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Review

Invasive fungal infections in liver transplantation

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SUMMARY

Invasive fungal infections (IFIs) in immunocompromised patients, particularly liver transplant recipients, are the subject of increasing clinical attention. Although the overall incidence of fungal infections in liver transplant recipients has declined due to the early treatment of high-risk patients, the overall mortality rate remains high, particularly for invasive candidiasis and aspergillosis. IFIs after liver transplantation are strongly associated with negative outcomes, increasing the cost to recipients. Numerous studies have attempted to determine the independent risk factors related to IFIs and to reduce the morbidity and mortality with empirical antifungal prophylaxis after liver transplantation. Unfortunately, fungal infections are often diagnosed too late; symptoms can be mild and non-specific even with dissemination. Currently, no consensus exists on which patients should receive antifungal prophylaxis, when prophylaxis should be given, which antifungal agents should be used, and what duration is effective. This review highlights the types of IFI, risk factors, diagnosis, antifungal prophylaxis, and treatment after liver transplantation. With the early identification of patients at high risk for IFIs and the development of new molecular diagnostic techniques for early detection, the role of antifungal compounds in fungal infection prophylaxis needs to be established to improve the survival rate and quality of life in liver transplant patients.

Introduction

To our knowledge, liver transplantation is one of the most effective therapeutic options for patients with certain acute and chronic end-stage liver diseases, such as acute liver failure, hepatocellular carcinoma, hepatolenticular degeneration, severe hepatitis, and decompensated cirrhosis. The steady increase in the number of liver transplant recipients means hospitals are treating more immunocompromised patients; this can be associated with increased infection-related morbidity and mortality and higher hospital care costs.[1–3] Fungal infections are well-recognized, life-threatening complications of liver transplantation.

Delayed diagnosis of fungal infections is a major complicating factor. Symptoms are not specific, and even patients with disseminated disease with multiple organ involvement might not present with organ-specific changes or clinical signs.

Fungal infections can be the major factor associated with a poor prognosis in liver transplant recipients. Prior studies have shown that between 5% and 42% of liver transplant patients develop at least one fungal infection after transplantation. *Candida* species are the most common, followed by *Aspergillus* species. The mortality associated with these infections ranges from 25% to 69%; however, Aspergillus-associated mortality has been found to approach 100% if untreated.[4–8]Given the high incidence and mortality rate of fungal infections after liver transplantation, the identification of risk factors and antifungal prophylactic agents is necessary and urgent. Although most fungal infections are caused by *Candida spp*, infections by *Aspergillus spp* have increased significantly in recent years and are associated with poor outcomes.[7,9] Numerous well-defined risk factors associated with fungal infections, especially invasive fungal infections (IFIs), have been documented in numerous studies and include renal insufficiency (particularly when dialysis is indicated), rejection treatment, cytomegalovirus (CMV) viremia or disease, acute hepatic insufficiency, early graft failure, lengthy operation time, retransplantation, prolonged preoperative hospitalization (particularly in the intensive care unit (ICU)), preoperative use of broad-spectrum antibiotics, substantial intraoperative infusions of cellular blood products, fungal colonization, and re-exploration after transplantation.[1–6,9–19] The identification of risk factors for IFIs in liver transplant recipients could facilitate the timely use of antifungal prophylactic agents, thereby preventing the development of an invasive mycosis or disseminated fungal infection. This might improve the prognosis for liver transplant recipients.

Fungal infection incidence and fungi

Fungal infections are a major cause of morbidity and mortality among patients undergoing orthotopic liver transplantation (OLT).

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Table 1
Risk factors for invasive fungal infections in liver transplant recipients

Candida species	Aspergillus species	Cryptococcus species
Prolonged and complicated liver transplantation surgery or choledochojejunostomy	Fulminant hepatitis as an indication for liver transplantation	Severe immunosuppression
Prolonged broad-spectrum antibiotics use	Preoperative broad-spectrum antibiotics use	CMV disease
Prolonged hospitalization, especially in the ICU	Renal failure, especially requiring dialysis	
Post-transplantation dialysis	Retransplantation	
Retransplantation	Severe immunosuppression	
Candida colonization	The presence of Aspergillus antigenemia	
CMV disease	CMV disease	

CMV, cytomegalovirus.

Improved surgical techniques and immunosuppressive regimens have reduced mechanical complications and rejection episodes in liver transplant recipients; however, as many as 42% of liver transplant recipients still develop IFIs. The mortality associated with these infections can reach 100%, especially in cases of invasive aspergillosis.[6,10,20,21]

Fungal infections most frequently affect the lung and urinary tract. *Candida* species, especially *Candida albicans*, account for the majority of all fungal infections, followed by *Aspergillus* species, *Cryptococcus neoformans*,[8,18,20] other molds, and *Histoplasma capsulatum*.[22] Fung found fungal infections in 55 (6.6%) of 834 adults who underwent OLT between 1989 and 1992: 65% had Candida, 16% had Aspergillus, 16% had Cryptococcus, and 2% had phaeohyphomycetes. The mortality for these infections was 54.5%.[23]

As surgical methods and techniques have become increasingly sophisticated and postoperative care has improved, the incidence of fungal infections has significantly decreased.[3,17,23] Advances in immunosuppressive management have reduced the use of corticosteroids or have even eliminated their use,[17] increasing the risk of opportunistic infections. However, IFIs after liver transplantation have been associated with adverse outcomes, with reported mortality rates as high as 92–100% for invasive aspergillosis and 70% for invasive candidiasis.[6,20,21,24] A recent meta-analysis showed that *C. albicans* infections account for the majority of fungal infections, and antifungal prophylaxis has a beneficial effect on total fungal infections but not on overall mortality. However, patients receiving prophylaxis experienced a higher proportion of non-*C. albicans* infections, so the selection of triazole-resistant Candida strains is of concern and needs to be carefully addressed in future trials.[20] Ultimately, determining the risk factors for IFIs and preemptive use of prophylactic antifungal agents should be priorities, because these efforts might improve the survival rate and prognosis of liver transplant recipients.

