Chapter 9
Emergence of Invasive Fungal Infection: Diagnosis and Treatment in Humans

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9.1 Introduction

Fungi are opportunistic pathogens (endogenous or exogeneous) that are freely present in the environment and cause either no or very mild symptoms in healthy individuals, but manifest a fatal physical morbid state in immunocompromised individuals. Invasive fungal infections are those infections wherein fungal pathogens have invaded and colonized the deep tissues, which results in prolonged illness (Badiee and Hashemizadeh 2014). The Invasive Fungal Infections Cooperative group (IFICG) of the European Organisation for Research and Treatment of Cancer (EORTC) and the Mycology Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAID) define invasive fungal infections as the presence of fungal elements either as mold or yeast in deep tissues or needle aspiration biopsy that is further confirmed by culturing and histopathological examination (Ramana et al. 2013). The innate immune system of healthy individuals combats the invasion from fungal infection and does not acquire invasive fungal infections, except exhibiting mild symptoms. The reason for this is that the skin barrier of healthy individuals and the innate and adaptive immunities provide defense against these infections in most normal situations. In the case of patients with severe burns or severely impaired immune system, the chances of invasive fungal infections increase significantly. Invasive fungal infections can affect any part of the body and result in morbidity as well as enhanced mortality chances in immunocompromised individuals due to weakened immune system in response to these infections. These individuals can also have multiple comorbidities (Hope et al. 2013). The number of

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patients at risk of suffering from invasive fungal infections is increasing worldwide. Research in this area has attracted attention due to: (a) the complex nature of infections; (b) often there being multiple infections occurring at the same time; and (c) its diagnosis and treatment. In addition to the immunocompromised individuals, the patients undergoing immunosuppressive therapy, major surgery, hematopoietic stem cell transplantation (HSCT), and the patients with neoplastic diseases, acquired immune deficiency syndrome (AIDS), chronic pulmonary diseases, etc. form a high-risk group of individuals (Fig. 9.1) (Badiee and Hashemizadeh 2014; Person et al. 2010). Invasive fungal infections have also been increasingly reported in individuals with grafts, prosthetic devices, and also in the patients who underwent any major aggressive surgery or organ transplantation (Person et al. 2010). There are several factors that can increase the chances of invasive fungal infections in individuals. One of the major reasons is the use of broad-spectrum antibiotics and antineoplastic and immunosuppressive agents (Enoch et al. 2006; Ravikumar et al. 2015). The increased predisposition of patients with pancreatitis, human immunodeficiency virus (HIV) infection, burns, and neutropenia to fungal infection are other reasons for increased risk of invasive fungal infections (Ruping et al. 2008). Various reports have demonstrated that the chances of invasive fungal infections are also determined by several factors, which include both genetic as well as environmental conditions (Maskarinec et al. 2016; Benedict and Park 2014).

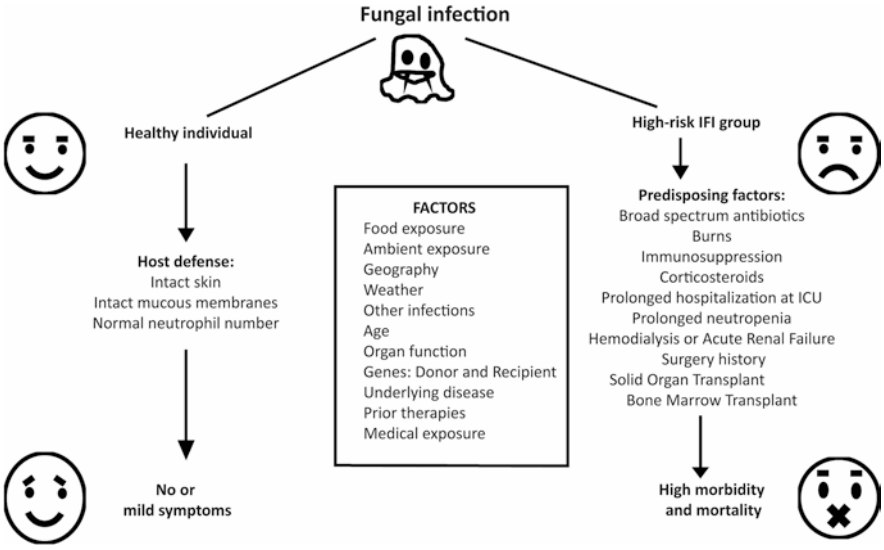

Fig. 9.1 Factors promoting invasive fungal infections and the formation of a high-risk invasive fungal infection group

