Review Article

Indian J Med Res 139, February 2014, pp 195-204

Opportunistic invasive fungal infections: diagnosis & clinical management

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Received May 14, 2013

Invasive fungal infections are a significant health problem in immunocompromised patients. The clinical manifestations vary and can range from colonization in allergic bronchopulmonary disease to active infection in local aetiologic agents. Many factors influence the virulence and pathogenic capacity of the microorganisms, such as enzymes including extracellular phospholipases, lipases and proteinases, dimorphic growth in some *Candida* species, melanin production, mannitol secretion, superoxide dismutase, rapid growth and affinity to the blood stream, heat tolerance and toxin production. Infection is confirmed when histopathologic examination with special stains demonstrates fungal tissue involvement or when the aetiologic agent is isolated from sterile clinical specimens by culture. Both acquired and congenital immunodeficiency may be associated with increased susceptibility to systemic infections. Fungal infection is difficult to treat because antifungal therapy for *Candida* infections is still controversial and based on clinical grounds, and for molds, the clinician must assume that the species isolated from the culture medium is the pathogen. Timely initiation of antifungal treatment is a critical component affecting the outcome. Disseminated infection requires the use of systemic agents with or without surgical debridement, and in some cases immunotherapy is also advisable. Preclinical and clinical studies have shown an association between drug dose and treatment outcome. Drug dose monitoring is necessary to ensure that therapeutic levels are achieved for optimal clinical efficacy. The objectives of this review are to discuss opportunistic fungal infections, diagnostic methods and the management of these infections.

Key words Antifungal treatment - diagnostic methods - immunodeficiency galactomannan - invasive fungal infections

Introduction

Invasive fungal infections (IFI) have significantly increased due to advances in medical care in the at-risk immunocompromised population. Fungal species are widely distributed in soil, plant debris and other organic substrates, and make up approximately 7 per cent (611,000 species) of all eukaryotic species on earth[1], although only about 600 species are human pathogens[2].

Major risk factors for IFI include neutropenia <500 neutrophils/ml for more than 10 days, haematological malignancies, bone marrow transplantation, prolonged (>4 wk) treatment with corticosteroids; prolonged (>7 days) stays in intensive care, chemotherapy, HIV

infection, invasive medical procedures, and the newer immune suppressive agents. Other risk factors are malnutrition, solid organ transplantation, severe burns or prolonged stays in intensive care (>21 days), systemic corticosteroids for >7 days, and major surgery[3,4]. There are also reports of the presence of infection in immunocompetent patients[5] without signs or symptoms of conditions associated with immunocompromised status.

Infection can be transmitted by the inhalation of spores (aspergillosis, cryptococcosis, histoplasmosis), percutaneous inoculation in cutaneous and subcutaneous infections (dermatophytosis, madura foot), penetration into the mucosa by commensal organisms such as *Candida albicans*, and the ingestion of a toxin in contaminated food or drink (gastrointestinal disease).

Infections may be mild and only superficial or cutaneous (*e.g.* dermatophytosis and *Tinea versicolor*) or may cause life-threatening, systemic illness (*e.g.* candidiasis, aspergillosis and mucormycosis). The clinical manifestations of the disease caused by a given fungal agent can be highly variable and related to host immunity and physiological condition. For example, *Candida* spp. can invade a local site (mucocutaneous or cutaneous candidiasis, onychomycosis) or cause systemic infections (renal, liver abscess, lung and nervous central system). Allergic symptoms were reported in infections with other fungi such as *Aspergillus* spp. (allergic bronchopulmonary aspergillosis). The isolation of these organisms from clinical samples may indicate colonization, infection or disease; consequently interpreting the results of diagnostic tests may be challenging for clinicians who treat these patients. Treatment requires early suspicion and is difficult because only a few antifungal agents are available, most usually have side effects, and some organisms have developed resistance[6]. Clinicians need drugs that are highly effective but have low toxicity.

In this review opportunistic fungal infections, diagnostic methods and the management of these infections are discussed.

Clinical manifestations and epidemiology

The standard definition of IFI was developed by members of the European Organization for Research in the Treatment of Cancer–Invasive Fungal Infection Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group[7].

