Review

Histoplasmosis in Liver Transplant Recipients: Case Reports and a Review of the Literature

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Abstract

We report two cases of histoplasmosis in orthotopic liver transplant (OLT) recipients to illustrate the variable presentations, complications encountered during treatment, the spectrum of diagnostic modalities, and case outcomes. Case 1 describes the insidious presentation of presumed gastrointestinal histoplasmosis 12 years after OLT, which was defined by months of intermittent diarrhea and focal colonic disease on colonoscopy. A diagnosis of *Histoplasma capsulatum* was ultimately made by broad range PCR performed on colonic tissue. Due to the patient's inability to tolerate itraconazole, treatment consisted of two weeks of liposomal amphotericin B with resolution of colonic ulcers on follow-up colonoscopy. Case 2 illustrates a case of severe pulmonary histoplasmosis with

dissemination in an OLT recipient one year after transplantation. Treatment was complicated by the development of acute respiratory distress syndrome requiring mechanical ventilation and hemodynamic shock requiring extracorporeal membrane oxygenation therapy. A review of the literature on histoplasmosis in OLT recipients accompanies our cases and emphasizes the epidemiology, clinical manifestations, diagnosis, treatment, and prevention.

Keywords
Histoplasmosis; liver; transplant

1. Introduction

The geographic distribution of *Histoplasma*, a thermally dimorphic fungus found in soil, is dynamic and is thought to have a worldwide distribution [1]. Although the exact prevalence is not known, several studies have highlighted Brazil, Thailand, China, India, French Guiana, Africa, and Australia as countries of high endemicity [2-7]. Within the United States, the Ohio, Mississippi, and St. Lawrence River valleys represent the geographical niches with the highest endemicity of *Histoplasma*; however, outbreaks in states outside of these river valleys do occur [6, 8]. Infection develops primarily through inhalation of infectious microconidia (Figure 1) [1, 3-5]. Alveolar macrophages then phagocytize the spores, allowing intracytoplasmic yeast forms to be seen on direct microscopy of Giemsa-stained macrophages (Figure 2). Donor-derived *Histoplasma* is a unique mode of infection that must be considered in transplant recipients [1, 9]. Disease severity is dependent on several factors, including the load of inhaled conidia and the host's cellular immune response [1, 9]. Hematogenous dissemination of *Histoplasma* occurs in most patients after acute infection but rarely manifests as disseminated infection due to the development of cell-mediated immunity [10]. In a healthy host, sensitized T-cells alert macrophages to kill the intracellular organism. In the immunosuppressed host, *Histoplasma* remains viable in macrophages leading to progressive disseminated histoplasmosis that is fatal if left untreated [10]. Even in immunocompetent hosts, *Histoplasma* may not always be eradicated by cell-mediated immune response, but instead be contained, leading to potential reactivation at a later time if there is a decline in cellular immunity, such as after orthotopic liver transplantation (OLT) [4, 9]. In this article, we describe two unique presentations of *Histoplasma capsulatum* infection occurring in OLT recipients, and we provide a review of the literature from 1996 through July 2019.
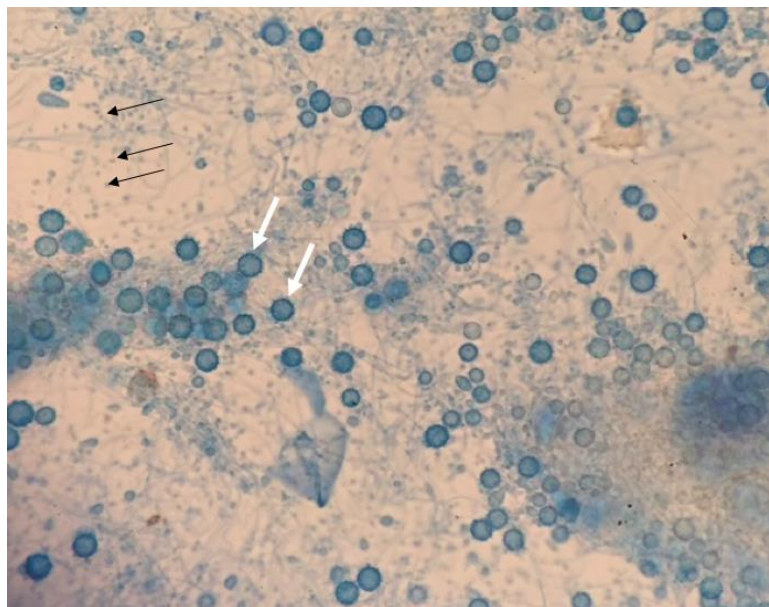
Figure 1 Present on hyaline and septate hyphae are abundant tuberculate, thick-walled macroconidia (white arrows) and numerous smooth-walled microconidia (black arrows) (Lactophenol cotton blue stain, magnification 40x). Figure courtesy of Dr. Todd M. Lasco, Microbiology Laboratory at Baylor St. Luke's Medical Center, Houston, TX 77030, USA.