9.2 Invasive Fungal Infections

The most important fungal pathogens causing invasive fungal infections are yeasts (*Candida* spp., *Cryptococcus* spp., and *Pneumocystis* spp.) and molds (*Aspergillus* spp., *Fusarium* spp., *Scedosporium prolificans*, *Mucor*, *Rhizopus*, *Rhizomucor*, *and Absidia*) (Ramana et al. 2013). Of these, *Cryptococcus*, *Candida*, *Aspergillus*, and *Pneumocystis* collectively cause more than 90% of the deaths due to invasive fungal infections globally (Brown et al. 2012). These infections can be transmitted by the inhalation of spores, penetration into the mucosa by commensal organisms, such as *Candida albicans*, and the ingestion of contaminated food (Badiee and Hashemizadeh 2014). The resulting infection might be mild or life-threatening, depending upon various factors, as shown in Fig. 9.1. Invasive fungal infections are associated with high morbidity and mortality rates. For instance, mortality associated with invasive candidiasis varies from 50 to 75%, while it can reach almost 100% in the case of invasive aspergillosis (Pfaller and Diekema 2007). Delay in antifungal therapy to treat invasive fungal infections also increases mortality and, therefore, requires early diagnosis and medical intervention, but both of which are very challenging (Badiee and Hashemizadeh 2014; Morrell et al. 2005). Table 9.1 lists the major invasive fungal infections and shows the statistics for invasive fungal infections worldwide.

In the following sections, the major invasive fungal infections and their diagnosis and treatment will be discussed.

9.2.1 Aspergillosis

Aspergillosis is the most common fungal infection in humans and is caused by a group of filamentous fungi, *Aspergillus* (Hartwick and Batsakis 1991; Schmiedel and Zimmerli 2016). *Aspergillus* is mostly found in soil, food, air, water, and

Table 9.1 Statistics of the most significant invasive fungal infections (Brown et al. 2012)

Disease	Causative agent	Mortality rates in infected populations (%)
Aspergillosis	*Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus flavus*	30–95
Candidiasis	*Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*	46–75
Cryptococcosis	*Cryptococcus neoformans* and *Cryptococcus gattii*	20–70
Mucormycosis	*Rhizopus oryzae*, *Rhizopus microsporus*, and *Rhizomucor pusillus*	30–90
Pneumocystis	*Pneumocystis jirovecii*	20–80

decomposing matter (Anaissie et al. 2002; Tamgadge et al. 2012) and cause a gradual destructive disease of the lungs called chronic pulmonary aspergillosis, which complicates other pulmonary-related conditions or diseases, such as tuberculosis, chronic obstructive pulmonary disease (COPD), and systemic inflammatory disease. There are several species of this genus but *A. fumigatus, A. niger*, and *A. flavus* are the most common causative agents of aspergillosis (Sethi et al. 2012). According to the guidelines of the Infectious Diseases Society of America (IDSA) for the treatment of aspergillosis, there are three major types of aspergillosis: invasive aspergillosis, pulmonary aspergilloma, and allergic bronchopulmonary aspergillosis (ABPA) (Stevens et al. 2000). Pulmonary aspergilloma, a saprophytic form of aspergillosis, usually develops in a pre-existing cavity in the lung and results from the ingrowth of colonized *Aspergillus*, while allergic bronchopulmonary aspergillosis is an immunological pulmonary disorder caused by hypersensitivity to *Aspergillus fumigatus* (Agarwal et al. 2013). However, there are also reports showing that, under certain circumstances, the colonization of airways by *Aspergillus* species can transform into an invasive disease (Shahi et al. 2015; Gago et al. 2018). Invasive aspergillosis is a devastating infection and is highly lethal in immunocompromised individuals. The lungs are the most common sites of primary invasive disease, while the central nervous system (CNS) is the secondary site of this invasive disease (Anagnostou et al. 2014; Mohammadi et al. 2015). Invasive aspergillosis leads to a mortality rate of around 50% even if diagnosed and treated, but delayed diagnosis may be 100% fatal (Latge 1999).