Among immunocompetent hosts, keratitis and onychomycosis are the most common infections. Other infections in immunocompetent patients include sinusitis, pneumonia, thrombophlebitis, peritonitis, fungemia, endophtalmitis, septic arthritis, vulvovaginitis and osteomyelitis[8].

In immunocompromised patients, any fungus present in the environment may be potentially pathogenic. *Aspergillus* and *Candida* spp. are the main organisms isolated most frequently from immunocompromised patients. The other most relevant aetiologic agents are *Cryptococcus* spp., *Fusarium* spp., *Zygomycete*, *Dematiaceous* fungi and opportunistic yeast-like fungi[9]. Colonization of the mucosal surfaces by *Candida* spp. is the first step in the process of systemic candidiasis. *Candida* is isolated from 84-88 per cent of mucocutaneous surfaces in hospitalized patients or even healthy adults[10]. Because of its stronger adherence capacity, *C. albicans* is found more often than other *Candida* species[10].

Central nervous system candidiasis may present with scattered brain microabscesses or larger lesions, vasculitis or thrombosis. *Candida* species are reportedly the third or fourth most frequently isolated organisms in nosocomial bloodstream infections, the fourth most common cause of hospital-acquired systemic infections in the USA in nosocomial candidemia[11], and the fifth most common cause of bloodstream infections in paediatric intensive care units[12]. The crude mortality rates in the USA were reported to be up to 50 per cent[13].

There are many species of *Aspergillus* in the environment but the most frequently recovered species depending on the geographical region are *Aspergillus fumigatus*, *A. flavus, A. niger* or *A. terreus*. Clinical manifestations can range from colonization in allergic bronchopulmonary disease to active disseminated infection, with mortality rates ranging from 40 to 90 per cent depending on the site of infection, host immune status, and the treatment regimen[14]. The crude mortality rate has been reported to be as high as 95 per cent in bone marrow transplant recipients[14]. Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to *Aspergillus* mycelia, which colonize the bronchi with a prevalence rate of 1 to 2 per cent in people with asthma, 7 to 14 per cent in steroid-dependent asthma patients, and 2 to 15 per cent in patients with cystic fibrosis[15]. Other clinical manifestations of aspergillosis are invasive pulmonary aspergillosis, cutaneous and wound infections, keratitis, and *Aspergillus* sinusitis.

Cryptococcus neoformans is a major cause of infection in immunocompromised patients[16]. Primary infections due to *C. neoformans* are common and most are asymptomatic in the lung. *Cryptococcus neoformans* can cause a severe form of meningitis and meningo-encephalitis in patients with AIDS. Disseminated cryptococcal infection can cause clinical manifestations in the skin, ocular, soft tissue or bones and joints[16].

Zygomycosis is the most lethal opportunistic fungal infection particularly among patients with diabetes mellitus, haematological malignancies, and patients receiving deferoxamine treatment. *Rhizopus*, *Mucor*, and *Rhizomucor* species account for up to 75 per cent of mucormycosis cases[17]. Infection with *Entomophthora* species has been reported in immunocompetent patients[18]. *Fusarium* species cause a broad spectrum of infections in humans, including superficial and disseminated infections, the latter with a mortality rate that approaches 100 per cent[19]. Members of this genus may also cause allergic diseases and mycotoxicosis following the ingestion of toxin-contaminated food[20].

Pathogenesis

Many factors are important in the virulence and pathogenic capability of microorganisms. Enzymes secreted from colonizing organisms (especially when *C. albicans* is involved), such as extracellular phospholipases, proteinases and hydrolytic enzymes, contribute to host tissue invasion[21]. Dimorphism in the ability of some *Candida* species to grow as a unicellular yeast at room temperature and in pseudohyphal and filamentous forms in the host's body or at 37 °C is another virulence factor[22], because the hyphal forms are able to release hydrolytic enzymes and can specifically invade epithelial and endothelial cells[23].

