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Fungal infections in the transplant recipient and laboratory methods for diagnosis

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Although the last twenty five years have produced tremendous technological advances in solid organ and bone marrow transplantation, a continuing problem for the transplant recipient is infection, with fungi playing a significant role. Risk of fungal infection varies with time following transplantation. The period of intense immunosuppression between the first and sixth months following solid organ transplantation is notable for infections caused by opportunistic fungi such as *Candida* spp. and *Aspergillus* spp. [1]. These pathogens continue to threaten the solid organ recipient in later phases if organ rejection occurs. In the early phase following bone marrow transplantation (BMT) the risk of infection is determined by the duration of neutropenia and, as in other neutropenic populations, infections with *Candida* spp. are common [2]. The next phase, which follows marrow engraftment and typically includes the second and third post-transplant months, is dominated by fungal as well as viral pathogens. If neutropenia has been prolonged, the risk for infection with opportunistic fungal pathogens such as *Aspergillus* spp. rises dramatically [3].

In addition to the immunosuppresive therapy inherent to transplantation, other predisposing factors to invasive fungal infection in the hospitalized patient such as broad spectrum antibiotics, long-term venous access lines, hyperalimentation, malnutrition, disruption of mucosal and skin surfaces, and recent major surgical procedures [4,5] certainly apply to many transplant recipients. The incidence of invasive fungal infection ranges from 5% in kidney recipients [6] to greater than 20% in liver [5,7] and pancreas recipients [8].

Candida spp. are the most common fungal pathogens among the transplant population and in kidney and heart transplant recipients the manifestations of infection include esophagitis, urinary tract infection, and line-related infections [9]. Dissemination from these sites may subsequently occur. The renal transplant patient, in whom the urinary tract is a more common site of infection than in the other organ recipients, is prone to urinary tract infection with *Candida* spp. with the associated complications of obstruction due to the formation of fungus balls, and candidemia [10]. Colonization of the respiratory tract by *Candida* is usually innocuous, even in the transplant recipient. An exception to this is in the lung or heart/lung recipient, in whom involvement of the bronchial mucosa can have the devastating complications of rupture of the surgical anastomosis or fungal mediastinitis [11].

Liver transplant patients are at particularly high risk for infection with *Candida*, which at times accounts for 30% or more of all infections [5]. In one large review, there was a 77% mortality associated with invasive candidiasis [12]. Most candidal infections occur in this group within the first 100 post-transplant days [5,7,12]. This may be attributed to the unique risk factors of major intra-abdominal surgery breaching the bowel and biliary tract and early deficient hepatic reticuloendothelial cell function, as well as the often poor pre-operative medical condition of the liver transplant candidate. Accordingly, the most common types of infection are intraabdominal and abdominal wound infections [5], often with subsequent dissemination. In the granulocytopenic BMT patient, candidiasis is often disseminated, with involvement of the liver, spleen, kidney, heart, gastrointestinal tract, lungs, and brain [13]. Another form of disseminated infection described in the neutropenic host is hepatosplenic candidiasis [13]. Typically, the patient will have unexplained fever during the period of granulocytopenia, then develop clinical signs of hepatic involvement after the return of functioning neutrophils. Occurring in 10 to 25 percent of BMT patients, infection with *Candida* is associated with a poor outcome - a mortality of 39% for candidemia alone, and 90% when tissue invasion occurs [14].

Infections due to *Aspergillus* spp. are less common among transplant recipients than *Candida*, bacterial or viral pathogens, typically occurring in less than 6 percent of patients [15,16]. Aspergillosis, however, is perhaps the most greatly feared of infectious complications in this group as mortality may approach 100% [15]. The pathogenesis of infection due to this organism, as well as underlying host immune defects contribute to this alarmingly high mortality. The major host defense against this organism are functioning neutrophils, and therefore it is not surprising that the groups at greatest risk for infection include neutropenic cancer patients and bone marrow transplant recipients. Among solid organ recipients, *Aspergillus* infection is associated with the intense immunosuppression required to treat rejection [17]. While immunosuppression is one prerequisite for invasive infection with *Aspergillus*, environmental exposure is another. Aspergillosis is perhaps the infection most closely related to nosocomial exposure. Sampling of hospital air has clearly shown a rise in spore counts during times of construction inside or outside the hospital, presumably due to agitation of dust and soil thus increasing the concentration of airborne organisms, and outbreaks within a hospital have been associated with periods of construction [18,19].