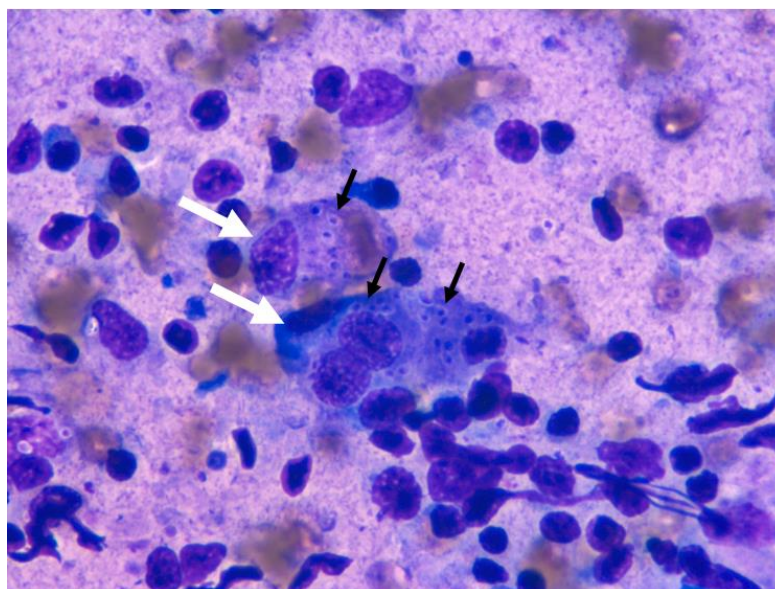**Figure 2** Fine needle aspiration smear showing intracellular yeast forms (black arrows) of *Histoplasma* within macrophages (white arrows) (Giemsa stain, magnification 1000x). Figure courtesy of Dr. Laila Woc-Colburn.

2. Cases

Per Baylor College of Medicine policies, case reports or case series describing interesting observations on three or fewer patients do not meet the definition of research. This submission is

a case report. This submission is not considered research under 45 CFR 46.102(d). Therefore, this manuscript was exempt from IRB review and approval.