Conidial size and count are factors that affect virulence in some aetiologic agents. *Aspergillus fumigatus*, with small conidia, is the main agent in invasive pulmonary aspergillosis, whereas *A. flavus*, with large conidia, is an important aetiologic agent in *Aspergillus* sinusitis, cutaneous and wound aspergillosis[24]. A low conidia count may not produce infection. According to Pasqualotto *et al*[25], *Aspergillus* species caused death when as few as 102 spores were inoculated. Other pathogenic factors detected in patients with invasive aspergillosis are sensory deprivation (environmental *p*H), mutational restriction of nutrient acquisition, siderophore and amino acid biosynthesis, extracellular elastolytic proteases, gliotoxin and hyaronic acid. *Aspergillus fumigatus* and *C. albicans* can support growth across a broad range of environmental *p*H values, and the biosynthesis of specific materials protects virulence factors involved in fungal pathogenicity.

The outer cell wall layer plays a major role in an organism's pathogenicity. Chitin can influence immune recognition by blocking dectin-1, leading to significant reductions in cytokine production[26]. In *Cryptococcus* species the polysaccharide capsule protects the organism against the host immune system. Other pathogenicity factors in this organism include melanin production, mannitol secretion, superoxide dismutase, proteases and phospholipases[27]. The synthesis of melanin and other conidial pigments in fungal organisms such as *A. fumigatus* reduces complement opsonization by camouflaging binding sites, and reduces the ability of C3 to bind with conidia, although this property is still controversial[28].

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[3], siderophore production and an efficient iron transport system are pathogenic agents in members of the Zygomycetes family[3]. Toxin production is another pathogenic factor in *Aspergillus* and *Fusarium* species, which can contaminate food and cause mycotoxicosis[20].

Immunity

The first-line anatomical barriers of defense are the skin and mucosal surfaces, which protect the human body with an acidic *p*H, enzymes, mucus and other antimicrobial secretions[29]. When these barriers are broken by surgery or indwelling catheters, radiotherapy, burns or chemotherapy, fungal agents can reach the deep tissues. The clinical forms and severity of the disease manifestations depend on the host's defenses and immune response. An active host immune response against fungal spores or hyphae leads to allergies and asthma, and to the collapse of immune system defenses in *Aspergillus* infection[30,31], which results in the invasive form of infection. Both acquired and congenital immunodeficiency may be associated with increased susceptibility to IFI, which frequently occurs in patients with phagocytic and cellular immune defects.

Once fungi enter, opsonization promotes fungal uptake and innate immune activation by a wide range of phagocytic and signaling receptors[31] which include

C-type lectin receptors and Toll-like receptors by host cell pattern recognition receptors[32]. In healthy and immunocompetent individuals, the innate immune system (neutrophils and macrophages) is an efficient sentinel that provides protection from thousands of fungal species through phagocytosis of the invading pathogens by cell surface receptors. Numerous cytokines, *e.g.* interleukin (IL)-1β, IL-12, IL-17, IL-23 and tumour necrosis factor (TNF)-α, are important in directing innate and adaptive responses to fungal pathogens[29].

Cell-mediated immunity through Th1-based responses to fungal infections, characterized by TNF-α, IL-12 and interferon (IFN)-γ production, is a protective factor, and Th2-based responses, characterized by IL-4, IL- 6 and IL-13 production[29], are maladaptive and deleterious factors[33,34]. Uncontrolled Th2 responses lead to chronic infections or allergic responses; in this connection, IL-4 and IL-13 play important pathogenic roles in allergic bronchopulmonary mycosis[35,36].

Diagnosis of infections

The clinical manifestations of fungal infection are not specific, and like other infective diseases, a high degree of suspicion is required for the early diagnosis and optimal management of these infections. Systemic fungal infections, according to standard criteria[7], are established when histopathologic examination with special stains confirms fungal tissue involvement or when the aetiologic agent is isolated from clinical sterile specimens by culture.

Radiological evidence from X-rays and high-resolution computed tomography is useful for the diagnosis of fungal infections. Pulmonary fungal infections such as aspergillosis, fusariosis, scedosporiosis or zygomycosis are characterized by central cavitation of pulmonary lesions, infiltration, pulmonary nodules, and halo or air-crescent signs[37,38]. Documentation of the diagnosis of infection requires serial high-resolution computed tomography; however, the risk of radiation exposure in children must be considered[39,40]. The specificity of these methods is lower in children than in adults[39].

