Chapter 6

Fungal Infections in Immunosuppressed Patients

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Additional information is available at the end of the chapter

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1. Introduction

Fungal infections, also called mycoses, are important causes of morbidity and mortality in humans. Some fungal infections are endemic, and these infections are usually caused by fungi that are present in the environment and whose spores enter humans. Other fungal infections are said to be opportunistic because the causative agents cause mild or no disease in healthy individuals but may infect and cause severe disease in immunodeficient persons. The human airway is continuously open to the nonsterile environment where fungal spores have the potential to reach lung tissue and produce disease. In the immunocompromised host, many fungi, including species of fungi typically considered nonpathogenic, have the potential to cause serious morbidity and mortality. Over the last several decades the advent of the human immunodeficiency virus (HIV) epidemic and the increasing use of immunosuppressive drugs for serious medical conditions have dramatically increased the number of persons who are severely immunocompromised. In addition, the range and diversity of fungi that cause disease have broadened. Although *Candida* and *Aspergillus* species continue to be the fungal pathogens that most frequently cause invasive fungal disease in immunocompromised persons overall, infections due to previously uncommon hyaline and dematiaceous filamentous fungi are being reported with increasing frequency. This is significant because, despite marked advances in antifungal therapy, infections caused by opportunistic fungal infections (rare and emerging) continue to be associated with high morbidity, high mortality, and poor patient outcomes. This results from a combination of drug-resistant strains, lack of robust clinical studies evaluating treatments, and severe underlying diseases in the patient [2].

The principal mediators of innate immunity against fungi are neutrophils and macrophages. Patients with neutropenia are extremely susceptible to opportunistic fungal infections. Phagocytes and dendritic cells sense fungal organisms by TLRs and lectin-like receptors called dectins . Neutrophils presumably liberate fungicidal substances, such as reactive oxygen species and lysosomal enzymes, and phagocytose fungi for intracellular

killing. Many extracellular fungi elicit strong TH17 responses, which are driven in part by the activation of dendritic cells by fungal products binding to the dectin receptor and resulting production of TH17- inducing cytokines (IL-6, IL-23) from the dendritic cells. The TH17 cells stimulate inflammation, and the recruited neutrophils and monocytes destroy the fungi. *Candida* infections often start at mucosal surfaces, and cell-mediated immunity is believed to prevent spread of the fungi into tissues. TH1 responses are protective in intracellular fungal infections, such as histoplasmosis, but these responses may elicit granulomatous inflammation, which is an important cause of host tissue injury in these infections. Fungi also elicit specific antibody responses that are of protective value [2].

2. Candidiasis

Candidiasis is caused by infection with species of the genus *Candida,* predominantly with *Candida albicans. Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for *Candida* species to invade tissues normally resistant to invasion. *Candida* species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues [3].

The increased prevalence of local and systemic disease caused by these yeasts has resulted in numerous new clinical syndromes, the expression of which depends primarily on the immune status of the host. *Candida* species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illnesses. The clinical manifestations may be acute, subacute or chronic to episodic. Involvement may be localized to the mouth, throat, skin, scalp, vagina, fingers, nails, bronchi, lungs, or the gastrointestinal tract, or become systemic as in septicemia, endocarditis and meningitis. In healthy individuals, Candida infections are usually due to impaired epithelial barrier functions and occur in all age groups, but are most common in the newborn and the elderly. They usually remain superficial and respond readily to treatment. Systemic candidiasis is usually seen in patients with cell-mediated immune deficiency, and those receiving aggressive cancer treatment, immunosuppression, or transplantation therapy. The management of serious and life-threatening invasive candidiasis remains severely hampered by delays in diagnosis and the lack of reliable diagnostic methods that allow detection of both fungemia and tissue invasion by *Candida* species [4].

The first step in the development of a candidal infection is colonization of the mucocutaneous surfaces. All of the factors outlined above are associated with increased colonization rates. The routes of candidal invasion include (1) disruption of a colonized surface (skin or mucosa), allowing the organisms access to the bloodstream, and (2) persorption via the gastrointestinal wall, which may occur following massive colonization with large numbers of organisms that pass directly into the bloodstream [5].

Candida species are the most common cause of fungal infection in immunocompromised persons. Oropharyngeal colonization is found in 30-55% of healthy young adults, and *Candida* species may be detected in 40-65% of normal fecal microbiota [6, 7].

Candida species are yeastlike fungi that can form pseudohyphae and some species can develop true hyphae, as *Candida albicans* do. For the most part, *Candida* species are confined to human and animal reservoirs; however, they are frequently recovered from the hospital environment, including on foods, countertops, air-conditioning vents, floors, respirators, and medical personnel. They are also normal commensals of diseased skin and mucosal membranes of the gastrointestinal, genitourinary, and respiratory tracts [8-10].

Candida species also contain their own set of well-recognized but not well-characterized virulence factors that may contribute to their ability to cause infection. The main virulence factors include the following [11]:

- Surface molecules that permit adherence of the organism to other structures (eg, human cells, extracellular matrix, prosthetic devices)
- Acid proteases and phospholipases that involve penetration and damage of cell envelopes
- Ability to convert to a hyphal form (phenotypic switching)

As with most fungal infections, host defects also play a significant role in the development of candidal infections. Host defense mechanisms against *Candida* infection and their associated defects that allow infection are as follows:

- Intact mucocutaneous barriers - Wounds, intravenous catheters, burns, ulcerations
- Phagocytic cells -Granulocytopenia
- Polymorphonuclear leukocytes - Chronic granulomatous disease
- Monocytic cells -Myeloperoxidase deficiency
- Complement -Hypocomplementemia
- Immunoglobulins -Hypogammaglobulinemia
- Bone marrow transplantation
- Solid organ transplantation (liver, kidney)
- Parenteral hyperalimentation
- Hematologic malignancies
- Foley catheters
- Solid neoplasms
- Recent chemotherapy or radiation therapy
- Corticosteroids
- Broad-spectrum antibiotics
- Burns
- Prolonged hospitalization
- Gastrointestinal tract surgery
- Central intravascular access devices
- Premature birth

- Hemodialysis
- Acute and chronic renal failure

Over 200 species of *Candida* exist in nature; thus far, only a few species have been associated with disease in humans.

- The medically significant *Candida* species include the following [12]:
  - *C albicans,* the most common species identified (50-60%)
  - Candida glabrata (previously known as Torulopsis glabrata) (15-20%)
  - C parapsilosis (10-20%)
  - Candida tropicalis (6-12%)
  - Candida krusei (1-3%)
  - Candida kefyr (< 5%)
  - Candida guilliermondi (< 5%)
  - Candida lusitaniae (< 5%)
  - *Candida dubliniensis,* primarily recovered from patients infected with HIV

C glabrata and *C albicans* account for approximately 70-80% of *Candida* species recovered from patients with candidemia or invasive candidiasis. *C glabrata* has recently become very important because of its increasing incidence worldwide, its association with fluconazole resistance in up to 20% of clinical specimens, and its overall decreased susceptibility to other azoles and polyenes.