2.1 Case 1

A 51-year-old Caucasian female from Houston, Texas with a past medical history of chronic kidney disease and autoimmune hepatitis status post OLT 12 years prior presented with intermittent diarrhea of several months' duration. Her immunosuppressive regimen included: mycophenolate mofetil (MMF) and sirolimus. She underwent colonoscopy, which revealed an ulcerated, friable mass suspicious for malignancy in the ascending colon. On histopathology, necrotizing granulomatous inflammation was seen, and special stains for fungal organisms, acid-fast bacilli (AFB), and immunohistochemical markers for cytomegalovirus, human herpesvirus 1 and 2 were negative. Colonic tissue microbiology studies were negative for organisms by Gram's stain and cultures for AFB, fungal organisms, and routine bacterial culture. Fungal serum studies for blastomycosis, coccidioidomycosis, and histoplasmosis were negative. Colonic tissue was sent for broad range 16S ribosomal ribonucleic acid gene sequencing (University of Washington, Seattle, Washington), which was positive for *Histoplasma capsulatum*. Given the clinical presentation, necrotizing granulomas on histopathology, and *Histoplasma* detected in tissue by broad range PCR, a presumptive diagnosis of colonic histoplasmosis was made. The patient was started on itraconazole, but due to intolerability to the medication, treatment was switched to daily infusions of liposomal amphotericin B (LAmB) at 3 mg/kg/day for two weeks. Her diarrhea resolved, and a repeat colonoscopy was performed one month after the completion of therapy showing normal mucosa throughout the entire colon. Histopathology from colonic mucosal biopsies revealed no significant diagnostic abnormalities. No further antifungal therapy was given due to patient preference, history of intolerance to itraconazole, chronic renal disease, clinically localized histoplasmosis without evidence of dissemination, and demonstration of resolved colonic lesions on colonoscopy. She maintains close medical follow up and has had no evidence of recurrence.

2.2 Case 2

A 34-year-old Hispanic male was admitted for worsening pancytopenia of a two-week duration. His past medical history was significant for end-stage liver disease secondary to alcoholic cirrhosis status post OLT one year prior. He lived in Houston, TX and recently started mowing the lawn and landscaping. Two weeks prior to the current hospitalization, he was admitted for fevers without associated symptoms and underwent an extensive infectious disease workup that only revealed Rhinovirus on a respiratory viral panel. He received supportive care at the time. Upon re-admission, he revealed that he had been having persistent, daily fevers, and dry cough since discharge. His immunosuppressant regimen included the following: prednisone and tacrolimus. MMF had been stopped a few days prior to admission due to worsening cytopenia. His laboratory values revealed leukopenia (0.8 K/μL; reference range: 4.0-10.0 K/μL) and anemia (7.5 g/dL; reference range: 13.0-16.8 g/dL). Chest computed tomography revealed diffuse reticulonodular markings and multifocal ground-glass opacities throughout the pulmonary parenchyma. The patient underwent bronchoscopy with lavage, which showed numerous predominantly intracellular yeast forms, suggestive of *Histoplasma sp*. Lavage fungal culture was positive for *Histoplasma capsulatum*, which was confirmed by deoxyribonucleic acid (DNA) probe. Urine

Histoplasma Antigen was >25.0 ng/mL (reference range: <0.5 ng/mL). Following a diagnosis of disseminated histoplasmosis, he was started on LAmB. Fevers and leukopenia resolved on LAmB; however, his hospital course was complicated by the development of acute respiratory distress syndrome (ARDS) requiring mechanical ventilation, hemodynamic shock requiring extracorporeal membrane oxygenation therapy and adjunctive corticosteroids, and acute kidney injury. His antifungal regimen consisted of 11 days of LAmB 3 mg/kg/day, followed by itraconazole 200 mg twice daily for a planned twelve months of treatment. He retained his graft and slowly improved.

3. Epidemiology

Histoplasmosis has an extensive range in North America, extending from Canada into South America [1]. It is the most common endemic mycosis in the United States (U.S.) with substantial population exposure, by evidence of skin testing, dating back to the mid-twentieth century [1, 8]. Even within the U.S., disease surveillance data has identified a growing number of cases in states not previously thought to be endemic, such as Michigan, New York, and Texas, among others [1, 8]. Experts postulate that this expanding endemicity could be due to multiple factors, including a growing population of vulnerable, immunosuppressed hosts [1, 8]. A product of mitigated immune-mediated rejection, OLT patients are at higher risk of severe infection with *Histoplasma* due to impaired cellular immunity. Surveillance studies estimate that the incidence of histoplasmosis in transplant recipients in endemic areas is <1% [1, 4, 5, 11, 12]. The true incidence may be higher due to a lack of available laboratory support for surveillance and testing in some areas. On review of the literature, we identified twelve comprehensive case reports, spanning several countries, describing histoplasmosis infection in OLT recipients in the past two decades (Table 1).