Conventional mycological methods include direct microscopic examination and the culture of samples in the mycology laboratory. Pathological examination and direct smears of samples with potassium hydroxide by an expert is the most rapid, cost-effective and sensitive method for the diagnosis of fungal infections. An expert can identify some genera of aetiologic agents such as yeasts or molds (with septated or nonseptated hyphae) and thus help ensure prompt, effective therapy. However, it may not be possible to identify fungal strains by this method, and fungal growth or the use of complementary methods such as hybridization in paraffin-embedded tissue may be necessary.

Culturing clinical specimens (tissue, sputum, urine, wound, or blood) to isolate the aetiologic fungal agent is the gold standard for the diagnosis of IFI, provided that the samples are from sterile sites such as blood, tissue or cerebrospinal fluid, in which fungal infection can be documented. The sensitivity of culture differs among published studies[41]. In cases with a specific fungal infection such as zygomycosis, the aetiologic agent loses its viability during tissue homogenization before culture[42]. In addition, this method can produce false negative results if the clinical specimens are obtained after treatment with antifungal agents. Collecting appropriate tissues and other sterile specimens for culture or histology from patients whose condition is unstable, especially when neutropenia is present or platelet count is low, is difficult because it requires invasive procedures. The sensitivity of blood culture for the diagnosis of fungal infection is controversial. Multiple or repeated blood cultures should be performed to increase the likelihood of detecting candidemia, and filamentous fungi are rarely isolated from blood[43]. Culture can be useful to determine the sensitivity of the isolated fungi to antifungal agents and identify resistant species, and are thus needed to optimize patient management[6]. However, given the limitations of conventional methods of diagnosis for IFI, negative result on direct or pathologic smears and cultures do not rule out infection, so it is essential to use other suggested methods.

There are many serological methods for the diagnosis of fungal infections, and the results of these tests become available sooner than culture. Skin tests and serum IgE level are suitable methods for the diagnosis of disorders such as ABPA. It should be noted that antibody assays are often negative in immunosuppressed patients. The detection of antigens in serum or cerebrospinal fluid is recommended for the diagnosis of *Cryptococcus* infections. In severely ill patients the detection of antigens by enzyme immunoassay or the latex agglutination test in blood, bronchoalveolar lavage (BAL) fluid or urine can be useful for a rapid diagnosis. Cross-reactions are seen in some infected patients. The cut-off values for the detection of antigens in different populations may be

different[44], therefore, it is important to interpret the results appropriately. The serial measurement of antigen titres is not a reliable indicator for the evaluation of antifungal therapy.

The detection of fungal cell wall markers in serum has been reported for galactomannan (GM), (1, 3)-beta-D-glucan (BDG) and mannan[7]. Galactomannan is relatively specific for *Aspergillus* species[45], and can be detected in urine, bronchoalveolar lavage fluid cerebrospinal fluid and other specimens with enzyme immunoassay. Various sensitivity rates from 30 to 100 per cent, and similarly wide-ranging specificities from 38 to 98 per cent have been reported for GM[46,47]. Factors that limit the specificity of this test are immune reactivity with other fungi such as *Penicillium* spp. and *Paecilomyces* spp., false positive results with antibacterial agents such as beta-lactam antibiotics, particularly piperacillin–tazobactam[48] and amoxycillin with or without clavulanate[49], and dietary GM in pasta, cereals and milk[50].

1,3-Beta-D-glucan (BDG) is present in the cell wall of most pathogenic fungi, including *Fusarium, Candida*, *Aspergillus* and *Trichosporon*, and is not species-specific or genus-specific for each organism[51,52]. However, *Mucorales*, *C. neoformans* and *Blastomyces dermatitidis* contain relatively small amounts of cell wall BDG; therefore, these assays may not be completely reliable in patients infected with these organisms[53]. Sensitivities of 55 to 100 per cent, specificities of 71 to 93 per cent, positive predictive values of 40 to 89 per cent and negative predictive values of 73 to 100 per cent have been reported with various cut-off values for positivity ranging from 6 to 120 pg/ml for this assay[53,54]. Depending on the type of assay (Fungitell or Fungitec) and population, the recommended cut-off levels are 7 pg/ml[55], 20 pg/ml[54] or 60-80 pg/ml[51]. False-positive BDG findings occur in the patients with fungal colonization or mucositis who have received empirical antifungal therapy[55]. The combined use of a BDG assay (Glucatell) and a GM enzyme immunoassay (Platelia *Aspergillus*) improves the specificity of diagnosis[56].