9.2.1.1 Diagnosis

Diagnosing aspergillosis remains an obstacle and early diagnosis in immunocompromised individuals is also one of the major challenges. *Aspergillus* is common in the environment and can be found in the saliva and sputum of healthy people. The symptoms of aspergillosis are similar to those of tuberculosis. Therefore, the isolation of *Aspergillus* spp. in sputum samples is highly determined by the immune status of the host (Garnacho-Montero et al. 2005). For instance, invasive aspergillosis cannot be ruled out by negative sputum samples; on the other hand, invasive disease can be easily established in immunocompromised patients. Apart from this, as high as 70% of negative sputum samples could still have invasive aspergillosis disease (Tang and Cohen 1992; Yu et al. 1986). Histopathological examination followed by bronchoalveolar lavage, transthoracic needle biopsy (TNB), or video-assisted thoracoscopic surgery are standard procedures for the diagnosis of invasive aspergillosis (Walsh et al. 2008). Chest X-ray and computed tomography (CT) scan are good tools to diagnose aspergilloma as well as the characteristic signs of invasive and allergic bronchopulmonary aspergillosis, but they have some limitations (Hope et al. 2013). Chest X-ray is of less significance in the early stages of invasive disease, while the CT scan in combination with high-resolution images (HRCT) is more useful (Caillot et al. 1997, 2001). A recent advancement in the diagnosis of

invasive aspergillosis is the detection of *Aspergillus* antigens, such as galactomannan and (1 → 3)-beta-D-glucan in the body fluids of patients. A double-sandwich enzyme-linked immunosorbent assay (ELISA) kit approved by the U.S. Food and Drug Administration (US FDA) for the detection of galactomannan that is released by *Aspergillus* during its growth in the patient's serum provides a non-culture-based diagnosis of invasive aspergillosis (Boutboul et al. 2002; Marr et al. 2004a). In this test, the patient's sputum is stained with a dye, which identifies the presence of *Aspergillus*.

9.2.1.2 Treatment

Oral corticosteroids are one of the best ways to treat bronchopulmonary aspergillosis (Rosenberg et al. 1978; Wang et al. 1979; Capewell et al. 1989). Antifungal medications are not very helpful in allergic bronchopulmonary aspergillosis, but combination with corticosteroids can improve lung condition and its functioning. In certain situations of aspergilloma, surgical resection is required to remove the fungal mass, but the surgical route should be avoided, as it is risky (Uflacker et al. 1985; Soltanzadeh et al. 1977; Massard et al. 1992).

The management of invasive pulmonary aspergillosis is difficult. Voriconazole is considered the first-line therapy for invasive aspergillosis infections caused by these pathogens (Johnson and Kauffman 2003; Sambatakou et al. 2006; Ghannoum and Kuhn 2002; Herbrecht et al. 2002; Limper et al. 2011; Walsh et al. 2008). Voriconazole, a broad-spectrum triazole, is available in both intravenous and oral formulations. Although voriconazole has a milder side-effect profile, it can potentially interact with many significant drugs, such as cyclosporine, warfarin, terfenadine, carbamazepine, quinidine, rifampin, statins, and sulfonylureas (Johnson and Kauffman 2003; Sambatakou et al. 2006; Ghannoum and Kuhn 2002; Herbrecht et al. 2002; Limper et al. 2011).