Considering the pulmonary portal of entry, it is not surprising that the lungs are the predominant site of infec-

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tion. Clinical findings of invasive pulmonary aspergillosis are non-specific, thus a high clinical suspicion is necessary to facilitate an early diagnosis. Fever, shortness of breath, and cough may be present [17]. Compared to the neutropenic BMT patient, solid organ recipients tend to have a more insidious onset of disease with a paucity of objective pulmonary findings [15]. Diagnosis is often made only when the infection is widely disseminated and the chance of cure is slim, or is unsuspected during life but found at the time of autopsy [17].

Localized upper airway involvement by *Aspergillus* has previously been described in immunocompromised patients and is manifested by pseudomembrane formation and airway obstruction [20]. More recently, the new entity of invasive bronchial aspergillosis has been described in lung transplant recipients [21]. In this population, although *Aspergillus* initially causes local invasion limited to the anastomosis site and large airways, there is potential for widespread dissemination.

Dissemination of *Aspergillus* from a primary pulmonary focus is frequent in the transplant patient, and when dissemination occurs, central nervous system involvement is commonly found [22]. Meningitis, meningoencephalitis, hemorrhagic abscesses, and granulomatous involvement are described [22]. In the brain, as elsewhere, the organisms tends to involve vascular structures with subsequent hemorrhage or infarction, and stroke-like symptoms and findings are possible.

Fusarium spp. is becoming more commonly recognized as an opportunistic pathogen infecting neutropenic patients. In the immunocompromised, it is now known to cause locally invasive or disseminated disease [23,24,25]. The most prominent risk factor for infection with *Fusarium* spp. is neutropenia, and thus infection in the BMT patient is expected. Indeed, in this population fusariosis is second only to aspergillosis as a non-candidal fungal pathogen [26]. Clinical manifestations of infection reported in BMT patients include invasive sinus infection, cutaneous and soft tissue infection, fungemia, pulmonary involvement, osteomyelitis, bone marrow involvement, and dissemination to multiple organs [24,26]. The patient with disseminated fusariosis typically presents with fever, myalgias, skin lesions and fungemia, with subsequent resolution - or death, depending upon the return of white blood cells or lack thereof [24].

Cryptococcus neoformans infection tends to occur late in the post-transplant period. Given the relatively low level of immunosuppression at this stage of the transplant process, the appearance of infection is thought to be due to new exposure rather than reactivation of a latent focus, which would be expected during a period of more intense immunosuppression, more typical of the early post-transplant period [27]. Although the portal of entry is pulmonary, the most common clinical manifestation of infection in this population occurs after hematogenous dissemination with the appearance of subacute meningitis, which occurs in up to 90% of cases [27]. Another common extra-pulmonary site of involvement is the skin with the finding of papules, nodules, plaques, ulcers, or nonspecific cellulitis [28].

Organ recipients living in areas endemic for histoplasmosis, coccidioidomycosis, and blastomycosis, are at risk for primary infection following immunosuppression. Recipients living anywhere may experience reactivation of a latent infection acquired during a distant exposure. *Histoplasma capsulatum* is the most frequently encountered pathogen in this group; infection results from exposure to microfoci of the organism within an endemic area [29]. It tends to be disseminated at the time of diagnosis in the transplant population, and may have a rapidly progressive or chronic, indolent clinical course. Although *Coccidioides immitis* is a less common cause of infection in transplant patients, when found, infection is also usually disseminated. It tends to occur earlier in the post-transplant course than histoplasmosis, suggesting reactivation as the most likely form of acquisition [27]. Dissemination occurs in most immunocompromised patients with involvement of skin, bone, CNS, liver, and kidney [27]. *Blastomyces dermatitidis* is an uncommon pathogen in transplant recipients.