Mannan is mainly found as a characteristic cell wall component in yeasts. The detection of circulating *Candida* mannan and anti-mannan antibodies has been used as a diagnostic marker for invasive candidiasis or candidemia caused by the most pathogenic species of *Candida* in adult patients with neutropenia[57,58] and after myeloablative chemotherapy[59]. 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 89 and 46.2 per cent compared to culture as the gold standard[60,61]. In neonates, this test has yielded promising results particularly for ruling out candidiasis, considering its high negative predictive value of 98 per cent[62].

Detection of fungal DNA

The polymerase chain reaction (PCR) assay may serve as a powerful non-culture method for the diagnosis of systemic fungal infection in high-risk patients. Qualitative methods are sensitive in detecting fungal DNA in human blood samples, tissues, bronchoalveolar lavage and other body fluids[63]. The sensitivity and specificity vary according to type of the tests. For example, nested PCR has a sensitivity of 92.8 per cent and a specificity of 94 per cent [64], and panfungal PCR has sensitivity 80 per cent and a specificity of 95.6 per cent[65]. Quantitative methods include PCR ELISA, with a sensitivity of about 83.3 per cent and a sensitivity of about 91.7 per cent[66], and real-time PCR, with the sensitivity about 100 per cent and a specificity about 97 per cent[67]. Molecular methods, which are rapid and can yield results within 6 h, have revolutionized the diagnosis of fungal infections because these enable diagnosis during the incubation period and early stage[68] of infection, and prior to bone marrow transplantation[67].

The diagnosis of infection based on molecular and serologic techniques can provide powerful tools for the early diagnosis of IFI. Because the fungi are common in the environment and opportunistic fungi in immunocompromised patients can cause high morbidity and mortality, the interpretation of positive or negative results with different laboratory methods is difficult for clinicians, so more than one method should be used for early diagnosis.

Prevention

To prevent infection in immunocompromised patients, exposure to fungal spores must be limited with high-efficiency particulate air filters and positive pressure in the patient's room, and high-risk patients should avoid contact with soil, tap water and shower facilities[69]. Decreasing the duration of neutropenia or discontinuing immunosuppressive agents should be considered in efforts to prevent fungal infection. Knowledge of the susceptibility pattern of current fungi in each region and the use of prophylactic doses of interferon-γ and azole antifungal agents can be useful to manage infection in high-risk patients; however, after

long-term prophylaxis with antifungal agents, infection by resistant aetiologic agents needs to be considered. In addition, fungal colonization of different sites in the same patient (mouth, rectum, nose, urinary tract and vagina) and cutaneous infections should be evaluated before antineoplastic therapy and major surgery[70].

Treatment

The timely initiation of antifungal treatment is a critical component in the outcome for the patient. Unfortunately, patients with fungal infection often die of complications attributed to the infection despite antifungal therapy[70,71]. Delays in antifungal treatment in candidemia infections are associated with a 20 per cent increase in mortality if more than 12 h have elapsed after a positive blood culture result, and the mortality rate increases significantly on each of the following three days[72].

Localized infection is usually treated with topical antifungal agents, whereas disseminated infection requires the use of systemic agents with or without surgical debridement, and in some conditions immunotherapy is also advisable. Fungal infections are difficult to treat because antifungal therapy in *Candida* infections is still controversial and based on clinical grounds, and in molds, the fungus isolated from the culture medium must be assumed to be the pathogen because these organisms are saprophytic in the environment. Some molds such as *Aspergillus terreus, Fusarium solani* and *Mocuralea* are intrinsically resistant to antifungal agents; therefore, mortality rates in the patients infected with these organism are high[3,73].