Isavuconazole, a newer triazole, has been approved for the treatment of invasive aspergillosis and is more tolerable than voriconazole (Spitzer et al. 2017). Amphotericin B products can be used as second-line agents in patients who cannot tolerate or are failing voriconazole therapy (Cornely et al. 2007). To reduce the side effects associated with amphotericin, which include nephrotoxicity, electrolyte disturbances, and hypersensitivity, a new lipid-based preparation of amphotericin B, such as liposomal amphotericin B and lipid complex amphotericin B, has been introduced and used for treatment (Cornely et al. 2007). Echinocandin derivatives such as caspofungin, micafungin and anidulafungin are also effective agents in the treatment of invasive aspergillosis and can be used as salvage therapy. Another broad-spectrum triazole, posaconazole, is effective and safe as salvage therapy in patients with invasive pulmonary aspergillosis refractory to standard antifungal therapy (Pitisuttithum et al. 2005; Spanakis et al. 2006; Cohen-Wolkowiez et al. 2006). The combined therapy of caspofungin and liposomal amphotericin B is more successful as a primary rather than a salvage therapy (Koulenti et al. 2014). In case

of failure of this therapy, the combination of caspofungin and voriconazole is also recommended (Marr et al. 2004b). Combinations of antifungal therapies have also been used as salvage therapy in many high-risk patients (Elizabeth et al. 2015).

9.2.2 Candidiasis

Candida species are the most common causative agents of fungal infections, which can range from a non-life-threatening state such as mucocutaneous illnesses to invasive infection that may affect many organs. There are more than 17 different species of *Candida* that have been identified to cause invasive candidiasis and, of these species, only five species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*) are responsible for more than 90% of invasive candidiasis (Pfaller and Diekema 2007). Among these *Candida* species, *C. albicans* is the most common infectious agent, while *C. glabrata* is the second most common species causing invasive candidiasis (Achkar and Fries 2010; Pfaller and Diekema 2007). *Candida* species form a highly structured biofilm under different environmental conditions. Surface-associated *Candida* can grow embedded in extracellular matrix or biofilm, which is composed of carbohydrates and proteins (Achkar and Fries 2010). *Candida albicans* is distinguished from many other fungal species by its ability to form both yeast cells and hyphae. Hyphae are an important structural component of *C. albicans* biofilm formation (Nobile and Johnson 2015). This is the most common member of the human microbiota, appearing in the oral cavity, gastrointestinal tract, female genital tract, or on the skin (Achkar and Fries 2010; Ganguly and Mitchell 2011; Nobile and Johnson 2015). In the healthy human population, *C. albicans* is harmless, while under the alteration of immune defenses, it overgrows and causes serious systemic disease and organ failure (Prieto and Pla 2015). *Candida albicans* also forms biofilm along with *Staphylococcus aureus* on the surface of implantable medical devices or organs (Peters et al. 2010). Such multispecies infection leads to higher mortality rates than single-species infection (Zago et al. 2015). In addition, infections caused by *C. albicans* can be acquired at hospitals and have become a cause of major health concerns. *Candida albicans* is identified as the fourth most common blood isolate in US hospitals, accounting for around 10% of hospital-acquired bloodstream infections (Horn et al. 2012).

9.2.2.1 Diagnosis

The diagnosis of invasive candidiasis is difficult and complicated because there are no specific clinical manifestations of the disease. Researchers have developed polymerase chain reaction (PCR)-based methods for the detection of *Candida* species in blood samples (Mirhendi and Makimura 2003), DNA-based fluorescence in-situ hybridization (FISH) (Bisha et al. 2011), as well as ELISA for the diagnosis of candidiasis infections (Lain et al. 2007).

9.2.2.2 Treatment

The treatment of *Candida* infections varies substantially and is determined by the anatomic location of the infection and the immunity status of the infected individual. Polyenes, triazoles (fluconazole, itraconazole, voriconazole, and posaconazole), echinocandins (caspofungin, anidulafungin, and micafungin), and flucytosine are the most common antifungal agents for the effective treatment of candidiasis (Pappas et al. 2007, 2009; Pfaller and Diekema 2007). Fluconazole is considered a first-line agent in non-neutropenic patients with invasive candidiasis. Amphotericin B is used for non-albicans species (e.g., *C. krusei*).