4. Modes of Acquisition

Histoplasma infection in transplant recipients living in endemic areas is thought to occur primarily through inhalation of spores from exposure to disrupted soil [13]. However, reactivation of infection in the setting of immune suppression after transplantation or rejection can occur, even in nonendemic regions [1, 13]. Sylvestre et al. published a collection of images that showcased histopathology from a patient who had presumptive latent histoplasmosis then received an OLT. Fungal organisms were then seen on core-needle biopsy of the graft nine days after transplantation, prompting treatment [14]. Donor-derived infection is rare and estimated to occur in 1:10,000 transplants [1, 2, 13, 15, 16]. Our review of the literature found two case reports that featured presumed donor-derived histoplasmosis after liver transplantation (Table 1). Niyazi et al. described a 61-year-old male who received an OLT from a donor that was observed to have granulomatous inflammation of the spleen at autopsy [17]. The OLT recipient was placed on itraconazole for prophylaxis but developed severe infection five months post-transplant after discontinuing his antifungal medication. Botterel et al. describe a fatal case of disseminated histoplasmosis occurring seven months after OLT in a patient in France [2]. The recipient had no known travel to an endemic area; however, the donor lived in French Guiana for several years, suggesting *Histoplasma* was present in the liver at the time of transplantation.

Table 1 Summary of case reports of Histoplasmosis in liver transplant recipients.

References	Age (yrs)	Presenting signs/symptoms	Onset post-transplant (mo.)	Organ(s) involved	Treatment ±	Method of diagnosis	Geographic location ‡	Immunosuppressive Treatments ¶
1. Case 1	51	Diarrhea	132	GI tract (colon)	Itraconazole◊, LAmB 3 mg/kg/day for 2 wks	+ Tissue PCR (– Serum *Histoplasma* Ab)	Texas, U.S.A.	MMF, sirolimus
2. Case 2	34	Fever, pancytopenia, cough	12	Lungs, bone marrow	LAmB 3 mg/kg/day for 11 days, itraconazole 200 mg twice daily for 12 mo.	+ BAL culture + Urine *Histoplasma* Ag	Texas, U.S.A.	Prednisone, tacrolimus, MMF
3. Nakamura, GP, et al., Autops Case Rep 2019 [3]	36	Fever, diarrhea, rash and oral lesions	96	GI tract (oral, intestinal, rectal)	Itraconazole 300 mg/day	+ Histopathology	Brazil	Prednisone 5 mg/day, MMF 360 mg
4. Gomez-Santana, LV, et al. Actas Dermosifiliogr 2018 (case 3) [18]	75•	Fever, skin lesions	N/A	Skin	LAmB 5 mg/kg/day	+ Skin tissue culture	Argentina	Methylprednisone 4 mg/day, tacrolimus 3 mg twice daily, MMF 360 mg twice daily
5. Agrawal, N, et al. World J Gastroenterol	74•	Diarrhea, abdominal pain, weight loss	180	GI tract (ileo-colonic)	N/A	+ Histopathology	United Kingdom	MMF 1g twice daily, tacrolimus 1 mg twice daily,