Earlier studies reported that fungal infections in liver transplant recipients occurred predominantly in the early post-transplantation period.[5,6,15,24,25] Grauhan et al. reported that most fungal infections developed during the first 2 months post-transplantation.[5] Rabkin et al. found IFIs in their patients in the first 120 days following liver transplantation.[14] The mean time interval between transplantation and the development of a fungal infection was 15 days. Husain et al. analyzed 35 IFI cases and found that the median time of infection was 13.5 days, with 72% of infections occurring within the first month after transplantation.[24] However, more recent data have suggested a shift in IFI epidemiology in liver transplant patients. According to Singh et al., 55% of Aspergillus infections in the 1998–2001 cohort occurred $\geq$90 days after transplantation,[21] a rate similar to that found in a study conducted in Spain which reported 43% of cases of late-onset invasive aspergillosis.[18] This shift has important implications for the selection and timing of approaches to prevent invasive aspergillosis.[21]

Risk factors for fungal infections

The identification of specific risk factors that predispose liver transplant recipients to fungal infections is of critical importance. This information would facilitate the selective targeting of certain patients for specific preventive treatments, thus reducing the incidence of fungal infections and their associated mortality and health care burden. The potent immunosuppressive agents used to prevent transplant rejection usually have adverse effects on the host's defenses; they impair cell-mediated immunity, thereby increasing a patient's susceptibility to opportunistic fungal infections.[5] Therefore, the incidence of IFI is strongly influenced by the patient's clinical condition, level of immunosuppression, surgical factors, and the technical complexity of the surgery.[4]

Many studies have identified a number of risk factors associated with IFIs in liver transplant recipients. In a study of 152 transplant recipients, Briegel et al. identified two independent significant risk factors for systemic fungal infections: the amount of fresh-frozen plasma transfused due to poor initial allograft function, and acute renal failure requiring hemofiltration or hemodialysis.[10] The likelihood of IFIs increased markedly in transplant recipients with two or more risk factors. Rosenhagen et al. studied the risk factors for invasive aspergillosis and found retransplantation, CMV infection, dialysis, renal insufficiency, thrombocytopenia, and leukocytopenia to be significant factors in the univariate analysis; multivariate analysis revealed an independent influence of CMV infection and dialysis.[2] Many investigators have reported other risk factors, including prolonged operation time, a lengthy stay in the ICU, rejection treatment, fulminant hepatic failure, the need for a transfusion of cellular blood products, preoperative use of broad-spectrum antibiotics, early graft failure, fungal colonization, and re-exploration after transplantation.[13–16,18,19,26] These risk factors should be considered before antifungal prophylaxis is contemplated. Risk factors for IFIs in liver transplant recipients are summarized in Table 1.

Diagnosis of fungal infections

The diagnosis of any infection relies on recognizing indicative symptoms and signs and the laboratory isolation of the pathogenic microorganisms. However, the early diagnosis of IFI is difficult, and patients who require prophylaxis are often undiagnosed until it is too late because symptoms are often few and subtle, and signs are not specific. Moreover, laboratory isolation of fungal pathogens is difficult because some contaminating fungi may originate from the environment in the absence of disease and because other pathogens grow very slowly. Therefore, there is substantial debate among clinicians about the optimal diagnostic criteria for these infections. The identification of factors that place liver transplant recipients at risk for IFIs should improve the diagnosis of infection and the identification of patients who may benefit from antifungal prophylaxis.[17]

According to the Invasive Fungal Infections Cooperative Group in Europe and the Mycoses Study Group in the USA,[27] a 'proven' IFI is defined as a positive fungal culture or histological analysis of a tissue specimen taken from a disease site, or the identification or appearance of fungal or hyphal elements in a biopsy from a sterile site. 'Probable' and 'possible' IFIs are further defined on the basis of specific host factors, clinical features of fungal infection, and mycological evidence from culture and microscopic analysis and indirect tests, such as antigen detection. Unfortunately, these criteria only apply to the enrollment of patients in clinical trials and are not meant to guide clinical practice.[4]

So far, the ideal diagnostic assay for fungal infections in liver transplant patients has not been defined because it can be affected by many factors. Pathogens are often cultured from non-sterile sites, which affect the diagnosis. Generally, the diagnosis is made with the use of high-resolution computed tomography (CT). Invasive pulmonary aspergillosis, for example, will manifest early as a nodular opacity with surrounding attenuation, or 'halo sign'.[28] In late invasive aspergillosis, nodular lesions, diffuse pulmonary infiltrates, consolidation, or ground-glass opacities can be observed. Notably, Aspergillus infections disseminate beyond the lungs in approximately 50–60% of liver transplant recipients.[9]

