Presence of blastoconidia with hyphae differentiates Trichosporon from Geotrichum.\cite{23}

Hansenula anomala (Pichia anomala) yeast has been reported neonatal, paediatric and surgical ICUs, and in IC patients. It can cause a wide range of invasive infections, but fungemia especially associated with a central venous catheter is most common.\cite{24}

Lipophilic Malassazia furfur causes fungemia which is related to lipid infusion in IC patients, but the organism is less virulent than other fungal pathogens.\cite{55}

Fungemia in ICUs and in patients with central venous catheter has been linked to use of live yeast capsules Saccharomyces cerevisiae (S. boulardii) which are taken for prevention of diarrhoea.\cite{56}

**Fusarium spp.**

One of the most frequent septate mould causing IFIs reported in immunocompromised patients is due to Fusarium Spp. Three important species clinically identified are F. solani, F. oxysporum, and F. verticillioidis. In IC patients fusarium causes fungemia with or without endocarditis, skin lesions, sinusitis and lung disease.\cite{27} Risk factors for invasive fusariosis include prolonged...
neutropenia, and T cell immunodeficiency particularly in stem cell recipients with severe graft versus host disease.\cite{28}

**Mucormycotina**
The subphylum Mucormycotina (formerly Zygomycetes) contains most frequently isolated species such as *Mucor*, *Rhizopus*, *Rhizomucor*, *Lichtheimia* (previously known as *Absidia*), *Cunninghamella*, *Berthotella*, and *Apophysomyces elegans*.\cite{29} These opportunistic fungi produce acute rhino-cerebral and pulmonary diseases with thrombosis, infarction and blood vessel invasion. Risk factors include profound and prolonged neutropenia, chronic high dose corticosteroid use, uncontrolled diabetes mellitus, hypertriglyceridemia, voriconazole prophylaxis in stem cell transplant, tissue iron excess, and previous skin and soft tissue trauma.\cite{30} Rapid growth and affinity to the blood stream, heat tolerance, the production of efficient proteolytic enzymes such as lipases, proteases, glycosidic and lipolytic extracellular enzymes, siderophore production and an efficient iron transport system are pathogenic factors in members of the Zygomycetes family.\cite{1}

**Pheohyphomycosis spp.**
Pheohyphomycosis consists of a group of mycotic infections characterized by the presence of dematiaceous (dark walled) septate hyphae, and sometimes yeast or a combination of both in tissue.\cite{51} They are responsible for subcutaneous and systemic (especially cerebral and pulmonary) infection in IC hosts. Agents associated are *Cladophialophora bantiana*, *Bipolaris hawaiensis*, *Exserohilum rostatum*, *Phialophora Spp.*, *Cladosporium cladosporioides*, *Exophiala spinifera*, and *Fonseca pedrosoi*.\cite{32,33}

**Pneumocystis jirovecii**
*Pneumocystis* is a genus of unicellular fungi found in respiratory tract of many mammals and humans.\cite{54} It is most common opportunistic infection in persons with HIV, followed by cancer patients receiving chemotherapy and solid organ transplant recipients receiving immunosuppressant.\cite{35} Clinical manifestations are pneumonia as well as systemic infection involving lymph nodes, spleen, bone marrow and liver.

**Scedosporium spp.**
*Scedosporium Spp.* is recently emerging as resistant life threatening opportunistic infection in IC hosts. The commonest sites of infections are lungs, sinuses, bones, joints, eyes and brain. *S. apiospermum* and *S. prolificans* are two medically significant species of this genus.\cite{36}

Endemic mycoses in immunocompromised patients
Among endemic mycoses, genus *Penicillium*, *Histoplasma* and *Blastomyces* are common in IC patients. Only dimorphic *Penicillium* species, *P.marneffei* appears late in the course of HIV infection, usually at CD4 lymphocyte count < 100cells/cumm.\cite{37} Histoplasmosis caused by *Histoplasma capsulatum* usually produces acute and chronic pulmonary infections. The other common features like fever with hepatospleenomegaly may be misdiagnosed as visceral leishmaniasis in endemic areas of histoplasmosis.

**Diagnosis of fungal infections in immunocompromised hosts**
As clinical manifestations of fungal infections are non-specific, high degree of suspicion is required for early diagnosis and optimal management of these infections. Conventional mycological detection methods include direct microscopic examination, culture of samples, use of chromogenic agar, biochemical analyses, germ tube examination, histopathological examinations using special stains. Although conventional methods are efficient approaches, but these routine methods can require 48 to 72 hours or longer arriving at definitive identification, thus can cause considerable delay in correct patient diagnosis and management.

**Antigen detection**
Commercially available ELISA kits for detection of fungal cell wall markers in serum has been reported for galactomannan (GM), mannann and 1,3-beta-D-glucan (BDG). Galactomannan is relatively specific for *Aspergillus Spp.* and it can also be detected in urine, bronchoalveolar lavage (BAL) fluid, cerebrospinal fluid (CSF), and other sterile specimens.\cite{19} For diagnosis of
invasive aspergillosis it has variable sensitivity rates ranging from 30 to 100 per cent and specificity from 38 to 98 per cent. Factors the sensitivity and specificity are false positive results in patients treated with beta-lactam antibiotics such as piperacillin-tazobactam, dietary GM in pasta, cereals, and milk and cross reactivity with moulds such as *Penicillium Spp.* and *Paecilomyces Spp.* Thus serial testing of patient’s sample is required to achieve acceptable sensitivity and specificity.