2017 [4]							prednisone 5 mg/day
6. *Washburn, L, et al. JSCR 2017* [19]	32	Body aches, fever, congestion, dysuria, transaminitis	132	Liver	LAmB for 11 days, itraconazole for 1 yr	+ Histopathology + Urine *Histoplasma* Ag	Texas, U.S.A.	MMF, tacrolimus
7. *Niyazi, F, et al. Am J Gastroenterol 2015†* [17]	61	Fever, pancytopenia, abdominal pain	5	Liver*	Itraconazole	+ Histopathology + Urine *Histoplasma* Ag	Michigan, U.S.A.	Prednisone, tacrolimus, MMF
8. *Wiederkehr, J, et al. Transplantation 2014†* [20]	18	Tonsillitis, oral ulcers, fever, weight loss	9	Inferior vena cava, liver, spleen, GI tract	LAmB, itraconazole	+ Histopathology	Brazil	Tacrolimus, MMF
9. *Colaiacovo, R, et al. Endoscopy 2011†* [21]	68	Abdominal pain	12	GI tract (stomach, duodenum, colon)	Itraconazole	+ Histopathology	Brazil	N/A
10. *Leite, CAC, et al. Liver Transpl 2009†* [22]	15	Knee pain	9	Left knee synovium	Itraconazole	+ Synovial fluid culture	Brazil	Tacrolimus, prednisone
11. *Oh, YS, et al. Liver Transpl 2006* [23]	61	Diarrhea	N/A	GI tract (colon), pleural fluid	LAmB 100 mg/day for 50 doses, itraconazole 200 mg twice daily¤	+ Histopathology + Urine *Histoplasma* Ag (– Serum *Histoplasma* Ab)	Missouri, U.S.A.	MMF 1g twice daily, tacrolimus 2 mg/day, prednisone 5 mg/day
12. *Botterel, F, et al. Eur J Clin*	46•	Acute respiratory failure, shock,	7	Lungs, GI tract, spleen,	AmB, LAmB	+ BAL cytology + Tissue culture	France	Tacrolimus, azathioprine,

Microbiol Infect Dis 1999 [2]		jaundice		adrenal glands, lymph nodes*				prednisone to methylprednisolone, muromonab-CD3
13. Vinayek, R, et al. Clin Transplant 1998 [24]	42	Pancytopenia, transaminitis, myalgia, fever, nausea, vomiting	6	Skin, liver, bone marrow, GI tract (prepyloric), blood, lungs	AmB 8.5g total dose, ABLC 35 mg/day for 2 wks then 50mg/twice weekly for 6 mo., itraconazole 200mg twice daily◊	+ Histopathology + Bone marrow culture + Blood culture + Urine *Histoplasma* Ag	West Virginia, U.S.A.	Prednisone 5 mg/day, tacrolimus 3 mg/day, MMF 500 mg twice daily
14. Shallot, J, et al. Liver Transpl Surg 1997 [25]	44	Respiratory symptoms, fever	6	Bone marrow, blood, lungs	AmB 0.5 mg/kg/day, itraconazole 400 mg/day	+ Histopathology + Blood culture + Bone marrow culture + Urine *Histoplasma* Ag	Illinois, U.S.A.	Tacrolimus 3-5 mg/day, prednisone 15-20 mg/day

‡ Authors' affiliated hospital location was used as case geographical location, unless otherwise explicitly noted in case report; *Donor derived infection suspected; † Poster abstract or case/procedure vignette; ¶ Medication names and doses listed as reported in publication; • Patient death reported; ± Treatment dose and duration included when specified in case report; ¤ Post-treatment prophylaxis with itraconazole continued indefinitely; ◊Medication discontinued due to intolerance.

N/A = not available; yr(s) = year(s); mo. = months; wk(s) = week(s); mg = milligram; kg = kilogram; LAmB = liposomal amphotericin B; AmB = amphotericin B; ABLC = amphotericin B lipid complex; MMF = mycophenolate mofetil; U.S.A. = United States of America; PCR = polymerase chain reaction; BAL = bronchoalveolar lavage; GI = gastrointestinal; Antigen = Ag; Antibody = Ab