9.2.3 Cryptococcosis

Cryptococcosis is the one of the invasive fungal infections that cause significant morbidity and mortality in both immunocompromised and immunocompetent individuals worldwide. Cryptococcosis is caused by basidiomycetous fungi, *Cryptococcus* species. The two most common species that cause cryptococcosis are *C. neoformans*, which mostly affects immunocompromised individuals, and *C. gattii*, which can infect both immunocompromised as well as immunocompetent individuals (Oladele et al. 2017). These species are further divided into different genotypes (VNI, VII, VNB, VNIV, and VGI-IV) (Perfect and Bicanic 2015). The capsule of *C. neoformans* is made up of two polysaccharides, glucuronoxylomannan (GXM) and galactoxylomannan (GalXM), which act as a virulent factor (Zaragoza and Casadevall 2004; Bose et al. 2003; Zaragoza et al. 2009). Infection is caused by inhalation of these fungal spores, so the primary localization site is the lung, from where it spreads through the bloodstream to the CNS. *Cryptococcus* species have the propensity to locate in the CNS and cause fungal meningitis. Pulmonary cryptococcosis may be asymptomatic but meningitis is a common feature of infection, especially in HIV patients and solid-organ transplant recipients (Enoch et al. 2006). For instance, the HIV-infected patients are at higher risk of contracting cryptococcal infection, while the non-HIV patients also get infected by *Cryptococcus* spp., more often if have a history of transplantation or immunosuppressive therapies.

9.2.3.1 Diagnosis

Histopathology varies according to the immunological status of the host. In immunocompetent individuals, typical granulomas with multinucleated giant cells are formed at the site of cryptococcal infection, while in immunosuppressed hosts, cryptococcosis infects the CNS, skin, and the oral mucous membrane. The definitive diagnosis of cryptococcosis is established with periodic acid Schiff (PAS),

mucicarmine stain, and Fontana-Masson stain. The fungal wall stains with the PAS stain, mucicarmine stains the fungal capsule, while Fontana-Masson stains the fungal growth itself because it contains melanin, which is characteristic of cryptococci (Guarner and Brandt 2011). Diagnosis can also be done by the detection of fungal growth in cerebrospinal fluid (CSF) with India ink examination, isolation in tissue culture, and detection of cryptococcal polysaccharide capsular antigen (CrAg) in the serum and in the CSF through latex agglutination or ELISA (Oladele et al. 2017; Amaral et al. 2016).

9.2.3.2 Treatment

For many decades, amphotericin B has been used for treatment in transplant recipients and HIV patients (Sloan and Parris 2014). At present, the combination of liposomal amphotericin B and flucytosine (5-FC) treatment is considered as standard treatment due to its better performance in treating cryptococcal meningitis as compared to amphotericin B treatment alone (Larsen et al. 1990; de Gans et al. 1992; Brouwer et al. 2004; Dromer et al. 2008; Day et al. 2013; Sloan and Parris 2014). This combination is also a good treatment of choice for HIV-infected patients. This combination therapy improved early fungicidal activity (EFA) as well as lowered toxicity. However, although the high doses of amphotericin B and 5-FC have improved fungicidal activity, an increase in serious side effects has been reported (Bicanic et al. 2008). Fluconazole is also recommended as an alternative to flucytosine (Bellmann and Smuszkiewicz 2017; Perfect et al. 2010; Elizabeth et al. 2015). During combination of amphotericin B and fluconazole, higher fluconazole doses gave better outcomes compared to lower fluconazole doses (Yao et al. 2014; Elizabeth et al. 2015)**.** Apart from this, other azoles, such as itraconazole, voriconazole, isavuconazole, and posaconazole, have also been tested for the treatment of cryptococcosis, but they seem unreliable (Chotmongkol and Jitpimolmard 1992; Pitisuttithum et al. 2005; Thompson 3rd et al. 2016; de Gans et al. 1992). Out of these, voriconazole has better absorption and penetration to the CNS than itraconazole and posaconazole and it has been used for cryptococcosis treatment in normal individuals (Yao et al. 2015). The survivors of cryptococcal meningitis HIV-infected patients have notably higher concentrations of cytokine stimulating factor, such as IFN-γ, TNF-α, IL-6 and -8, than the non-survivors of cryptococcal meningitis HIV-infected patients (Siddiqui et al. 2005). Researchers also found that the combination of liposomal amphotericin B, voriconazole, and IFN-γ is successful in the treatment of cryptococcal meningitis in HIV patients (Gamaletsou et al. 2012). In addition to all the antifungal treatments, a new technology is in progress for cryptococcal meningitis treatment. This technology, in combination with the antifungal therapies, would prove to be better for cryptococcal meningitis management (Cutshaw et al. 2016).