Along with developments in immunology and molecular biology, new laboratory methods for detecting IFIs have also been established. Several molecules can be used as markers of Aspergillus infection, and two are of special interest: Aspergillus galactomannan (GM) and (1→3)-β-glucan (BG). The GM test is an enzyme-linked immunosorbent assay (ELISA) that detects galactomannan, an antigen released from Aspergillus hyphae upon host tissue invasion. The test's sensitivity ranges from 30% to 100%, with a specificity of approximately 85%.[28–31] However, because its sensitivity is decreased in patients receiving mold-active drugs, false-positive results are a major drawback of this test; therefore, its utility for prophylaxis has not been determined. BG, a main cell wall polysaccharide component of Aspergillus,[32] can be colorimetrically detected and is useful in diagnosis, with a sensitivity ranging from 50% to 87.5%. This component is specific for fungi other than zygomycetes and cryptococci; however, false-positive results are also a problem. GM and BG detection are useful for diagnosing invasive aspergillosis in high-risk patients after liver transplantation; moreover, a combination of the two tests can be useful for identifying false-positive reactions.[28,33]

Because universal fungal PCR primers that enable the detection of a broad range of fungi have been identified, the specific Aspergillus PCR assay has also been used to diagnose invasive aspergillosis with very good outcomes (100% sensitivity and 89% specificity). Quantitative real-time PCR for diagnosing invasive aspergillosis has shown sensitivity and specificity values of 67% and 100%, respectively, and can be used to monitor the fungal response to infection management.[28] Full advantage of this type of early laboratory diagnostic information should be taken for liver transplant recipients.

Antifungal prophylaxis

Prevention and management of IFIs in the immunocompromised patient has proven remarkably challenging. The number of antifungal agents has increased substantially in the past decades. The most commonly used are the triazole antifungals (e.g., fluconazole, itraconazole, and voriconazole) and the polyene antifungals (e.g., conventional amphotericin B and amphotericin B lipid complex). Triazole antifungals can lead to ergosterol depletion and the accumulation of aberrant sterols in the cell membrane by inhibiting the C-14 demethylation of lanosterol. Polyene antifungals achieve fungicidal activity by binding to ergosterol and disrupting the fungal cell membrane. Recently, a new family of antifungal agents, the echinocandins, has become available. Caspofungin and micafungin were approved for use in 2001 and 2005, respectively. These compounds inhibit the integrity of fungal cell walls by interfering with (1→3)-β-glucan synthase.[17]

Typically, attempts to prevent fungal infections have used both universal and preemptive prophylactic strategies. The use of selective digestive decontamination regimens, including nystatin, clotrimazole, and oral amphotericin B, could be used to maintain healthy anaerobic flora while neutralizing the overgrowth of Candida. Unfortunately, many trials of selective digestive decontamination for liver transplant recipients have failed to report the extremely low incidence of fungal infections, and none of the trials targeted only high-risk patients.[34–37] Consequently, the clinical effectiveness of selective digestive decontamination in reducing systemic Candida infections remains unknown.[4,26]

Fluconazole and other triazoles are routine prophylaxis in some transplant centers, while the amphotericin B formulation is used in others. Given the potential of antifungal agents, the emerging threat of drug resistance and the increased costs, preemptive or targeted antifungal prophylaxis should be reserved for patients with an obvious increased risk of IFIs.[17] Prime candidates for antifungal prophylaxis might include transplant recipients whose surgery was especially complicated, those who received multiple blood transfusion products, those affected by renal failure or who required dialysis, and those infected with CMV.

Antifungal prophylaxis use has reportedly reduced the incidence of IFIs in liver transplant recipients. A recent randomized, double-blind, placebo-controlled trial was performed in 71 consecutive liver transplant recipients who received either itraconazole (5.0 mg/kg orally preoperatively and 2.5 mg/kg orally twice a day postoperatively) or a placebo. They found a reduced proportion of patients with IFI in the itraconazole group (4% and 24%, respectively).[38] Another randomized trial of 232 high-risk liver transplant recipients who either received prophylactic amphotericin B or no prophylaxis found an increased incidence of fungal infections in those without prophylaxis, which was associated with increased overall hospital costs.[1] In addition, to evaluate the effectiveness of targeted antifungal prophylaxis, Singhal et al. administered 1 to 5 mg/kg doses of amphotericin B lipid complex to 30 high-risk transplant recipients. They showed no proven IFIs. Amphotericin B lipid complex appears to prevent IFIs and is well tolerated.[39]

Although antifungal prophylaxis has reduced the incidence of fungal infections, some studies have shown no improvement in overall mortality.[20,40] Winston et al. showed that prophylactic fluconazole (40 mg/day for 10 weeks after transplantation) decreased fungal colonization and prevented IFIs in a double-blind, placebo-controlled trial with 212 liver transplant recipients, but it did not improve overall survival.[41] Another randomized trial with 188 transplant recipients who received either oral itraconazole solution (200 mg every 12 h) or intravenous/oral fluconazole (400 mg every 24 h) found a similar incidence of proven IFI (7% and 3%, respectively) and no significant difference in mortality.[42] Similarly, Cruciani et al. performed a meta-analysis and determined that the beneficial effect of antifungal prophylaxis was associated with a reduction in Candida infections, and with mortality attributable to *C. albicans* in liver transplant recipients, but not with overall mortality.[20] Fortunately, a recent multicenter, non-comparative, open-label trial evaluated the prophylactic use of caspofungin (50 mg/day) for ≥21 days in 71 adult liver transplant recipients at high-risk of developing IFIs. In the modified intention-to-treat analysis, a successful treatment outcome was obtained in 88.7% of patients.[43]