C krusei is important because of its intrinsic resistance to ketoconazole and fluconazole (Diflucan); it is also less susceptible to all other antifungals, including itraconazole (Sporanox) and amphotericin B.

Another important *Candida* species is *C lusitaniae;* although not as common as other *Candida* species, *C lusitaniae* is of clinical significance because it may be intrinsically resistant to amphotericin B, although it remains susceptible to azoles and echinocandins.

C parapsilosis is also an important species to consider in hospitalized patients. It is especially common in infections associated with vascular catheters prosthetic devices. Additionally, *in vitro* analyses have shown that echinocandins have a higher minimum inhibitory concentration (MIC) against *C parapsilosis* than other *Candida* species. The clinical relevance of this *in vitro* finding has yet to be determined [13].

C tropicalis has frequently been considered an important cause of candidemia in patients with cancer (leukemia) and in those who have undergone bone marrow transplantation.

The diagnosis of almost any form of *Candida* disease requires an integration of clinical, epidemiological, and laboratory findings. Unfortunately, results from the routine laboratory studies are often nonspecific and not very helpful. Clinicians are required to act definitively and early based on a high index of suspicion. In the past, many patients with life-threatening candidiasis died without receiving antifungal therapy. Systemic candidiasis should be suspected in patients with persistent leukocytosis and either persistent neutropenia or other risk factors and who remain febrile despite broad-spectrum antibiotic

therapy. To be effective, antifungal therapy should be provided early and empirically in such high-risk patients. Cultures of nonsterile sites, although not useful for establishing a diagnosis, may demonstrate high degrees of candidal colonization. Always consider positive culture results from sterile sites to be significant and evidence of infection [14].

Candidemia and disseminated candidiasis [14, 15].

- Blood cultures are helpful but yield positive results in only 50-60% of cases of disseminated infection.
- Urinalysis may be helpful and may show either colonization or renal candidiasis.
- The serum (1,3)β-D-glucan detection assay (Glucatell, Fungitell) is a nonculture assay that was approved for use in the United States in May 2004. This assay measures the level of β-glucan (a fungal cell wall component). In a large multicenter study, the assay yielded a high specificity and positive predictive value with highly reproducible results [15].
- Cultures of nonsterile sites, although not useful for establishing a diagnosis, may be useful for initiating antifungal therapy in patients with fever that is unresponsive to broad-spectrum antimicrobials. Therefore, appropriate interpretation is required. Positive results from blood cultures and cultures from other sterile sites always imply the presence of invasive disease. Positive results from sterile sites should always be taken as significant and should always prompt treatment.
- Gastrointestinal, respiratory, and urinary tract cultures that are positive for *Candida* may not always represent invasive disease. However, these should be considered sites of colonization.

- Species identification [14]
  - *C albicans, C dubliniensis,* can be identified morphologically via germ-tube formation (hyphae are produced from yeast cells after 2-3 h of incubation) or biochemical assays.
  - CHROMagar *Candida* allows for the presumptive identification of several *Candida* species by using color reactions in specialized media that demonstrate different colony colors depending on the species of *Candida.*
  - API20C and API32C are biochemical assays that allow for the identification of the different *Candida* species with more precision. These assays evaluate the assimilation of numerous carbon substrates and generate profiles used in the identification of different fungal species.
  - *The C albicans* peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) test can be used to identify *C albicans in* 24-48 hours when the probe is added to smears that are made directly from the blood culture bottle and followed by hybridization. A newer version of this test now allows for the simultaneous identification of either *C albicans* or *C glabrata* [16, 17].
- Antifungal susceptibility testing [18]
  - In vitro susceptibility testing for *Candida* species is now standardized using the Clinical Laboratory Standards Institute (CLSI) microbroth dilution (CLSI M27-A2, 2002) or the disk diffusion (CLSI M44-P, 2003) methodology. This was formerly

known as the National Committee for Clinical Laboratory Standards (NCCLS) microbroth dilution.
    - These methods may be helpful in guiding difficult therapeutic decisions. Most of the difficult decisions involve antifungal-refractory oral or esophageal candidiasis in patients with advanced HIV disease.
- Nonculture *Candida* detection assays [18-20]
    - The *Candida* mannan assay yields a sensitivity of 31-90% (less for non-*albicans Candida* species).
    - The *Candida* heat labile antigen assay yields a sensitivity of 10-71%.
    - The D-arabinitol assay yields a sensitivity of 50% but is not useful for infection with *C krusei* or *C glabrata*.
    - The enolase assay yields a sensitivity of 55-75%, which improves with serial testing.
    - The (1,3)β-D-glucan assay is an amebocyte lysis assay with a sensitivity of 75-100% and a specificity of 88-100%. It is a broad-spectrum assay that detects *Aspergillus, Candida, Fusarium, Acremonium,* and *Saccharomyces* species. β-D-glucan is a cell wall component in a wide variety of fungi and can be detected based on its ability to activate factor G of the horseshoe crab coagulation cascade. The Fungitell assay may be used in the evaluation of invasive fungal infections caused by the fungi mentioned above. The assay does not detect infections caused by *Cryptococcus neoformans* or Zygomycetes.
    - Molecular assays such as the polymerase chain reaction (PCR) assay and DNA probes are still under development and in the early investigational phases, but they appear promising.

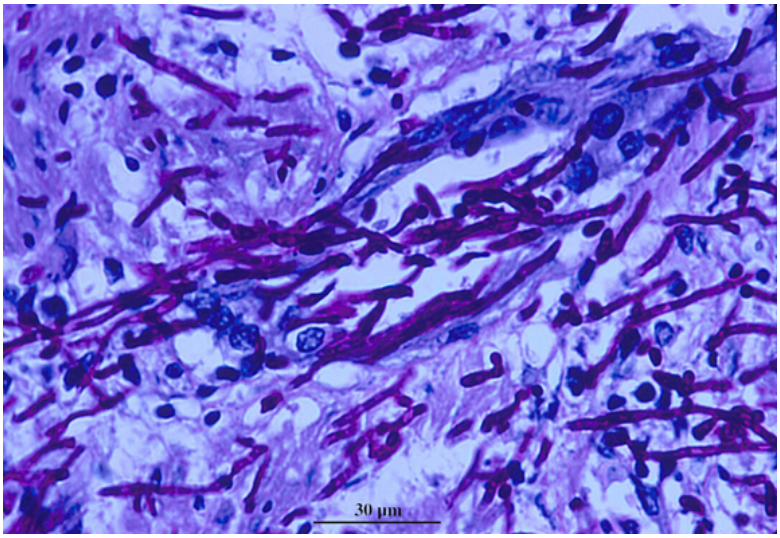

Figure 1. Periodic Acid-Schiff (PAS) stained section of post-mortem oesophagus showing invasion of blood vessel by *C. albicans*. Note blastoconidia and branched pseudohyphae. (Courtesy: www.mycology.adelaide.edu.au)

The treatments used to manage *Candida* infections vary substantially and are based on the anatomic location of the infection, the patients' underlying disease and immune status, the patients' risk factors for infection, the specific species of *Candida* responsible for infection, and, in some cases, the susceptibility of the *Candida* species to specific antifungal drugs. There have been significant changes in the management of candidiasis in the last few years, particularly related to the appropriate use of echinocandins and expanded-spectrum azoles for candidemia, other forms of invasive candidiasis, and mucosal candidiasis [21]. These latest recommendations include the echinocandins caspofungin, micafungin, and anidulafungin, along with voriconazole and posaconazole, as well as lipid formulations of amphotericin B in various situations. Fluconazole is still considered a first-line agent in nonneutropenic patients with candidemia or suspected invasive candidiasis [22].