9.2.4 Mucormycosis

Mucormycosis is an infection caused by members of the order Mucorales (Ibrahim et al. 2012). Mucorales are distributed into six families and all members of the six families cause cutaneous and deep infections. *Rhizopus oryzae*, *R. microsporus*, and *R. pusillus* are the most common species isolated from patients with mucormycosis and are responsible for around 70% of all cases of mucormycosis. This causative agent is ubiquitous in nature and mostly found in soil and decaying organic matter. Because of the abundance of this fungus, the mouth and the nose inhale the spores very often. In a normal person, the macrophage cells phagocytize the spores, while in immunocompromised individuals, the spores germinate into the hyphae and cause infections in the sinuses and lungs.

9.2.4.1 Different Forms of Mucormycosis

Mucormycosis invades the blood vessels, producing thrombosis and tissue infarction. It causes five major forms of infections (Prabhu and Patel 2004): (i) rhino-orbitocerebral, (ii) pulmonary, (iii) disseminated, (iv) cutaneous, and (v) gastrointestinal. Rhino-orbitocerebral is the most common form of mucormycosis, while pulmonary mucormycosis is a rare pulmonary fungal infection and difficult to diagnose. After aspergillosis and candidiasis, mucormycosis is the third most common invasive fungal infection (Hammond et al. 2011).

9.2.4.1.1 Rhino-orbitocerebral Mucormycosis

This is the most common form of mucormycosis and is mostly found in diabetic patients. The disease starts from the nose and progresses into the sinuses, orbit, and then intracranially. The initial symptoms are sinusitis or periorbital cellulitis, facial pain, blurry vision, and soft tissue swelling. If untreated, infection spreads from the ethmoid sinus to the orbit, resulting in loss of extraocular muscle function (Spellberg et al. 2005). This is also divided into two major forms: (i) highly fatal rhino-orbitocerebral and (ii) less fatal rhino-orbitocerebral. The former involves ophthalmic and internal carotid arteries, while the latter involves the sphenopalatine and greater palatine arteries, resulting in thrombosis of turbinates and necrosis of the palate (Yanagisawa et al. 1977).

9.2.4.1.2 Pulmonary Mucormycosis

Pulmonary mucormycosis is the most common form found in neutropenic and stem cell-transplant patients. It may develop as a result of inhalation or by hematogenous or lymphatic spread. Its symptoms include dyspnea, cough, and chest pain. Patients

with untreated pulmonary mucormycosis usually die from disseminated disease before respiratory failure occurs (Tedder et al. 1994). The overall mortality rate of pulmonary mucormycosis is approximately 50–70%, but is >95% if the pulmonary mucormycosis is part of a disseminated process (Spellberg et al. 2005).

9.2.4.1.3 Disseminated Mucormycosis

Disseminated mucormycosis may originate from any primary site of infection. Pulmonary mucormycosis has the highest incidence of dissemination. The most common site of dissemination is the brain, but metastatic lesions may also be found in the spleen, heart, skin, and other organs. The mortality associated with dissemination to the brain is about 100%. Even without CNS involvement, disseminated mucormycosis has a mortality of >90%. The diagnosis of disseminated disease is difficult because patients are usually severely ill from multiple diseases. When disseminated mucormycosis is suspected, a careful search should be made for cutaneous lesions that can be biopsied for diagnostic purposes (Spellberg et al. 2005).