Although antifungal prophylaxis for liver transplant recipients remains complex and controversial, many studies indicate that

Table 2
Prevention strategies and recommendations for invasive fungal infections in liver transplant recipients

Candida species	Aspergillus species	Cryptococcus species
Fluconazole, at least 400 mg daily for 4–8 weeks after transplantation	Lipid-associated amphotericin B, 1–5 mg/kg, or itraconazole 400 mg daily for 4 weeks before and after liver transplantation in patients with high-risk factors, especially those with two or more risk factors	Microbiological surveillance and prevention of CMV disease
Lipid-associated amphotericin B, 1 mg/kg for 5 days after transplantation	Microbiological surveillance and antifungal preemptive treatment in immunocompromised individuals	Rational use of antibiotics
Rational use of antibiotics	Rational use of antibiotics	High index of suspicion in severely immunocompromised individuals
Selective digestive decontamination	CMV disease prevention	
CMV disease prevention		
Targeted therapy with fluconazole, based on the presence of risk factors		

CMV, cytomegalovirus.

universal prophylaxis for liver transplant recipients (fluconazole in doses of at least 400 mg/day for more than 4 weeks) results in a clear but limited reduction in proven IFIs, but has no effect on overall mortality. Moreover, prophylaxis has been shown to lead to a significantly higher proportion of non-*C. albicans* infection and an increased potential for antifungal drug resistance, drug interactions, and drug-associated toxicity.[4,20,44] Given the present research evidence, preemptive or targeted antifungal prophylaxis should be offered promptly to high-risk patients (e.g., those with acute liver failure, complicated transplant surgery, or dialysis), and an appropriate antifungal agent should be selected according to the patient's condition. Recommendations regarding IFI prevention are outlined in Table 2.

Candida infection

Candidiasis is the most common fungal infection in liver transplant patients and accounts for more than 50% of IFIs.[6,8,13,24] *C. albicans* is the most frequently isolated species, followed by *Candida glabrata* and *Candida tropicalis*.[6,14,15,26] Candida is a known colonizer of the human gastrointestinal tract. Candida infection may arise after liver transplantation because conditions that support supercolonization or Candida overgrowth in the gut could promote the translocation of fungus to the extraluminal areas, resulting in subsequent intra-abdominal infections and further dissemination. Candida infections usually present as intra-abdominal abscesses, recurrent cholangitis due to biliary strictures, and peritonitis, all of which may be accompanied by fungemia.[15,17,26]

Husain et al. found that the risk factors for invasive candidiasis include the use of antibiotics to prevent spontaneous bacterial peritonitis, post-transplantation dialysis, and retransplantation.[24] Other risk factors include lengthy and complicated transplantation surgery, intraoperative transfusion,[3] antibiotic use, prolonged hospitalization (especially in the ICU), repeated intra-abdominal surgery after transplantation, Candida colonization, and CMV disease.[26]

Selective digestive decontamination with non-absorbable antibiotics, including nystatin, clotrimazole, and oral amphotericin B, can maintain anaerobic bacterial growth and reduce overgrowth of Candida. The clinical effectiveness of this technique, however, remains unknown.[4,26] At present, fluconazole is the most commonly used antifungal agent. The majority of studies have shown that antifungal prophylaxis clearly reduces fungal colonization, the overall incidence of proven fungal infections, and mortality attributable to fungal infection.[4,6,17,20,45] Growing evidence shows that fluconazole plays an important role in shifting infections toward non-*C. albicans* species.[20,46,47] A randomized, controlled study demonstrated that lipid-associated amphotericin B, when administered in the first 5 days after liver transplantation at a dose of 1 mg/kg, was also effective in reducing Candida infections during the first months after transplantation;[48] however, the value of preemptive treatment remains to be proven.[26] The current Infectious Diseases Society of America (IDSA) guidelines for managing candidiasis also recommend that only patients with two or more key risk factors (retransplantation, preoperative creatinine >2.0 mg/dl, choledochojejunostomy, intraoperative requirement of ≥ 40 units of blood products, prolonged intraoperative time, and fungal colonization at least 2 days prior to and 3 days after transplantation) be considered for antifungal prophylaxis.[49] CMV disease is a clear risk factor for all types of IFIs, and effective prophylaxis of patients at high risk for CMV disease, such as those who are CMV D+/R− (donor positive, recipient negative), has been shown to significantly decrease the incidence of invasive Candida infection in the absence of specific anti-Candida prophylaxis.[50]

Aspergillus infection

Aspergillus spp are the second most common fungal pathogens responsible for infections in liver transplant recipients; they account for up to one quarter of IFIs.[26] *Aspergillus spp* are airborne in all environments, both inside and outside the hospital. The inhalation of airborne spores results in pulmonary infection, with extrapulmonary dissemination to the central nervous system (CNS) and virtually any other organ.[17] Nevertheless, only a few species cause illness in humans; the individual's immunological status and pulmonary condition determine the disease pattern. The diagnosis of aspergillosis can be elusive because the fungus is very difficult to isolate and symptoms and signs are not specific.