3. Cryptococosis

Cryptococcus neoformans is an encapsulated yeast. In 1894, Busse, a pathologist, first described the yeast in a paper he presented to the Greifswald Medical Society. Busse isolated the yeast from the tibia of a 31-year-old woman, noted its resistance to sodium hydroxide, and published the case report that same year [23]. The following year, a surgeon named Buschke reported the same isolate from the same patient, thus establishing the early eponym of Busse-Buschke disease [24].

Since the initial reports, researchers have identified the diverse spectrum of host responses to cryptococcal infection. The responses range from a harmless colonization of the airways and asymptomatic infection in laboratory workers (resulting in only a positive skin test finding) to meningitis or disseminated disease. Although virulence in animals and, possibly, humans varies among strains of cryptococci, virulence probably plays a relatively small role in the outcome of an infection. The crucial factor is the immune status of the host. The importance of host immunity to the development of cryptococcosis is the single most critical feature of this infection from diagnosis to prognosis [25, 26]. A major pathological principle in understanding of cryptococcosis is that many individuals are infected with this yeast but their immune system controls the infection with minor and insignificant symptoms. However, like tuberculosis, the yeast can persist in tissue (dormancy) for long periods of time to then reactivate and produce disease during an immunosuppressive event. Furthermore, this reactivation scenario has been supported by recent observations with HIV infection progression to low CD4 counts (50-100 CD4 cells/*ul*) and this immunosuppressive lymphopenia directly linked with higher risk of cryptococcosis as the reduction of cell-mediated immune cells occurs [27].

There are a series of well-known risk factors associated with cryptococcal disease. The two highest risk factors are HIV infection and corticosteroid use. The corticosteroid use as a risk factor incorporates most of the transplant recipients and particularly, the solid organ transplants with their long term corticosteroid exposure and relatively high daily doses (> 20mg/day of prednisone) [28]. Among the other risk factors that require some discussion are the lymphomas/chronic leukemias and the connective tissue diseases in which most of these

cases are aided by corticosteroid usage. Diabetes continues to be present in a large number of patients with cryptococcosis [29].

It should be noted, however, that not all patients with cryptococcosis have an underlying disease. In fact, if you exclude HIV- infected patients, approximately 20-30% of patients with disseminated cryptococcosis will present with no apparent underlying diseases or known risk factors [29]. Since the host is classically protected by a vigorous Th_1 response, it is likely that those with no apparent underlying disease but who develop disseminated cryptococcosis do, in fact, have some undetected alterations in their protective immune responses. A small genetic susceptibility study through identification of DNA polymorphisms in certain immune genes has been started [30], but clearly, further comprehensive studies in genetic susceptibility and immune functions will be necessary for us to get a true appreciation for what is really going on in the apparently normal host with disseminated cryptococcosis. In fact, at times, the dysregulated immune systems and heterogenerous populations of patients makes this group of patients (apparently normal hosts)the hardest to manage since they may have late disease (prolonged disease because of delayed diagnosis) with either a high burden of organisms before treatment and/or prone to the development of a very aggressive immune reconstitution inflammatory syndrome (IRIS). In this group of apparently normal hosts with cryptococcosis, it is reasonable to check for underlying HIV infection since it is a treatable illness and also it is probably reasonable to obtain a total CD4 lymphocyte count to identify patients with idiopathic CD4 lymphocytopenia which may require prolonged antifungal suppressive therapy, although present data suggest that this idiopathic syndrome with cryptococcosis actually has a good prognosis [31].

The general theme of immunity for this disease is that prevention of disseminated cryptococcosis is controlled by an efficient cell-mediated immunity. This fact is supported by many *in vitro* studies, animal models and all present cytokine studies in humans [32]. For instance, defective production of interferon gamma and TNF-alpha but not IL-10 occurs in patients who have cryptococcosis which indicates a shifting to a predominant Th_2 host response [33]. Furthermore, during effective treatment at the CNS site of a cryptococcal infection, an up-regulated Th_1 response occurs as measured by higher CSF interferon gamma levels and lower CSF yeast counts [34].

Much of the host issues for diagnosis and initial management of cryptococcosis are based around a deficiency in host responses and the risk groups identify this focus. However, it is essential that clinicians realize that the total management of cryptococcosis must deal with the total immune dysregulation that occurs and not just its early deficiencies. This is emphasized by the many pleomorphic effects of the lingering cryptococcal polysaccharide can have on host immune functions. Thus, even the yeast and its products can modulate the host environment. This immune dysregulation was clearly identified during the AIDS epidemic with cryptococcosis and the use of HAART for HIV infection during cryptococcosis [35, 36].

Cryptococcus neoformans has become a major human pathogen and a common infection in certain immunocompromised hosts [36]. Cryptococcosis, the disease resulting from infection with *C. neoformans*, varies from a localized skin lesion or asymptomatic colonization of the respiratory tree to a widely disseminated life-threatening infection, which may infect all organs of the body. However, *C. neoformans* has a special propensity for invading the central nervous system and cryptococcal meningoencephalitis is the primary clinical presentation for the life-threatening stage of this infection [37].

Although the genus *Cryptococcus* contains more than 50 species, only *C neoformans* and *Cryptococcus gattii* are considered principal pathogens in humans. Previously, *C neoformans* was defined as having two varieties—var *neoformans* and var *gattii*. However, based on the elucidation of the genomic sequences, *C neoformans* and *C gattii* are now considered two distinct species. These two species have 5 serotypes based on antigenic specificity of the capsular polysaccharide; these include serotypes A, D, and AD (*C neoformans*) and serotypes B and C (*C gattii*). *C neoformans* is the most common species in temperate climates throughout the world and is found in aged pigeon droppings. Until recently, *C gattii* was found principally in tropical and subtropical climates. *C gattii* is not associated with birds but grows in the litter around certain species of eucalyptus trees (ie, *Eucalyptus camaldulensis, Eucalyptus tereticornis*) [38].