The management of fungal infections is different depending on the type of infection and aetiologic agents. Antifungal agents have varying spectrums of activity, dosing, safety profiles and costs. Furthermore, many confounding factors such as the aetiologic agent, age, underlying diseases and surgical complications can influence the outcome[74]. For example, for invasive aspergillosis voriconazole is superior to deoxycholate amphotericin B as the primary treatment in most patients[6].

Many studies have shown an association between drug dose and outcome[75,76]. Therapeutic drug monitoring (TDM) is necessary to ensure that therapeutic levels are achieved[77,78]. Evidence supports TDM to optimize clinical efficacy when the drug is used for prophylaxis and therapy in IFI. The criteria for treatment response include the disappearance of clinical and radiological symptoms attributed to the infection. In some cases, the interpretation of radiologic images may be problematic because lesions may remain in the lungs and sinuses. Because fungal pathogens are eukaryotes and many of their biological processes are similar to those in humans, many antifungal drugs can cause toxicity when used at therapeutic doses[79]. Monitoring the clinical response to individualized dose regimens can be helpful in interpreting the patient's condition with regard to treatment efficacy and toxicity, disease progression during therapy, drug absorption in patients with suspected poor oral absorption, signs or symptoms of significant toxicity or improper compliance with therapy.

Serum drug concentration is influenced by many factors. Fluconazole and flocytosin in most preparations are excreted by the kidney as active drugs, and itraconazole and voriconazole metabolism in the liver involves specific enzymes, predominantly CYP3A4, CYP2C9, CYP3A4 and CYP2C19[80-83]. Genetic polymorphisms of the enzyme[84] involved and complications in the liver or kidney can reflect the serum level of these antifungal agents.

The absorption of some antifungal agents requires special conditions. For example, absorption of itraconazole from capsule formulations is *p*H-dependent and requires an acidic environment, which is favoured by a full meal or a cola drink[84,85]. In contrast, absorption of the oral solution is enhanced in the fasted state[86]. Several patient-specific factors have been demonstrated to affect azole absorption including food (especially fat), gastric *p*H and the use of proton pump inhibitors, diseases associated with chemotherapy, and the frequency of administration (because of saturable absorption)[87,88]. Differences in serum drug levels between healthy volunteers and patients have been reported in connection with these factors[89,90]. Another important pharmacokinetic variable is drug interaction between azoles and rifampicin, carbamazepine, long-acting barbiturates, ritonavir, efavirenz and rifabutin[91], and most notably with cytochrome P450-inducing drugs[88].

The clinical use of flucytosine is limited due to its gastrointestinal, haematological and neurological toxicity, the rapid development of resistance when used as monotherapy, and the lack of parenteral formulations[92]. Accordingly, serum flucytosine concentration should be monitored to identify clinical indications, drug interactions and toxicity[93-95].

Fluconazole is primarily eliminated via renal excretion, with approximately 80 per cent of the unchanged drug appearing in the urine. Thus, dosage adjustments are warranted in patients with a creatinine clearance <50 ml/min[96]. When the susceptibility pattern of the drug is dose-dependent and in patients with renal dysfunction, monitoring can help improve the response.

Routine monitoring methods are available for lipophilic triazoles, itraconazole, voriconazole and posaconazole[78]. High performance liquid chromatography and bioassays have been developed to monitor the serum concentrations of triazoles, although the former method is the assay of choice when available[97]. Additional studies are needed to measure serum levels and determine the best timing to achieve optimum concentrations of different antifungal agents in different populations. The efficacy and safety of antifungal agents are influenced by serum concentrations, clinical factors and the patient's physiological condition; all these factors can therefore, provide clinically useful information to monitor steady-state concentrations in patients with serious IFI.

Conclusion

The best approach to the optimal management of fungal infection is early detection and identification of the causal agent, so that appropriate treatment can be initiated as soon as possible, especially in immunocompromised patients. Clinicians should be familiar with the use of diagnostc methods and suitable antifungal agents, because these are the factors with the greatest impact on the outcome for patients. The serum concentration of antifungal agents should be monitored to record both the efficacy and toxicity of these drugs.

Acknowledgment

The authors thank Dr Hassan Khajehei and K. Shashok for their help in writing the manuscript.

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