Surgical and medical improvements have not been associated with a decrease in the frequency of invasive aspergillosis,[3] and the invasive aspergillosis-related mortality rate for these patients exceeds 90%.[51] Many risk factors for invasive aspergillosis have been studied, including renal insufficiency, dialysis, retransplantation,[12] CMV infection, thrombocytopenia, leukocytopenia,[2] repeated bacterial infections, allograft dysfunction,[18] preoperative ICU stay, preoperative steroid administration,[16] fulminant hepatic failure,[15] the presence of Aspergillus antigenemia,[19] laparotomies, and the use of OKT3 monoclonal antibody.[52] Additionally, the severe immunosuppression conditions caused by anti-rejection drugs in liver transplant patients contribute to invasive Aspergillus infections.[6,26]

The identification of potential risk factors may reduce the morbidity and mortality rates of invasive aspergillosis. Unfortunately, except for patients with acute fulminant failure before transplantation, patients at risk (e.g., those who are severely immunocompromised) are difficult to identify. Antifungal therapy should be instituted upon any clinical suspicion of aspergillosis without waiting for microbiology results. Linden et al. reported

that first-line or early salvage for invasive aspergillosis with appropriate amphotericin B lipid complex, particularly at doses of 1.0 mg/kg daily, reduced mortality and improved survival.[53] Voriconazole, which should be available in the near future, could also be an alternative, but further research is needed. To evaluate the efficacy and safety of voriconazole in acute invasive aspergillosis, Denning et al. treated invasive aspergillosis patients with intravenously administered voriconazole 6 mg/kg twice daily, then 3 mg/kg three times daily for 6–27 days, followed by 200 mg twice daily, administered orally for up to 24 weeks. They reported a good response, proving that voriconazole is efficacious in treating acute invasive aspergillosis.[54] For severely immunocompromised patients after liver transplantation, we recommend an enhanced level of suspicion for IFI development and a prompt and aggressive search for the infection.[26]

Cryptococcus neoformans infection

Cryptococcosis is the third most common IFI in liver transplant recipients. *C. neoformans* is a ubiquitous saprophytic fungus with worldwide distribution. It is found in nature primarily in bird excrement, but non-avian sources have also been described.[55] The organism is tropic to the CNS and is the most common cause of meningitis in transplant recipients.[17,55] Cryptococcosis is thought to result from the failure of the host's defenses to contain the organism after inhaling aerosolized spores from an environmental source, and it manifests as symptomatic pneumonia or asymptomatic infection.[55] The median time before disease onset usually ranges from 16 to 21 months after transplantation.[55–57] The major *C. neoformans* infection sites in organ transplant recipients include the CNS and lungs, but this microorganism can also infect other organs and disseminate to multiple sites. CNS involvement and disseminated infections (involvement of two or more sites) have been documented in 52–61% of patients.[58] Recently, a longitudinal study of cryptococcosis in adult solid-organ transplant recipients suggested that cryptococcal infection occurs in 12 cases per 1000 transplant recipients. Symptoms emerged a mean of 30 months after transplantation. Clinical manifestations of infection included pneumonia only (46%), meningitis only (36%), dissemination to multiple distant organs (11%), or involvement of another single organ (e.g., lymph node) (7%). Cryptococcosis-associated mortality was 25%.[59]

The liver transplant patients most at risk for Cryptococcus infection are those who are severely immunosuppressed, which can contribute to a high level of CMV replication. In fact, CMV can increase not only the risk of Cryptococcus infection, but also the risk of Aspergillus and Candida infection.[15,26] Culture detection is necessary to diagnose cryptococcosis; however, a negative culture does not rule out cryptococcosis because small numbers of the organism may be present in the patient's cerebrospinal fluid (CSF) and in other clinical samples, or the cultured organism may not grow. Although serum cryptococcal antigen is helpful for diagnosing meningitis or disseminated disease, its sensitivity in patients with pneumonia is only about 40%.[17] Microbiological surveillance and CMV disease prevention are necessary to inhibit disease progress. Guidelines for cryptococcal disease management in solid-organ transplant recipients have been published by the IDSA.[60] Once diagnosed, cryptococcal meningitis is treated with a combination of liposomal amphotericin B or amphotericin B lipid complex and flucytosine (5-FC) for at least 2 weeks for the induction regimen, followed by fluconazole for 8 weeks for consolidation therapy, and fluconazole for 6–12 months for maintenance treatment.

Histoplasma capsulatum infection

H. capsulatum is endemic in the soil of the Ohio and Mississippi River Valleys and also prevalent in certain areas of South America, where it triggers several hundred thousand new infections each year.[61,62] The opportunistic dimorphic fungus can switch from a filamentous spore-forming mold in the soil to a pathogenic budding-yeast form in the human host, while inhalation of fungal conidia frequently results in subclinical infection or mild pulmonary illness in the normal human host.[63,64] Disseminated histoplasmosis most likely results from primary or secondary exposure or reactivation of latent disease usually induced by immunosuppressive therapy.[65] The incidence of histoplasmosis among liver transplant recipients is estimated to be low, with only a few case series.[22,65–67] The first case report of disseminated histoplasmosis after OLT was reported by Shallot et al. in the USA.[61] Following this report, the incidence of post-transplant histoplasmosis has gradually increased over the years. The clinical features of disseminated histoplasmosis in liver transplant recipients are nonspecific and similar to many other disseminated infections, and consist primarily of fever, cough, shortness of breath, and malaise or fatigue, usually resulting in a self-limited or latent disease.[68] Botterel et al. found evidence of disseminated histoplasmosis in the lungs, digestive tract, spleen, adrenal glands, and mesenteric lymph nodes at the time of autopsy in a liver transplant recipient initially presenting with respiratory failure and shock.[65]