Worldwide, *C neoformans* serotype A causes most cryptococcal infections in immunocompromised patients, including patients infected with HIV. For unknown reasons, *C. gattii* rarely infects persons with HIV infection and other immunosuppressed patients. Patients infected with *C gattii* are usually immunocompetent, respond slowly to treatment, and are at risk for developing intracerebral mass lesions (eg, cryptococcomas). Naturally occurring cryptococcosis occurs in both animals and humans, but neither animal-to-human transmission nor person-to-person respiratory transmission via the respiratory route has been documented. Transmission via organ transplantation has been reported when infected donor organs were used. *C neoformans* causes the vast majority of cryptococcal infections in immunosuppressed hosts, including patients with AIDS, whereas *C gattii* causes 70%-80% of cryptococcal infections among immunocompetent hosts [39].

C neoformans reproduces by budding and forms round yeastlike cells that are 3-6 μm in diameter. Within the host and in certain culture media, a large polysaccharide capsule surrounds each cell. *C neoformans* forms smooth, convex, yellow or tan colonies on solid media at 20-37°C (68-98.6°F). This fungus is identified based on its microscopic appearance, biochemical test results, and ability to grow at 37°C (98.6°F); most nonpathogenic *Cryptococcus* strains do not grow at this temperature. In addition, *C neoformans* does not assimilate lactose and nitrates or produce pseudomycelia on cornmeal or rice-Tween agar. Most strains of *C neoformans* can use creatinine as a nitrogen source, which may partially explain the growth of the organism in creatinine-rich avian feces. Another useful biochemical characteristic of *C neoformans,* which distinguishes it from nonpathogenic strains, is its ability to produce melanin. The fungal enzyme phenol oxidase acts on certain substrates (eg, dihydroxyphenylalanine, caffeic acid) to produce melanin [40, 41].

C gattii contains genotypes VGI and the more commonly identified VGIIa and VDIIb. *Cryptococcus* species can reproduce via same-sex mating, and VGIIa may have arisen from the same-sex mating of VGIIb and another strain that has yet to be identified [38].

In 1976, Kwon-Chung described the perfect (ie, sexual, teleomorphic) form of *C neoformans,* which was named *Filobasidiella neoformans*. Prior to the identification of *F neoformans,* which is mycelial, *C neoformans* was considered a monomorphic yeast. *F neoformans* results from the mating of suitable strains of serotypes A and D. The perfect state of *C gattii* is *Filobasidiella bacillispora* and results from the mating of serotypes B and C. Some strains of A and D can mate with strains of B and C [42].

Following inhalation, the yeast spores are deposited into the pulmonary alveoli, where they must survive the neutral-to-alkaline pH and physiologic concentrations of carbon dioxide before they are phagocytized by alveolar macrophages. Glucosylceramide synthase (GCS) has been identified as an essential factor in the survival of *C neoformans* in this extracellular environment. Although GCS is a critical factor in extracellular survival of the yeast, the yeast no longer requires GCS to survive the intracellular, more acidic, environment within the macrophage once it is phagocytized by alveolar macrophages. Unencapsulated yeast are readily phagocytosed and destroyed, whereas encapsulated organisms are more resistant to phagocytosis. The cryptococcal polysaccharide capsule has antiphagocytic properties and may be immunosuppressive. The antiphagocytic properties of the capsule block recognition of the yeast by phagocytes and inhibit leukocyte migration into the area of fungal replication [43].

The host response to cryptococcal infection includes both cellular and humoral components. Animal models demonstrate that natural killer cells participate in the early killing of cryptococci and, possibly, antibody-dependent cell-mediated killing. In vitro monocyte-derived macrophages, natural killer cells, and T lymphocytes can inhibit or kill cryptococci. A successful host response includes an increase in helper T-cell activity, skin test conversion, and a reduction in the number of viable organisms in the tissues. In addition to cellular mechanisms, anticryptococcal antibodies and soluble anticryptococcal factors have been described. Antibodies to cryptococcal antigens play a critical role in enhancing the macrophage- and lymphocyte-mediated immune response to the organism. Researchers have used monoclonal antibodies to capsular polysaccharide to passively immunize mice against *C neoformans* [44, 45].

C neoformans infection is usually characterized by little or no necrosis or organ dysfunction until late in the disease. Organ damage may be accelerated in persons with heavy infections. The lack of identifiable endotoxins or exotoxins may be partly responsible for the absence of extensive necrosis early in cryptococcal infections. Organ damage is primarily due to tissue distortion secondary to the expanding fungal burden. Extensive inflammation or fibrosis is rare. The characteristic lesion of *C neoformans* consists of a cystic cluster of yeast with no well-defined inflammatory response. Well-formed granulomas are generally absent. *C neoformans* can cause an asymptomatic pulmonary infection followed later by the development of meningitis, which is often the first indication of disease. If limited to the

lungs, *C neoformans* infection may cause pneumonia, poorly defined mass lesions, pulmonary nodules, and, rarely, pleural effusion. Although immune defects are common in patients with meningitis or disseminated infection, patients with disease that is confined to the lungs are usually immunocompetent [46].

Once cryptococcosis is considered in the differential diagnosis of an infectious disease there are very good tools to diagnose it. Histopathology can be relatively distinctive with the capsule around the yeasts identified by alcian blue or mucicarmine stains and a Fontana-Masson stain can identify melanin production. These 5-20um budding or single yeasts can clearly be seen in the low-cost colloidal medium of an initial India ink examination on cerebrospinal fluid that can be positive in up to 80% of HIV-infected patients with cryptococcal meningoencephalitis and with careful examination can be positive in 50% of non-HIV infected patients. This is generally related to burden of yeasts and frequently patients with AIDS and disseminated cryptococcosis will have a large burden of yeasts in CSF that can range between 10^6-10^7CFU of yeasts/ml. The India ink examination has difficulty in identifying yeasts when their concentrations drop to 10^3 CFU of yeasts/ml or lower. There are some clinical cases and this is more commonly reported in the lung where the histopathology has some difficulty in detecting small capsules. However, it is still likely that a capsule is present since an acapsular mutant is uniformly avirulent. There are a series of non -specific stains which might identify this yeast and particularly calcofluor and Gomori's methenamine silver stains which represent classic fungal stains will identify this yeast in tissue. There are culture, molecular, and antibody methods to distinguish *C. neoformans* from *C. gattii* but at present except for epidemiological purposes it is not clear that separating an isolate into specific species is a necessary requirement of the laboratory to help clinical management of the patient [47, 48].