5. Clinical Features

Progressive disseminated infection, with evidence of extrapulmonary tissue involvement, is the most common manifestation of *Histoplasma* infection in transplant recipients [1, 10, 26]. Shallot et al. illustrated this in the first published case of disseminated histoplasmosis after OLT [25]. Regarding presenting signs and symptoms, a wide spectrum has been reported in cases of histoplasmosis in OLT; however, febrile illness was one of the most common among the published cases we reviewed (Table 1; cases 2-4, 6-8, 13, and 14). The average time to infection post-transplant is typically within the first two years, but this can vary from a few months to several years [1]. In our review of published case reports, we found that the mean onset of infection occurred at 46.6 months post-liver transplant, with a range of 5 to 180 months (Table 1). With dissemination and progression of histoplasmosis, hepatosplenomegaly, respiratory symptoms, gastrointestinal (GI) tract involvement (e.g. oral ulcers and diarrhea), pancytopenia, anorexia, weight loss, and transaminitis commonly develop. Case 2 highlighted disseminated histoplasmosis with severe, life-threatening symptoms in an OLT recipient. Case 1 illustrated an insidious presentation of presumed histoplasmosis in an OLT recipient, defined by months of intractable diarrhea without other symptoms and focal colonic disease found on colonoscopy. Interestingly, colonic histoplasmosis was first described in 1908 by Samuel T. Darling during a postmortem examination and has since been described in multiple case reports in immunocompromised hosts, including multiple OLT recipients [23]. We identified six case reports with prominent GI symptomatology, and all were subsequently found to have histoplasmosis involvement of the GI tract (Table 1; cases 3, 5, 7, 9, 11, and 13). Less common, but more severe, manifestations of histoplasmosis, such as thrombotic microangiopathy, hemophagocytic lymphohistiocytosis, adrenal insufficiency, osteoarticular, peritoneal, and genitourinary infection, have been described in transplant recipients [1]. Leite et al. presented an abstract of a 15-year-old female with left knee pain who was discovered to have *Histoplasma capsulatum* in the synovial fluid nine months after OLT (Table 1, case 10) [22]. Although central nervous system (CNS) histoplasmosis has been described in immunocompromised hosts, we did not find any published cases occurring in OLT recipients.

6. Diagnosis

A combination of the different diagnostic modalities is often needed to diagnose histoplasmosis. Identification of the tuberculate macroconidia (Figure 1) in culture allows a presumptive diagnosis of histoplasmosis; however, a definitive test to verify the fungus as *Histoplasma* should always be performed [27]. A commercially available chemiluminescent DNA probe for *Histoplasma capsulatum* (AccuProbe; GenProbe, Inc., San Diego, CA) is the confirmatory test most often used for definitive diagnosis [27]. In the presence of a compatible clinical presentation, identification of intracellular yeast forms, consistent with *Histoplasma*, on histopathology is an alternative to culture diagnosis (Figure 2). Grocott's (Gomori's) methenamine silver (GMS) and periodic acid Schiff (PAS) stains are useful in identifying *Histoplasma* in tissue. Wright-Giemsa stain can help identify intracellular yeast in peripheral blood [28].

Antigen detection in urine and blood is another commonly used method for diagnosis and is particularly helpful, as culture can take up to 3-4 weeks to provide a result. Urine antigen

detection has been reported to be the most sensitive test in solid organ transplant (SOT) patients (>90%). In a study on histoplasmosis after SOT, Assi et al. reported that the urine *Histoplasma* antigen was positive in 132/142 (93%) patients, including 24 OLT recipients [26]. The sensitivity of *Histoplasma* urine antigen was 100% among all SOT recipients tested [12]. In a multicenter study that included immunocompetent and immunocompromised patients, the degree of antigenuria correlated with immune status and the severity of disseminated histoplasmosis [29]. The presence of *Histoplasma* antigen in samples other than blood and urine can be helpful in establishing a diagnosis. Antigen detection in bronchoalveolar lavage (BAL) fluid, for example, can provide a means of rapid diagnosis of pulmonary histoplasmosis [30]. In cases of CNS histoplasmosis, data from a large, retrospective, multicenter study, which included transplant (13%) and non-transplant patients, *Histoplasma* antigen was detected in the cerebrospinal fluid (CSF) in 53 (68.8%) patients, these patients were more likely to be immunocompromised patients or those with severe disease [31].