9.2.4.1.4 Cutaneous Mucormy**c**osis

The mucormycosis agent cannot penetrate the skin but individuals with a disrupted protective cutaneous barrier are at high risk of cutaneous mucormycosis. Cutaneous mucormycosis can be very invasive and penetrate from the cutaneous and subcutaneous tissues into the adjacent fat, muscle, and even bone (Spellberg et al. 2005).

9.2.4.1.5 Gastrointestinal Mucormycosis

Gastrointestinal mucormycosis is a rare form of mucormycosis. It mainly occurs in extremely malnourished individuals, especially infants or children. The stomach, colon, and ileum are the most commonly involved sites during gastrointestinal mucormycosis. Non-specific abdominal pain and distention associated with nausea and vomiting are the most common symptoms (Spellberg et al. 2005).

9.2.4.2 Diagnosis

The diagnosis of mucormycosis is challenging and treatment should start as early as possible in order to decrease mortality. Although the diagnosis is difficult, some tests such as CT scan or magnetic resonance imaging (MRI) may be helpful to define the infections or tissue destruction. Histopathology is the gold standard for this diagnosis. The detection of fungal DNA in tissue samples by PCR is a non-culture-based method that may allow improved diagnosis of mucormycosis (Hammond et al. 2011).

9.2.4.3 Treatment

There are some important factors for the eradication of mucormycosis : rapidity of diagnosis, appropriate surgical removal of infected tissue, and appropriate antifungal therapy. For patients with mucormycosis, surgical treatment plus antifungal therapy is better than the use of antifungal therapy alone (Spellberg et al. 2005). Polyene-based antifungal therapy, such as amphotericin B, is the primary therapy for mucormycosis treatment. Amphotericin B deoxycholate (d-AmB) is the only antifungal agent for the treatment of mucormycosis that has been approved by the US FDA. d-AmB has been replaced by the various lipid formulations of amphotericin. Posaconazole is better than itraconazole and isavuconazole. Fluconazole and voriconazole have no meaningful activity against agents of mucormycosis. No other azoles except for posaconazole are recommended in the treatment of mucormycosis. Flucytosine lacks activity against agents of mucormycosis (Skiada et al. 2013). Liposomal amphotericin B is frequently used and is the most effective drug against mucormycosis. Lipid amphotericin B is also combined with echinocandins to treat mucormycosis (Spellberg and Ibrahim 2010).

9.2.5 Pneumocystis Pneumonia

Pneumocystis pneumonia is an opportunistic infection that occurs in immunosuppressed individuals, primarily individuals with HIV infections, caused by the invasion of the unicellular fungus, *Pneumocystis jirovecii*. Initially, it was misclassified as protozoan, on the basis of its morphology, but, later, it was considered as fungi (Thomas Jr. and Limper 2004). *Pneumocystis* isolated from one species cannot infect another species, meaning that the microbe is host-specific. There are five major species-specific *Pneumocystis* species: *P. carinii* and *P. wakefieldiae* in rats, *P. murina* in mice, *P. oryctolagi* in rabbits, and *P. jirovecii* in humans (Aliouat-Denis et al. 2008; Sokulska et al. 2015). Transmission occurs via aerosols from patients with pneumonia or from early-life contact with family or community members who carry the organism in their lungs (Brown et al. 2012). Its presence in lungs is asymptomatic. Pneumocystis pneumonia has been one of the main causes of morbidity and mortality among HIV-infected people (Sokulska et al. 2015), but the death rate in non-HIV individuals (30–60%) is significant higher than that in HIV-infected individuals (Sokulska et al. 2015; Schmiedel and Zimmerli 2016).