The serologies of cryptococcosis for diagnosis have been very well studied. Serum cryptococcal antibodies are not particularly helpful in diagnosing and deciding treatment for cryptococcosis and therefore are not used clinically. These antibodies, however, are useful for epidemiological studies of exposure and their presence may actually suggest a good prognostic sign. On the other hand, the detection of cryptococcal capsular polysaccharide antigen in serum or CSF has performed extraordinarily well in diagnosis for many years . It is one of the premier diagnostic tests in all of medical mycology. There is also some correlation between antigen load and burden of viable yeasts in the host prior to treatment so the height of the antigen titer may have some prognostic features. There are primarily two types of commercial tests, latex agglutination and ELISA systems. The sensitivity and specificity of these tests are above 90% and although there are occasional false positives or false negatives, these results can frequently be sorted out with careful repeat testing or confirmatory culture results. Therefore, in areas where this fungus is endemic, any subacute or chronic meningitis case should have a CSF cryptococcal polysaccharide antigen test performed. It is rapid and accurate. Despite its diagnostic utility and possessing some general prognostic features on initial titer, the cryptococcal antigen test is not very precise for use in following therapy. It is a large molecule with many immunological effects but the exact kinetics of its elimination from the body are

variable and not precisely predictive of success. All clinicians would like to see the antigen eliminated from the host with successful therapy but treatment decisions cannot be directly linked to the quantitative antigen measurement. The term isolated cryptococcal polysaccharidemia describes a condition in very high-risk patients primarily those with HIV infection who have a positive cryptococcal antigen titer and no positive cultures or prominent symptoms. In these patients the incidence of eventually developing cryptococcosis is very high and in most patients an examination of CSF is warranted and even if negative, administration of empiric antifungal therapy should be considered [49, 50].

The use of the cryptococcal antigen in a high prevalence area such as sub-Saharan Africa as a screening device has great appeal if it could be cheap and easily performed. There is now a Lateral Flow Test being studied and marketed which has the great potential to be cheap and could use finger stick blood or urine. It is very low tech as a simple dipstick test and has an easy storage requirement. There are now prospective data that in those HIV-infected patients that have a negative cryptococcal antigen test are unlikely to develop cryptococcosis over the next year. A positive cryptococcal antigen test will need a lumbar puncture work up to rule out CNS disease and even with negative CNS disease but a positive test, it probably supports antifungal treatment until HAART returns improved host immunity. This simple Lateral Flow Test could have a profound effect on new management strategies for the vast number of patients with this disease. It simply needs to be integrated into the health care systems in resource-limited environments. All these issues are being examined at present [51].

Finally, the yeast is relatively easy to culture on standard media with growth in 2-10 days and it can be isolated from blood culture systems. Although quantitative CSF yeast counts have excellent predictive value for therapeutic outcome in research studies, they have yet to be incorporated into clinical practice. In fact, future strategies should consider measurement of the rate of yeast decline in CSF as a judge to success of induction therapy and its length [52, 53].

The demonstration of encapsulated yeast cells in CSF, biopsy tissue, blood or urine should be considered significant, even in the absence of clinical symptoms. Positive sputum specimens should be considered potentially significant, even though *Cryptococcus* may also occur in respiratory secretions as a saprophyte. Basically, all patients with a positive microscopy for cryptococci, from any site should be investigated for disseminated disease, especially by culture and antigen detection.

In patients who are co-infected with HIV and *C neoformans,* the therapeutic goal may differ from that in patients with cryptococcal infection uncomplicated by HIV infection. For cryptococcal infections in patients with concomitant HIV infection who have a CD4 count of less than 200 cells/μL, the therapeutic goal is to control the acute infection, followed by life-long suppression of *C neoformans*. For patients infected with HIV who have successfully completed an initial course of therapy, remain free of symptoms of cryptococcal disease, and reconstitute their CD4 count to more than 200 CD4 cells/μL for more than 6 months, some authorities suggest that suppressive therapy may be discontinued. However, if the patient's CD4 count falls to less than 200 cells/μL, suppressive therapy should be reinstituted [54].

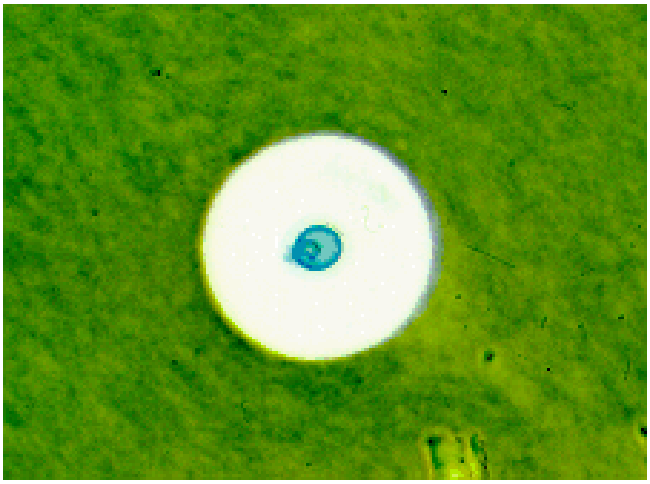

Figure 2. India ink preparation of CSF showing a typical yeast cell of *C. neoformans* surrounded by a characteristic wide gelatinous capsule. (Courtesy: www.mycology.adelaide.edu.au)

4. Pneumocystosis

Pneumocystis is a genus of unicellular fungi found in the respiratory tracts of many mammals and humans. Distinct genomic variability exists between host-specific members of the genus. The organism was first described in 1909 by Chagas and then a few years later by Delanöes, who ultimately named the organism in honor of Dr. Carini after isolating it from infected rats. Years later, Dr. Otto Jirovec and his group isolated the organism from humans, and the organism responsible for PCP was renamed after him [55].

The taxonomic classification of the *Pneumocystis* genus was debated for some time. It was initially mistaken for a trypanosome and then later for a protozoan. In the 1980s, biochemical analysis of the nucleic acid composition of *Pneumocystis* rRNA and mitochondrial DNA identified the organism as a unicellular fungus rather than a protozoan. Subsequent genomic sequence analysis of multiple genes including elongation factor 3, a component of fungi protein synthesis not found in protozoa, further supported this notion.

The organism is found in 3 distinct morphologic stages, as follows:

- The trophozoite (trophic form), in which it often exists in clusters
- The sporozoite (precystic form)
- The cyst, which contains several intracystic bodies (spores)

Pneumocystis organisms are commonly found in the lungs of healthy individuals. Most children are believed to have been exposed to the organism by age 3 or 4 years, and its occurrence is worldwide [56].

Pneumocystis carinii pneumonia (PCP), as the condition is commonly termed (although the causative organism has been renamed *Pneumocystis jiroveci*, is the most common opportunistic

infection in persons with HIV infection. *Pneumocystis* first came to attention as a cause of interstitial pneumonia in severely malnourished and premature infants during World War II in Central and Eastern Europe. Before the 1980s, fewer than 100 cases of PCP were reported annually in the United States, occurring in patients who were immunosuppressed (eg, cancer patients receiving chemotherapy and solid-organ transplant recipients receiving immunosuppressants). In 1981, the Centers for Disease Control and Prevention reported PCP in 5 previously healthy homosexual men residing in the Los Angeles area [57].

P jiroveci is now one of several organisms known to cause life-threatening opportunistic infections in patients with advanced HIV infection worldwide. Well over 100,000 cases of PCP were reported in the first decade of the HIV epidemic in the United States in people with no other cause for immunosuppression.