The *Histoplasma* antigen assay is susceptible to cross-reactivity with other organisms and, thus, is not sufficient to diagnose histoplasmosis in the absence of additional supporting diagnostics. This has been described with *Blastomyces*, *Coccidioides*, *Paracoccidioides*, *Sporothrix*, *Aspergillus,* and *Talaromyces marneffei* [27, 29, 32-34]. Similarly, a false-positive *Aspergillus* antigen test may be seen in patients with histoplasmosis, especially those with high titers. In the presence of a compatible clinical presentation, a positive *Aspergillus* antigen should foster consideration for *Histoplasma* antigen testing [35]. False-positive antigenemia has also been described in SOT recipients who receive antithymocyte globulin (ATG) [36]. This is suspected to be due to human-anti-rabbit antibodies causing interference with the assay. Similar interferences have been reported in other tests in patients receiving rabbit ATG, and clinicians should be aware of possible false-positive immunoassay results [36]. Specifically, a positive serum *Histoplasma* antigen with a concurrent negative urine *Histoplasma* antigen should raise suspicion of a false-positive result [27].

Like in other SOT patients, it is difficult to discern if histoplasmosis in OLT recipients represents a primary infection, reactivation of prior infection, or donor-derived infection. Molecular testing has been utilized in such cases for epidemiological and monitoring purposes. Limaye et al. reported on the use of molecular diagnostic methods to link two cases of disseminated histoplasmosis in renal transplant recipients who received organs from the same donor [16]. The DNA-finger print of the *Histoplasma* isolate, identified by random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR), was identical between the two recipients, suggesting donor-derived histoplasmosis [16].

Histoplasma antigen works by detecting *Histoplasma* galactomannan that is present in its cell wall. As an additional component of the cell wall, (1-3)-β-D-glucan (BG) has had little data on its use as a marker of histoplasmosis infection. A study by Egan et al. comparing BG to *Histoplasma* antigen tests in patients with presumptive histoplasmosis found positive results in 20/23 (87%) cases [37]. Due to its presence in the cell wall composition of several fungal pathogens (e.g. *Candida sp.*, *Aspergillus sp.*, and *Pneumocystis jirovecii*), the specificity of the BG test is low [37].

Modern imaging modalities can provide excellent resolution for visualization of infectious processes, but imaging is usually limited to detecting advanced disease states. There are isolated case reports of diagnosing disseminated histoplasmosis with 18-Fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET/CT) [38-41]. In areas where *Histoplasma* is endemic, certain

findings, such as adrenal masses with intense FGD uptake, should raise suspicion for histoplasmosis but does not provide a definitive diagnosis of histoplasmosis [41].

Early diagnosis of histoplasmosis in OLT recipients is key to preserving graft function and achieving good patient outcomes. This is accomplished through providers maintaining a high index of suspicion and providing prompt clinical evaluations at the onset of symptoms. Intervention with multiple diagnostic modalities, including early invasive procedures to obtain material for histopathology and culture, may be required to make a definitive diagnosis.

7. Treatment

Treatment of histoplasmosis is driven primarily by the severity and extent of disease. The severity of infection is determined clinically and graded by the need for hospitalization, presence of hemodynamic instability, respiratory status, and extent of infection; which is ranked as mild, moderate, or severe. The most common manifestation of histoplasmosis in OLT recipients, as part of the SOT population, is progressive disseminated disease [1]. The American Society of Transplantation Infectious Diseases Community of Practice (AST IDCOP) guidelines for the treatment of endemic fungal infections in SOT patients recommends itraconazole monotherapy in mild to moderate cases [1]. In moderately-severe to severe infections, initial treatment with amphotericin for one-to-two weeks, followed by step-down therapy with itraconazole is recommended [1]. For disseminated disease, Amphotericin B products are preferred, and the choice of formulation is determined by availability, tolerability, and cost [1, 10]. Ultimately, the duration of therapy is determined by clinical response and adequate improvement. When possible, immunosuppression should be reduced as it is an essential adjunctive to treatment [1]. In progressive disseminated histoplasmosis, select patients may be placed on indefinite therapy with itraconazole in cases where immunosuppression cannot be reduced [10]. Patients with a history of histoplasmosis within two years preceding transplantation should be considered for itraconazole prophylaxis to prevent infection reactivation [10]. There is limited data available on salvage therapy in those who fail amphotericin B or itraconazole.