As a cause of subacute pneumonia in the transplant recipient, *Pneumocystis carinii* is seen in the early and mid post-transplant periods, and in the later period if the patient is suffering with rejection or CMV infection [30]. Infection is more common and more severe in lung transplant recipients, perhaps due to impaired local defense mechanisms in addition to general immunosuppression [31]. It has also been suggested that *Pneumocystis* infection may predispose the lung transplant recipient to chronic rejection [31]. Fortunately, preventative therapy with trimethoprim-sulfamethoxazole is highly effective, and should be administered for the first six post-transplant months [1].

The clinical microbiology laboratory plays a vital role in assisting the physician in the medical management of the transplant patient with suspected or confirmed mycotic disease. The diagnosis of fungal infection in the solid organ transplant or BMT recipient includes three approaches: 1) the isolation of the organism, 2) serologic detection of antibody or antigen, and 3) histopathologic evidence of invasion [32]. Lysis centrifugation enhances the recovery of fungi from blood, the exception being *Aspergillus* spp. which is rarely recovered from blood. Studies have shown that lysis centrifugation is superior to broth-based blood culture systems for the recovery of fungi as well as mycobacteria [33,34]. While lysis centrifugation may be too expensive and labor intensive for the laboratory to use on all blood cultures, consideration should be given to reserving the method for use on immunosuppressed patients. Fungi may also be isolated from otherwise sterile sites or fluid collections by aspiration. In some cases these cultures may be positive in patients with no evidence of fungemia [35]. The laboratory diagnosis of fungal infection in the transplant recipient can be facilitated by the use of established protocols for specific specimen types or specific clinical presentations [36]. The use of established protocols ensures that critical specimens will be processed appropriately, and are especially compatible with invasive procedures that obtain limited quantities of tissue, as these procedures may be risky to the patient. Protocols should provide testing for both common and uncommon pathogens, and generally combine histological examination, special stains and culture [36].

A plethora of fungal serologic tests have yielded variable success in the early diagnosis of fungal infections in transplant recipients. Detection of host antibody to *Candida* spp. is of little value in the diagnosis of invasive disease [37]. Tests to detect circulating antigens of *Candida* spp. have been studied extensively but their value remains controversial [37,38]. The detection of circulating antibodies to *Aspergillus* spp. is useful in allergic bronchopulmonary disease and aspergilloma but not in the early detection of invasive disease [39]. Detection of circulating antigen may correlate with invasive disease but even after years of extensive investigation such tests are still considered experimental [39,40]. Conversely, the latex agglutination test for detection of circulating polysaccharide antigen of *Cryptococcus neoformans* is a relia-

ble means of diagnosis and can also provide an indication of the patient's response to therapy [41]. Likewise, the detection of *Histoplasma capsulatum* antigen in serum and urine is also useful for the early diagnosis of disseminated histoplasmosis [42].

As the diagnosis of candidiasis prior to death is possible in less than half of immunocompromised patients [43], the utility of surveillance cultures for earlier prediction or diagnosis of infection with *Candida* spp. has been an area of great interest. Sites and substances commonly cultured include blood, sputum, stool, urine, throat, nasopharynx, rectum, and skin. Although surveillance methods are utilized in many organ transplant programs, their ability to predict future invasive disease remains debatable. In bone marrow transplant and leukemic patients, it has been shown that patients who develop disseminated candidiasis have a very high incidence of positive surveillance cultures for *Candida* prior to diagnosis of disseminated disease [44,45]. Colonization without invasion, however, is also common in this population and many patients with positive surveillance cultures never develop invasive disease. This is supported by studies in bone marrow patients and patients with hematological malignancies which show a poor positive predictive value for the isolation of *Candida albicans* from surveillance cultures [46,47]. The poor sensitivity for the early recovery of *Aspergillus* from routine culture specimens limits the use of surveillance methods for the early detection of infection. When *Aspergillus* is found in a respiratory specimen its significance is often questioned, as it may represent contamination. The conclusion drawn from several studies is that a positive respiratory culture for *Aspergillus* spp. in a host with predisposing factors is highly suggestive of invasive disease [48,49,50]. It has also been noted that although false positive sputum cultures are common, multiple positive cultures are more indicative of true infection than a single positive culture [17]. Therefore, a positive sputum culture in a neutropenic BMT patient or in a solid organ recipient undergoing treatment for rejection should prompt a thorough evaluation and institution of empiric antifungal therapy.

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