While officially classified as a fungal pneumonia, PCP does not respond to antifungal treatment. Although a histopathologic demonstration of the organism is required for a definitive diagnosis (see Histologic Findings), treatment should not be delayed [58].

Treatment of PCP may be initiated before the workup is complete in severely ill high-risk patients. Treatment of PCP depends on the degree of illness at diagnosis, determined on the basis of the alveolar-arterial gradient. Antibiotics are primarily recommended for treatment of mild, moderate, or severe PCP. Trimethoprim-sulfamethoxazole (TMP-SMX) has been shown to be as effective as intravenous pentamidine and more effective than other alternative treatment regimens. Corticosteroids are used as adjunctive initial therapy only in patients with HIV infection who have severe PCP. Preventive measures (eg, smoking cessation and chemoprophylaxis) can play an important role in disease management [59].

4.1. Transmission of Pneumocystis

Animal studies have suggested that *Pneumocystis* organisms are communicable; airborne transmission has been reported. Human evidence of this is provided by molecular analysis of *Pneumocystis* isolates obtained from groups of patients involved in hospital outbreaks [60, 61].

Further evidence of human transmission has been found in cases of recurrent pneumonia in which the genotype of *Pneumocystis* organisms in the same person differed in prior episodes. Despite this, barrier precautions are not required for patients hospitalized with *P carinii* pneumonia (PCP) except to protect other patients with depressed immunity.

4.2. Development of PCP

Disease occurs when both cellular immunity and humoral immunity are defective. Once inhaled, the trophic form of *Pneumocystis* organisms attach to the alveoli. Multiple host immune defects allow for uncontrolled replication of *Pneumocystis* organisms and development of illness. Activated alveolar macrophages without $CD4^+$ cells are unable to eradicate *Pneumocystis* organisms. Increased alveolar-capillary permeability is visible on electron microscopy.

Physiologic changes include the following:

- Hypoxemia with an increased alveolar-arterial oxygen gradient
- Respiratory alkalosis
- Impaired diffusing capacity
- Changes in total lung capacity and vital capacity

There have been reports of PCP occurring as part of the immune reconstitution syndrome [62, 63].

4.3. Risk factors for PCP

PCP is caused by infection with *P jiroveci.* The following groups are at risk for PCP [64, 65]:

- Persons with HIV infection whose $CD4^+$ cells fall below 200/μL and who are not receiving PCP prophylaxis (In addition, in patients with HIV infection, findings of other opportunistic infections [eg, oral thrush] increases the risk of PCP, regardless of $CD4^+$ count).
- Persons with primary immune deficiencies, including hypogammaglobulinemia and severe combined immunodeficiency (SCID).
- Persons receiving long-term immunosuppressive regimens for connective-tissue disorders, vasculitides, or solid-organ transplantation (eg, heart, lung, liver, kidney)
- Persons with hematologic and nonhematologic malignancies, including solid tumors and lymphomas
- Persons with severe malnutrition

Before the widespread use of prophylaxis for *P carinii* pneumonia (PCP), the frequency of *Pneumocystis* infection in lung transplant patients alone was as high as 88%. Now, with the routine use of prophylaxis, PCP is very rare in solid-organ transplant patients and has significantly decreased in patients infected with HIV. Prior to the widespread use of highly active antiretroviral therapy (HAART), PCP occurred in 70-80% of patients with HIV infection. The frequency of PCP is decreasing with the use of PCP prophylaxis and HAART. PCP is still the most common opportunistic infection in patients with HIV infection. Patients with HIV infection are more prone to PCP recurrence than patients not infected with HIV. In developing regions of the world, the prevalence of PCP was once thought to be much lower, but studies have shown that the lower reported incidence is likely a failure to accurately diagnose PCP. An accurate diagnosis requires access to modern medical care, which is not available worldwide [66].

Currently, the frequency of documented *Pneumocystis* infection is increasing in Africa, with *Pneumocystis* organisms found in up to 80% of infants with pneumonia who have HIV infection. In sub-Saharan Africa, tuberculosis is a common co-infection in persons with PCP [67].

A lactic dehydrogenase (LDH) study is performed as part of the initial workup. LDH levels are usually elevated (>220 U/L) in patients with *P carinii* pneumonia (PCP). They are elevated in 90% of patients with PCP who are infected with HIV. The study has a high

sensitivity (78-100%); its specificity is much lower because other disease processes can result in an elevated LDH level. LDH levels appear to reflect the degree of lung injury. They should decline with successful treatment. Consistently elevated LDH levels during treatment may indicate therapy failure and a worse prognosis [68].

Quantitative PCR for pneumocystis may become useful in distinguishing between colonization and active infection, but these assays are not yet available for routine clinical use [69].

In one study, patients with positive quantitative PCR but negative immunofluorescence for pneumocystis had a higher 1-year mortality but only in the context of systemic inflammatory conditions. There was no significant difference for patients with solid-organ or hematologic malignancy [70].

β-D-Glucan (BDG) is a cell-wall component of many fungi, including candida, aspergillus, and pneumocystis (but not the zygomycetes). It has been shown to be a sensitive test to detect PCP in a meta-analysis of 12 studies assessing the sensitivity, specificity and overall accuracy of the test [71].

5. Aspergilosis

Aspergillus species are ubiquitous molds found in organic matter. Although more than 100 species have been identified, the majority of human illness is caused by *Aspergillus fumigatus* and *Aspergillus niger* and, less frequently, by *Aspergillus flavus* and *Aspergillus clavatus*. The transmission of fungal spores to the human host is via inhalation [72].

Aspergillus may cause a broad spectrum of disease in the human host, ranging from hypersensitivity reactions to direct angioinvasion. *Aspergillus* primarily affects the lungs, causing 4 main syndromes, including allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing *Aspergillus* pneumonia (or chronic necrotizing pulmonary aspergillosis [CNPA]), aspergilloma, and invasive aspergillosis. However, in patients who are severely immunocompromised, *Aspergillus* may hematogenously disseminate beyond the lung, potentially causing endophthalmitis, endocarditis, and abscesses in the myocardium, kidney, liver, spleen, soft tissue, and bone. *Aspergillus* is second to *Candida* species as a cause of fungal endocarditis. *Aspergillus* -related endocarditis and wound infections occur in the context of cardiac surgery [73].

ABPA is a hypersensitivity reaction to *A fumigatus* colonization of the tracheobronchial tree and occurs in conjunction with asthma and cystic fibrosis (CF). Allergic fungal sinusitis may also occur alone or with ABPA. Bronchocentric granulomatosis and malt worker's lung are 2 hypersensitivity lung diseases that are caused by *Aspergillus* species, but they are rare [74].

An aspergilloma is a fungus ball (mycetoma) that develops in a preexisting cavity in the lung parenchyma. Underlying causes of the cavitary disease may include treated tuberculosis or other necrotizing infection, sarcoidosis, CF, and emphysematous bullae. The ball of fungus may move within the cavity but does not invade the cavity wall; however, it may cause hemoptysis [75].