A diagnosis of proven post-transplantation histoplasmosis is established with culture, including blood cultures and bone marrow aspiration, as well as biopsy cultures from possibly affected organs, or histopathology. However, other tests, such as the Histoplasma urine antigen test or the Histoplasma serological test, may provide more rapid results. Many previous studies have reported that the sensitivity of the Histoplasma urine antigen test for the diagnosis of disseminated disease is approximately 90% for the immunocompromised patient.[69] A recent study in solid organ transplant recipients demonstrated that 69% were positive by Histoplasma urine antigen test and 33% were positive by Histoplasma serological test .[22] Disseminated histoplasmosis is a potentially lethal event but is relatively uncommon among liver transplant recipients. Because timely diagnosis may be aided by the use of urinary and serum Histoplasma antigen tests and by aggressive bronchoscopic evaluation of lesions seen on a CT scan of the chest, with appropriate treatment, the prognosis appears to be good.[22]

According to the IDSA guidelines for the management of histoplasmosis, the therapy of histoplasmosis in general should start with liposomal amphotericin B. Studies have shown this to cure histoplasmosis more often than itraconazole. However, with a favorable course, therapy can later be switched to oral itraconazole in many cases.[64,70] The duration of treatment varies from 12 weeks for acute disease to more than 12 months for progressive disseminated disease. Blood and urine antigenemia can be used for monitoring, especially after the end of therapy, which often lasts a year, since disease can recur. However, treatment should also be individualized on the basis of diagnosis, the state of immunosuppression, and potential consequences of diseases (e.g., CNS).[64] As antifungal prophylaxis evolves over time, to reduce IFI complications after liver transplantation, we should continue to pay attention to the regional epidemiology of histoplasmosis.[22]

Conclusions

Given the increased risk and poor outcomes in liver transplant recipients who develop fungal infections, early diagnosis and aggressive antifungal prophylaxis should be considered upfront in high-risk patients. The early identification of patients at high risk of developing fungal infections may improve outcomes. Further research is needed to determine the benefits of new molecular and immunological diagnostic assays. Concerns about identifying high risk transplant recipients and selecting appropriate antifungal

agents are very relevant, and the potential advantages of prophylaxis should be measured against the potential harm.

Ethical approval

Our study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, Zhejiang Province, China.

Conflict of interest

The authors have no conflicts of interest to disclose.