Mannan is found as a characteristic cell wall component in yeasts especially *Candida Spp.* The detection of circulating Candida mannan and anti-mannan antibodies has been used as diagnostic marker for invasive candidiasis or candidemia in adult IC patients with neutropenia. The overall sensitivity of mannan antigen detection in patients with candidemia has been reported to be between 69 and 90.9 per cent and specificity between 46.2 and 89 per cent when compared to culture as gold standard.

1,3-beta-D-glucan (BDG) is present as cell wall polysaccharide in most pathogenic fungi including *Candida Spp.*, *Aspergillus Spp.*, *Fusarium Spp.*, *Trichosporon Spp.*, *Saccharomyces Spp.*, and *Acremonium Spp.* and it is not species or genus specific for each organism. However, *Mucorales*, *Cryptococcus Spp.* and * Blastomycesdermatitidis* which either lack the glucan or produce it at minimal levels. The sensitivity has been reported to be 50 to 100 per cent and specificity 71 to 93 per cent. False positive BDG findings occur in the patients with fungal colonization or mucositis who have received empirical antifungal therapy. The combined use of BDG assay (Fungitell™Associates of Cape Code Inc., East Falmouth, MA, USA) and GM enzyme immunoassay (PlateliaAspergillus™ EIA; Bio-Rad Laboratories, Hemel Hempstead, UK) improves the specificity of diagnosis.

Molecular tests

Polymerase chain reaction (PCR) assay constitutes one of the recent progresses in identification of fungal genomics in high-risk immunocompromised patients. Both qualitative and quantitative PCR methods are available for the detection of fungal DNA. The nested PCR and pan-fungal PCR have sensitivities of 92.8 and 80 per cent and specificities of 94 and 95.6 per cent respectively. The quantitative real-time PCR has shown the sensitivity of 100 per cent and the specificity of 97 per cent.

Pyrosequencing is another rapid DNA sequencing method that requires novel chemistry to sequence pre-selected regions of the genome to short sequences (> 70 bp). Molecular methods are rapid (within 6 hours) that enable diagnosis during the incubation period, very early stage of infection and prior to bone marrow transplantation. Since opportunistic fungi can cause high morbidity and mortality in IC patients, thus serial tests and more than one method should be employed for early diagnosis.

Radiological imaging

The radiographic patterns obtained from X-rays, high-resolution computed tomography (HRCT) and magnetic resonance imaging (MRI) can be useful for detection of both pulmonary and extra-pulmonary IFIs with some degree of certainty. These tests are often non-specific and require the existence of macroscopic lesions which are usually occur late in the course of disease and are markers of poor prognosis. HRCT of chest can detect classic halo sign and macronodules are considered as early indicators of invasive disease.

Treatment with antifungal drugs based on these non-specific early CT findings have been associated with improved survival. Serial HRCT in neutropenic patients has shown to be more sensitive than single test in non-neutropenic patients.

Treatment and prevention

The early and prompt initiation of antifungal treatment is most crucial in the outcome for the patient. It has been reported that in Candidemia infections a 20 per cent increase in mortality if there was more than 12 hour have elapsed in antifungal treatment after positive blood culture result and the mortality rate increases significantly on each of the following three days.

The treatment of fungal infections depends on the type of infection and its
etiolgic agent. Also the antifungal agents have varying spectrums of activity, dosing, safety profiles and costs. Broadly, the systemic antifungal agents have grouped into azoles (i.e., fluconazole, voriconazole), polyenes (i.e., amphotericin B, deoxycholate, amphotericin lipid formulations and liposomal amphotericin B) and echinocandins (i.e., caspofungin, micafungin and anidulafungin). The echinocandins exhibit potent in vitro and in vivo fungicidal activity against Candida species, including azole-resistant pathogens and are approved for the treatment of oesophageal candidiasis, Candidemia and other select forms of invasive candidiasis.

To improve outcomes for patients with invasive fungal infections, complimenting strategies such as immunomodulators and combination therapies can be used. Adjuunctive interferon gamma could be suggested for refractory cryptococcosis. Combination therapy is highly recommended for candida endocarditis, cryptococcal meningitis and other difficult to treat cases.

To prevent infection in IC patients, exposure to fungal spores must be limited with high-efficiency particulate air filters and positive pressure ventilation in the patient’s room. Also high-risk patients should avoid contact with soil, tap water and shower facilities. Decreasing the duration of neutropenia or discontinuing immunosuppressive agents should be considered in efforts to prevent infection. Adequate information about susceptibility pattern of current fungi that are prevalent in a particular area should be available. Routine monitoring of serum concentrations of antifungal agents are required to know the efficacy and toxicity of antifungals by using high performance liquid chromatography and bioassay methods. This may also prevent the development of drug resistance. Antifungal prophylaxis is recommended for high-risk patients undergoing pancreas, liver, and small bowel transplantations, for chemotherapy induced neutropenic patients, haemopoitic stem cell transplantation recipients with neutropenia and adults admitted in ICUs who are at risk for developing candidiasis.

CONCLUSION
The best approach to optimal management of fungal infection in IC patients is early detection and identification of causative fungus, so that appropriate treatment can be initiated as soon as possible. Regular surveillance testing combined with clinical and radiological information is required to arrive at early diagnosis. The message to both clinicians and clinical microbiologists is that there are no uniformly non-pathogenic fungi. Any fungus can cause a lethal infection in an IC host and should never be rejected as a contaminant provided they are detected from sterile specimen in sterile condition. Broad and injudicious use of any antifungal agent in a severely IC host may result in superinfections due to organisms that are both unusual and drug resistant.

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Conflict of Interest: None declared

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