9.2.5.1 Diagnosis

Pneumocystis pneumonia is difficult to diagnose because of the non-specific symptoms and signs of infection. The diagnosis of pneumocystis pneumonia requires microscopic detection of *P. jirovecii* in respiratory tract specimens, followed by staining with dyes or antibodies. Cysts or trophozoites are morphologically

identified by methenamine silver nitrate or Giemsa stains (Sowden and Carmichael 2004). Pneumocystis from sputum, bronchoalveolar fluid, or lung tissue can also be identified by reverse transcription (RT)-PCR. Microscopic methods have limited sensitivity, while RT-PCR has high sensitivity and can be implemented as a rapid routine diagnostic test. (1 → 3)-beta-D-glucan in association with lactate dehydrogenase has been used as a biomarker in the case of HIV-infected patients (Esteves et al. 2014; Held et al. 2011). Up to now, attempts to culture *P. jirovecii* have been failures, except for recently, when a three-dimensional air–liquid interface culture system made up of differentiated pseudostratified airway epithelial cell line named as CuFi-8 cells has been demonstrated for culturing *P. jirovecii* (Schildgen et al. 2014).

9.2.5.2 Treatment

Typical antifungal drugs have been found to be not effective for *Pneumocystis* infection. Folic acid is needed for the synthesis of purines, glycine, and thymidylate, which are necessary for the proper functioning of an organism. The lifecycle on *P. jirovecii* also depends on folic acid synthesis but disruption of the folic acid synthesis pathway results in failed acquisition of the folic acid from the environment. Thus, the folic acid synthesis pathway has been used as a target for therapeutic agents for pneumocystis pneumonia treatment. Dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) are two important enzymes involved in the folic acid synthesis pathway (Volpe et al. 1993). Trimethoprim (TMP) and a sulfa drug named sulfamethoxazole (SMZ) are inhibitors of the DHFR and DHPS enzymes, respectively, and have been used as agents to treat pneumocystis pneumonia (Lobo et al. 2013). The most effective treatment for pneumocystis pneumonia is the combination of these two drugs, as has been shown in HIV-negative individuals (Thomas Jr. and Limper 2004). This drug can be taken either orally or by intravenous infusion. However, in HIV-positive individuals, sulfamethoxazole causes allergic reactions, so sulfamethoxazole should be replaced by dapsone. Apart from this drug, pentamidine, prednisone, a combination of clindamycin and primaquine, and a combination of trimetrexate and leucovorin are also used. The inhibition effect of TMP-SMZ can be further enhanced by the addition of low doses of caspofungin, which is an inhibitor of the (1 → 3)-beta-D-glucan synthase, an enzyme required for the synthesis of cell walls of many fungi. Caspofungin and clindamycin have been reported to be a salvage therapy to treat immunocompromised patients having allergies or adverse reactions to TMP-SMZ (Li et al. 2016).

9.3 Conclusion and Future Implications

Fungi are opportunistic infectious agents and most of them are usually not pathogenic to normal healthy individuals. However, when they infect individuals at high risk of invasive fungal infection, they cause a wide range of diseases, ranging from superficial infections to disseminated infections of the vital internal organs. Invasive fungal infections continue to be a major problem due to improved medical care among individuals at high risk of invasive fungal infections. Despite the recent efforts, early diagnosis and intervention still need to be addressed due to their high associated mortality and morbidity rates. There have been significant advances in therapeutic options for invasive infections caused by *Aspergillus* and *Candida* species. Successful treatment of these opportunistic infections requires prompt diagnosis and aggressive therapy with antifungal agents. Inhalation of spores of these microorganisms is the most common mode of infection in a susceptible host (Deepa et al. 2014). To better understand the problem, we need to define accurate epidemiological data, the socioeconomic impact of the disease, and also stimulate scientific interest in this field. To tackle these challenges, we need to focus on: (i) development of better and rapid diagnostics tools which have an immediate impact on the mortality rate and (ii) development of more effective and less toxic antifungal drugs/vaccines, especially for the treatment of immunocompromised patients. By the use of combination drug therapy, we can increase the effectiveness of the drug and minimize the risk of development of resistance and decrease toxicity (Elizabeth et al. 2015). During vaccination development against invasive fungal infections, efforts should be focused on identifying epitopes with immunogenic T cell and adjuvants. Attention directed towards these goals would have a significant effect in reducing the global burden and negative impact of invasive fungal infections.

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