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References

1. Reed A, Herndon JB, Ersoz N, Fujikawa T, Schain D, Lipori P, et al. Effect of prophylaxis on fungal infection and costs for high-risk liver transplant recipients. *Liver Transpl* 2007;**13**:1743–50.
2. Rosenhagen M, Feldhues R, Schmidt J, Hoppe-Tichy T, Geiss HK. A risk profile for invasive aspergillosis in liver transplant recipients. *Infection* 2009;**37**:313–9.
3. Singh N, Wagener MM, Marino IR, Gayowski T. Trends in invasive fungal infections in liver transplant recipients: correlation with evolution in transplantation practices. *Transplantation* 2002;**73**:63–7.
4. Eschenauer GA, Lam SW, Carver PL. Antifungal prophylaxis in liver transplant recipients. *Liver Transpl* 2009;**15**:842–58.
5. Grauhan O, Lohmann R, Lemmens P, Schattenfroh N, Keck H, Klein E, et al. Fungal infections in liver transplant recipients. *Langenbecks Arch Chir* 1994;**379**:372–5.
6. Paya CV. Fungal infections in solid-organ transplantation. *Clin Infect Dis* 1993;**16**:677–88.
7. Singh N. Fungal infections in the recipients of solid organ transplantation. *Infect Dis Clin North Am* 2003;**17**:113–34. viii.
8. Wajszczuk CP, Dummer JS, Ho M, Van Thiel DH, Starzl TE, Iwatsuki S, et al. Fungal infections in liver transplant recipients. *Transplantation* 1985;**40**:347–53.
9. Singh N, Paterson DL. Aspergillus infections in transplant recipients. *Clin Microbiol Rev* 2005;**18**:44–69.
10. Briegel J, Forst H, Spill B, Haas A, Grabein B, Haller M, et al. Risk factors for systemic fungal infections in liver transplant recipients. *Eur J Clin Microbiol Infect Dis* 1995;**14**:375–82.
11. Verma A, Wade JJ, Cheeseman P, Samaroo B, Rela M, Heaton ND, et al. Risk factors for fungal infection in paediatric liver transplant recipients. *Pediatr Transplant* 2005;**9**:220–5.
12. Singh N, Pruett TL, Houston S, Munoz P, Cacciarelli TV, Wagener MM, et al. Invasive aspergillosis in the recipients of liver retransplantation. *Liver Transpl* 2006;**12**:1205–9.
13. Shi SH, Lu AW, Shen Y, Jia CK, Wang WL, Xie HY, et al. Spectrum and risk factors for invasive candidiasis and non-Candida fungal infections after liver transplantation. *Chin Med J (Engl)* 2008;**121**:625–30.
14. Rabkin JM, Oroloff SL, Corless CL, Benner KG, Flora KD, Rosen HR, et al. Association of fungal infection and increased mortality in liver transplant recipients. *Am J Surg* 2000;**179**:426–30.
15. Patel R, Portela D, Badley AD, Harmsen WS, Larson-Keller JJ, Ilstrup DM, et al. Risk factors of invasive Candida and non-Candida fungal infections after liver transplantation. *Transplantation* 1996;**62**:926–34.
16. Osawa M, Ito Y, Hirai T, Isozumi R, Takakura S, Fujimoto Y, et al. Risk factors for invasive aspergillosis in living donor liver transplant recipients. *Liver Transpl* 2007;**13**:566–70.
17. Kusne S, Blair JE. Viral and fungal infections after liver transplantation—part II. *Liver Transpl* 2006;**12**:2–11.
18. Gavalda J, Len O, San Juan R, Aguado JM, Fortun J, Lumbreras C, et al. Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case–control study. *Clin Infect Dis* 2005;**41**:52–9.
19. Fortun J, Martin-Davila P, Moreno S, De Vicente E, Nuno J, Candelas A, et al. Risk factors for invasive aspergillosis in liver transplant recipients. *Liver Transpl* 2002;**8**:1065–70.
20. Cruciani M, Mengoli C, Malena M, Bosco O, Serpelloni G, Grossi P. Antifungal prophylaxis in liver transplant patients: a systematic review and meta-analysis. *Liver Transpl* 2006;**12**:850–8.
21. Singh N, Avery RK, Munoz P, Pruett TL, Alexander B, Jacobs R, et al. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. *Clin Infect Dis* 2003;**36**:46–52.
22. Cuellar-Rodriguez J, Avery RK, Lard M, Budev M, Gordon SM, Shrestha NK, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009;**49**:710–6.
23. Fung JJ. Fungal infection in liver transplantation. *Transpl Infect Dis* 2002;**4**(Suppl 3):18–23.
24. Husain S, Tollemar J, Dominguez EA, Baumgarten K, Humar A, Paterson DL, et al. Changes in the spectrum and risk factors for invasive candidiasis in liver transplant recipients: prospective, multicenter, case-controlled study. *Transplantation* 2003;**75**:2023–9.
25. Karchmer AW, Samore MH, Hadley S, Collins LA, Jenkins RL, Lewis WD. Fungal infections complicating orthotopic liver transplantation. *Trans Am Clin Climatol Assoc* 1995;**106**:38–47. discussion 47–8.
26. Paya CV. Prevention of fungal and hepatitis virus infections in liver transplantation. *Clin Infect Dis* 2001;**33**(Suppl 1):S47–52.
27. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
28. Muñoz P, Guinea J, Bouza E. Update on invasive aspergillosis: clinical and diagnostic aspects. *Clin Microbiol Infect* 2006;**12**:24–39.
29. Maertens J, Van Eldere J, Verhaegen J, Verbeken E, Verschakelen J, Boogaerts M. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. *J Infect Dis* 2002;**186**:1297–306.
30. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001;**97**:1604–10.
31. Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. *Lancet Infect Dis* 2004;**4**:349–57.
32. Latge JP, Moutaouakil M, Debeaupuis JP, Bouchara JP, Haynes K, Prevost MC. The 18-kilodalton antigen secreted by *Aspergillus fumigatus*. *Infect Immun* 1991;**59**:2586–94.
33. Pazos C, Ponton J, Del Palacio A. Contribution of (1–>3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005;**43**:299–305.
34. Arnow PM, Carandang GC, Zabner R, Irwin ME. Randomized controlled trial of selective bowel decontamination for prevention of infections following liver transplantation. *Clin Infect Dis* 1996;**22**:997–1003.
35. Hellinger WC, Yao JD, Alvarez S, Blair JE, Cawley JJ, Paya CV, et al. A randomized, prospective, double-blinded evaluation of selective bowel decontamination in liver transplantation. *Transplantation* 2002;**73**:1904–9.
36. Rosman C, Klompmaker IJ, Bonsel GJ, Bleichrodt RP, Arends JP, Slooff MJ. The efficacy of selective bowel decontamination as infection prevention after liver transplantation. *Transplant Proc* 1990;**22**:1554–5.
37. Zwaveling JH, Maring JK, Klompmaker IJ, Haagsma EB, Bottema JT, Laseur M, et al. Selective decontamination of the digestive tract to prevent postoperative infection: a randomized placebo-controlled trial in liver transplant patients. *Crit Care Med* 2002;**30**:1204–9.
38. Sharpe MD, Ghent C, Grant D, Horbay GL, McDougal J, David Colby W. Efficacy and safety of itraconazole prophylaxis for fungal infections after orthotopic liver transplantation: a prospective, randomized, double-blind study. *Transplantation* 2003;**76**:977–83.
39. Singhal S, Ellis RW, Jones SG, Miller SJ, Fisher NC, Hastings JG, et al. Targeted prophylaxis with amphotericin B lipid complex in liver transplantation. *Liver Transpl* 2000;**6**:588–95.
40. Fortun J, Martin-Davila P, Moreno S, Barcena R, de Vicente E, Honrubia A, et al. Prevention of invasive fungal infections in liver transplant recipients: the role of prophylaxis with lipid formulations of amphotericin B in high-risk patients. *J Antimicrob Chemother* 2003;**52**:813–9.
41. Winston DJ, Pakrasi A, Busuttil RW. Prophylactic fluconazole in liver transplant recipients. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1999;**131**:729–37.
42. Winston DJ, Busuttil RW. Randomized controlled trial of oral itraconazole solution versus intravenous/oral fluconazole for prevention of fungal infections in liver transplant recipients. *Transplantation* 2002;**74**:688–95.
43. Fortun J, Martin-Davila P, Montejo M, Munoz P, Cisneros JM, Ramos A, et al. Prophylaxis with caspofungin for invasive fungal infections in high-risk liver transplant recipients. *Transplantation* 2009;**87**:424–35.
44. Playford EG, Webster AC, Sorrell TC, Craig JC. Systematic review and meta-analysis of antifungal agents for preventing fungal infections in liver transplant recipients. *Eur J Clin Microbiol Infect Dis* 2006;**25**:549–61.
45. Singh N, Wagener MM, Cacciarelli TV, Levitsky J. Antifungal management practices in liver transplant recipients. *Am J Transplant* 2008;**8**:426–31.
46. Nguyen MH, Peacock Jr JE, Morris AJ, Tanner DC, Nguyen ML, Snydman DR, et al. The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. *Am J Med* 1996;**100**:617–23.
47. Rocco TR, Reinert SE, Simms HH. Effects of fluconazole administration in critically ill patients: analysis of bacterial and fungal resistance. *Arch Surg* 2000;**135**:160–5.
48. Tollemar J, Hockerstedt K, Ericzon BG, Jalanko H, Ringden O. Liposomal amphotericin B prevents invasive fungal infections in liver transplant recipients. A randomized, placebo-controlled study. *Transplantation* 1995;**59**:45–50.
49. Pappas PG, Kauffman CA, Andes D, Benjamin Jr DK, Calandra TF, Edwards Jr JE, et al. Clinical practice guidelines for the management of candidiasis: 2009