CNPA is a subacute process usually found in patients with some degree of immunosuppression, most commonly that associated with underlying lung disease, alcoholism, or long-term corticosteroid therapy. Because it is uncommon, CNPA often remains unrecognized for weeks or months and can cause a progressive cavitary pulmonary infiltrate [74].

Invasive aspergillosis is a rapidly progressive, often fatal infection, associated with significant mortality, with a rate of 30-95%, that occurs in patients who are severely immunosuppressed, including those who are profoundly neutropenic, those who have received bone marrow or solid organ transplants, and patients with advanced AIDS or chronic granulomatous disease. This infectious process is characterized by invasion of blood vessels, resulting in multifocal infiltrates, which are often wedge-shaped, pleural-based, and cavitary. Dissemination to other organs, particularly the central nervous system, may occur [76].

Aspergillus causes a spectrum of disease, from colonization to hypersensitivity reactions to chronic necrotizing infections to rapidly progressive angioinvasion, often resulting in death. Rarely found in individuals who are immunocompetent, invasive *Aspergillus* infection almost always occurs in patients who are immunosuppressed by virtue of underlying lung disease, immunosuppressive drug therapy, or immunodeficiency [73].

Aspergillus hyphae are histologically distinct from other fungi in that the hyphae have frequent septae, which branch at 45° angles. The hyphae are best visualized in tissue with silver stains. Although many species of *Aspergillus* have been isolated in nature, *A fumigatus* is the most common cause of infection in humans. *A flavus* and *A niger* are less common. Likely, this relates to the ability of *A fumigatus,* but not most other *Aspergillus* species, to grow at normal human body temperature. Human host defense against the inhaled spores begins with the mucous layer and the ciliary action in the respiratory tract. Macrophages and neutrophils encompass, engulf, and eradicate the fungus. However, many species of *Aspergillus* produce toxic metabolites that inhibit macrophage and neutrophil phagocytosis. Corticosteroids also impair macrophage and neutrophil function. Underlying immunosuppression (eg, HIV disease, chronic granulomatous disease, pharmacologic immunosuppression) also contributes directly to neutrophil dysfunction or decreased numbers of neutrophils. In individuals who are immunosuppressed, vascular invasion is much more common and may lead to infarction, hemorrhage, and necrosis of lung tissue. Persons with CNPA typically have granuloma formation and alveolar consolidation. Hyphae may be observed within the granulomata [77].

Because *Aspergillus* infection may cause colonization, allergy, or invasive infection, its manifestations are quite variable and are best considered based on the disease process. Allergic bronchopulmonary aspergillosis is defined by several abnormalities, including asthma, eosinophilia, a positive skin test result for *A fumigatus,* marked elevation of the serum immunoglobulin E (IgE) level to greater than 1000 IU/dL, fleeting pulmonary infiltrates, central bronchiectasis, mucoid impaction, and positive test results for *Aspergillus* precipitins (primarily immunoglobulin G [IgG], but also immunoglobulin A and immunoglobulin M, antibodies). Minor criteria for diagnosis include positive *Aspergillus* radioallergosorbent assay test results and culture findings for *Aspergillus* in sputum [74].

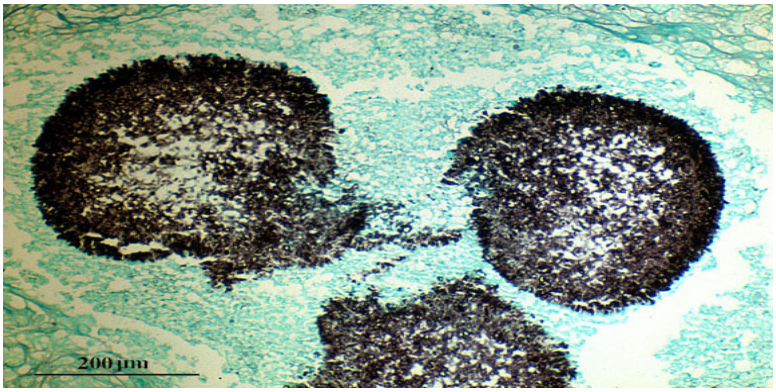

Figure 3. Grocott's methenamine silver (GMS) stained tissue section of lung showing fungal balls of hyphae of *Aspergillus fumigatus*. (Courtesy: www.mycology.adelaide.edu.au)

Diagnostic criteria for ABPA in persons with CF were revised by the Cystic Fibrosis Foundation. ABPA is considered a definite diagnosis requiring treatment if the following are noted: (1) clinical deterioration, including cough, wheeze, increased sputum production, diminished exercise tolerance, or diminished pulmonary function; (2) total serum IgE level greater than 1000 IU/mL or a greater than 2-fold rise from baseline; (3) positive serology results for *Aspergillus* (*Aspergillus* precipitins or *Aspergillus* -specific IgG or IgE); and (4) new infiltrates on chest radiographs or CT scans. Treatment for ABPA is also recommended in patients with CF who have new radiographic findings and symptoms and a change in baseline IgE level to greater than 500 IU/mL [79].

Definitive diagnosis of invasive aspergillosis or chronic necrotizing *Aspergillus* pneumonia depends on the demonstration of the organism in tissue [76].

In the appropriate clinical setting of pulmonary infiltrates in a patient who is neutropenic or immunosuppressed, visualization of the characteristic fungi using Gomori methenamine silver stain or Calcofluor or a positive culture result from sputum, needle biopsy, or bronchoalveolar lavage (BAL) fluid should result in the prompt institution of therapy. This is especially important after bone marrow transplantation because a positive *Aspergillus* culture result from sputum has a 95% positive predictive value for invasive disease. However, a negative fungus result from culture of sputum or BAL fluid does not exclude pulmonary aspergillosis because *Aspergillus* is cultured from sputum in 8-34% of patients and from BAL fluid in 45-62% of patients eventually found by biopsy or autopsy to have invasive disease [80].

An assay to detect galactomannan, a major component of the *Aspergillus* cell wall, is available. Patients who are at high risk, such as those who have received stem cell transplants or who have prolonged neutropenia, may be screened for the development of invasive *Aspergillus* infection by monitoring serum galactomannan levels weekly.The presence of an elevated galactomannan level in BAL fluid may also be helpful in the diagnosis of pulmonary aspergillosis in patients in whom compatible radiographic changes

are present and BAL testing is performed in the suspicious area. A meta-analysis and systematic review determined that the measurement of BAL-galactomannan levels may help in diagnosing invasive aspergillosis early [81, 82].

A study by Luong et al of 150 BAL samples from lung transplant recipients concluded that real-time polymerase chain reaction (PCR) assays could be useful in diagnosis of invasive aspergillosis in high-risk populations. Pan-*Aspergillus* PCR combined with BAL galactomannan testing was 97% specific and 93% sensitive for invasive pulmonary aspergillosis. Species-specific real-time PCR assays for *A fumigatus* and for *A terreus* could be used to rule out or identify the common *A fumigatus* and the amphotericin B-resistant *A terreus* [83].