update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
50. Badley AD, Seaberg EC, Porayko MK, Wiesner RH, Keating MR, Wilhelm MP, et al. Prophylaxis of cytomegalovirus infection in liver transplantation: a randomized trial comparing a combination of ganciclovir and acyclovir to acyclovir. NIDDK Liver Transplantation Database. *Transplantation* 1997;**64**:66–73.
51. Singh N, Arnow PM, Bonham A, Dominguez E, Paterson DL, Pankey GA, et al. Invasive aspergillosis in liver transplant recipients in the 1990s. *Transplantation* 1997;**64**:716–20.
52. Kusne S, Torre-Cisneros J, Manez R, Irish W, Martin M, Fung J, et al. Factors associated with invasive lung aspergillosis and the significance of positive Aspergillus culture after liver transplantation. *J Infect Dis* 1992;**166**:1379–83.
53. Linden PK, Coley K, Fontes P, Fung JJ, Kusne S. Invasive aspergillosis in liver transplant recipients: outcome comparison of therapy with amphotericin B lipid complex and a historical cohort treated with conventional amphotericin B. *Clin Infect Dis* 2003;**37**:17–25.
54. Denning DW, Ribaud P, Milpied N, Caillot D, Herbrecht R, Thiel E, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 2002;**34**:563–71.
55. Vilchez RA, Fung J, Kusne S. Cryptococcosis in organ transplant recipients: an overview. *Am J Transplant* 2002;**2**:575–80.
56. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in solid organ transplant recipients: current state of the science. *Clin Infect Dis* 2008;**47**:1321–7.
57. Singh N, Forrest G. Cryptococcosis in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):S192–8.
58. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis* 2001;**7**:375–81.
59. Vilchez R, Shapiro R, McCurry K, Kormos R, Abu-Elmagd K, Fung J, et al. Longitudinal study of cryptococcosis in adult solid-organ transplant recipients. *Transpl Int* 2003;**16**:336–40.
60. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
61. Shallot J, Pursell KJ, Bartolone C, Williamson P, Benedetti E, Layden TJ, et al. Disseminated histoplasmosis after orthotopic liver transplantation. *Liver Transpl Surg* 1997;**3**:433–4.
62. Kauffman CA. Histoplasmosis: a clinical and laboratory update. *Clin Microbiol Rev* 2007;**20**:115–32.
63. Wheat LJ, Conces D, Allen SD, Blue-Hnidy D, Loyd J. Pulmonary histoplasmosis syndromes: recognition, diagnosis, and management. *Semin Respir Crit Care Med* 2004;**25**:129–44.
64. Wheat J, Sarosi G, McKinsey D, Hamill R, Bradsher R, Johnson P, et al. Practice guidelines for the management of patients with histoplasmosis. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:688–95.
65. Botterel F, Romand S, Saliba F, Reynes M, Bismuth H, Samuel D, et al. A case of disseminated histoplasmosis likely due to infection from a liver allograft. *Eur J Clin Microbiol Infect Dis* 1999;**18**:662–4.
66. Vinayek R, Balan V, Pinna A, Linden PK, Kusne S. Disseminated histoplasmosis in a patient after orthotopic liver transplantation. *Clin Transplant* 1998;**12**: 274–7.
67. Oh YS, Lisker-Melman M, Korenblat KM, Zuckerman GR, Crippin JS. Disseminated histoplasmosis in a liver transplant recipient. *Liver Transpl* 2006;**12**:677–81.
68. Freifeld AG, Iwen PC, Lesiak BL, Gilroy RK, Stevens RB, Kalil AC. Histoplasmosis in solid organ transplant recipients at a large Midwestern university transplant center. *Transpl Infect Dis* 2005;**7**:109–15.
69. Kauffman CA. Diagnosis of histoplasmosis in immunosuppressed patients. *Curr Opin Infect Dis* 2008;**21**:421–5.
70. Wheat LJ, Freifeld AG, Kleiman MB, Baddley JW, McKinsey DS, Loyd JE, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:807–25.