Histopathology and silver staining for persons with invasive aspergillosis demonstrates the characteristic septate hyphae, branching at acute angles, and acute inflammatory infiltrate and tissue necrosis with occasional granulomata and blood vessel invasion. The airways of patients with ABPA contain mucus filled with degenerating eosinophils and typical fungal hyphae. ABPA may occur on a background of chronic eosinophilic pneumonia and bronchiolitis, granulomatous bronchitis, bronchocentric granulomatosis, and, occasionally, BOOP [84].

Selection of therapy also needs to consider the certainty of the diagnosis. Voriconazole, itraconazole, the investigational azoles with anti-mould activity, and amphotericin B all possess a reasonably broad-spectrum of activity against *Aspergillus* and the related hyaline moulds. Their activity does, however, vary for the agents of zygomycosis, with posaconazole being the azole with the most reliable activity against this class of fungi. The echinocandin glucan synthesis inhibitors (caspofungin, FK463, and anidulafungin) possess a narrower spectrum of activity and should only be used if the infection is known to be due to *Aspergillus* spp [85, 86].

6. Zygomycosis

Zygomycosis is an infection caused by fungi in the orders Mucorales and Entomophthorales. The Mucorales order contains 2 families exist—Mucoraceae and Cunninghamellaceae. Mucormycosis is another common name applied to this same group of diseases. This designation reflected the predominance of the Mucorales in causing disease in humans. However, this term ignored the role of the Entomophthorales (*Conidiobolus* species and *Basidiobolus* species). The currently accepted designation is zygomycosis, reflecting all disease processes caused by the members of the class Zygomycetes. During the past decade, the Zygomycetes have emerged as common causes of invasive fungal infections [87].

The pathogens that cause zygomycosis are commonly found in the environment on fruit, on bread, and in soil and are common components of decaying organic debris. These organisms are ubiquitous and generally saprophytic, rarely causing disease in immunocompetent hosts, but they are the third-most-common cause of invasive fungal infection in

immunocompromised patients, especially stem cell transplant recipients and patients with underlying hematologic malignancies [88].

Fungi are ubiquitous in the natural world, often found in association with plants, mammals, and insects. Accordingly, humans are continually exposed to multiple genera of fungi via various routes, including the respiratory and gastrointestinal routes, which allow the possibility of colonization. Depending on the interaction between host mucosal defense mechanisms and fungal virulence factors, colonization may be transient or persistent, or local disease may ensue [89].

Overall, *Rhizopus* species from the Mucoraceae family are the most commonly identified etiologic agents of zygomycosis in humans. Of the *Rhizopus* species, the most common agent associated with zygomycosis is *Rhizopus arrhizus* (*Rhizopus oryzae*), followed by *Rhizopus rhizopodiformis*. Other causes include *Mucor* species, *Cunninghamella bertholletiae, Apophysomyces elegans, Absidia* species, *Saksenaea* species, *Rhizomucor pusillus, Entomophthora* species, *Conidiobolus* species, and *Basidiobolus* species [90].

Zygomycosis caused by *R arrhizus* is acute and rapidly fatal despite early diagnosis and treatment. These organisms have a particular predilection for invading major blood vessels, with ensuing ischemia, necrosis, and infarction of adjacent tissues, resulting in the production of black pus. Persons at particular risk include those with granulocytopenia and acidosis. For unknown reasons, the Zygomycetes have a propensity to affect patients with acidosis, particularly those with diabetes. They also infect patients with acidosis secondary to renal insufficiency, diarrhea, and aspirin intake. Patients who are receiving glucocorticoids or deferoxamine and those who have undergone splenectomy also are at risk [90].

The overall mortality rate associated with zygomycosis is approximately 50% and has remained at this level for the past 50 years. Rhinocerebral zygomycosis carries a mortality rate of approximately 85%. Mortality rates are very high because, by the time zygomycosis is suspected and diagnosed, it has frequently spread diffusely and caused extensive tissue destruction. However, the risk of mortality varies depending on the characteristics of the host, the type of infection, the site of infection, and the use of surgical intervention. In general, antifungal therapy and surgical management independently decrease the likelihood of death [91].

Zygomycosis manifests as a spectrum of diseases, depending on the portal of entry and the predisposing risk factors of the patient. The 5 major clinical forms include rhinocerebral zygomycosis, pulmonary zygomycosis, abdominopelvic and gastric (gastrointestinal) zygomycosis, primary cutaneous zygomycosis, and disseminated zygomycosis [91].

Most persons who develop zygomycosis are immunocompromised, although 15-20% of patients have no evidence of any underlying condition at the time of the diagnosis.Thus, sporadic cases in immunocompetent hosts are not uncommon. The most common risk factors include the following [91, 92] :

- Stem cell transplantation
- Poorly controlled diabetes mellitus, either type 1 or type 2
- Hematologic malignancy (eg, leukemias, lymphomas)
- Solid organ transplants
- Steroid use
- Metabolic acidosis
- Deferoxamine therapy
- Severe and prolonged neutropenia
- Intravenous drug use
- Renal failure
- Peritoneal dialysis
- Burns
- Penetrating trauma (rare)

Unfortunately, findings from laboratory studies are nonspecific for zygomycosis. Diagnosis requires a high index of suspicion, a host with appropriate risk factors, and evidence of tissue invasion with the characteristic appearance of broad nonseptate hyphae with right-angle branches. No serologic tests are available, and blood cultures are of no benefit [93].

7. Conclusion

Opportunistic fungal infections of the body which occur almost exclusively in debilitated patients whose normal defence mechanisms are impaired. The organisms involved are cosmopolitan fungi which have a very low inherent virulence. The increased incidence of these infections and the diversity of fungi causing them, has paralleled the emergence of Acquired Immune Deficiency Syndrome (AIDS), more aggressive cancer and post-transplantation chemotherapy and the use of antibiotics, cytotoxins, immunosuppressives, corticosteroids and other macro disruptive procedures that result in lowered resistance of the host allowing fungi to invade tissues and produce pathological changes that can cause death. Compromised immunity is the most important predisposing factor for clinically significant fungal infections. Neutrophil deficiency as a result of bone marrow suppression or damage is frequently associated with such infections. Different fungi infect humans and may live in extracellular tissues and within phagocytes. Therefore, the immune responses to these microbes are often combinations of the responses to extracellular and intracellular bacteria. However, less is known about antifungal immunity than about immunity against bacteria and viruses. This lack of knowledge is partly due to the paucity of animal models for mycoses and partly due to the fact that these infections typically occur in individuals who are incapable of mounting effective immune responses. Improved diagnostic methods have been developed for an early diagnostic of opportunistic mycosis in order to control the disease and save more lives.

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