Itraconazole levels should be checked after steady-state has been reached, generally two weeks after initiating therapy to identify sufficient levels and to optimize therapy. A random itraconazole blood concentration level that is recommended for adequate therapy is at least 1.0 µg/ml [10]. The oral solution formulation of itraconazole is absorbed better than the capsule formulation, reaching approximately a 30% higher blood concentration [10]. Azole blood level monitoring is available through most large reference laboratories [10].

In cases of itraconazole intolerance, serious drug interactions, or inadequate blood levels, newer azoles (e.g. voriconazole, isavuconazole, and posaconazole) have been used as an alternative to first-line therapy but are limited to case reports and small series [1, 12, 42, 43]. Posaconazole and voriconazole have demonstrated in vitro activity against *Histoplasma* and have been successfully used in a small number of patients with histoplasmosis; however, studies are lacking in transplant recipients [44, 45]. Isavuconazole was used as primary therapy in a study by Thompson et al. with limited success [46]. Fluconazole is the recommended second-line azole for histoplasmosis; however, it is less effective than itraconazole [10, 47, 48]. Lastly, ketoconazole is effective in mild cases of histoplasmosis, but it is not used regularly for histoplasmosis in the U.S. due to an increased number of adverse events [10].

Itraconazole is a potent cytochrome P450 3A4 inhibitor. Interactions with calcineurin inhibitors like tacrolimus, sirolimus, and cyclosporine are common, leading to increased serum drug concentrations. Their levels must be monitored closely in OLT recipients with appropriate dose adjustments, particularly at the time of initiation and discontinuation of antifungal therapy [49].

Histoplasma antigen levels typically fall with effective therapy and can be used as a marker of treatment response. Per Infectious Diseases Society of America (IDSA) guidelines, monitoring of serum and urine *Histoplasma* antigen levels is recommended before treatment initiation and then at two weeks, one month, and every three months thereafter while on therapy [1, 10]. In case 2, the patient's urine *Histoplasma* antigen measurement at diagnosis and at two weeks after starting therapy was >25.0 ng/mL (reference range: <0.5 ng/mL); however, after one month of treatment, his urine antigen level decreased to 20 ng/mL. After completion of therapy, *Histoplasma* antigen monitoring is recommended for 12 months [10]. Levels of serum and urine antigen usually fall to undetectable levels in most patients after the first few months; however, SOT recipients clear antigen levels slowly and can have persistently positive antigen levels after ten months of treatment [1, 42]. Relapse has been documented to occur more often after discontinuation of therapy when urine Histoplasma antigen (MiraVista Ag®) levels are >2 ng/mL [1]. A rise in antigen levels during monitoring should prompt consideration for histoplasmosis relapse and reinitiating treatment.

8. Outcomes, Screening, and Prophylaxis

Treatment of moderate-to-severe histoplasmosis in SOT recipients with amphotericin followed by prolonged itraconazole is highly effective, with most case series in the SOT population reporting treatment efficacy from 80%-100% [1, 12, 42, 47]. Mortality rates in three series that included SOT recipients with histoplasmosis were between 15% - 27% with attributable rates of 10%, 9% (2 cases), 10.4% (5 cases), respectively [15, 26, 42]. Most deaths occurred within a month of diagnosis. Rarely, IRIS has been described in SOT patients with disseminated histoplasmosis, often in the setting of concomitant immunosuppression reduction [1]. Jazwinski et al. reported a case of IRIS occurring in a SOT patient with severe disseminated histoplasmosis [50]. Management is predominately supportive but can include corticosteroids to dampen the inflammatory reaction in severe cases. Currently, the use of primary prophylaxis in transplant patients is not recommended; however, secondary prophylaxis with itraconazole can be considered in patients with previously reported histoplasmosis within two years before transplantation [1]. In some select cases, OLT patients may receive secondary prophylaxis with itraconazole to prevent-donor histoplasmosis (Table 1, case 7). The optimal duration of secondary prophylaxis is not known. Pre-transplant serological screening in endemic areas for prior *Histoplasma* exposure is not recommended due to poor predictive value [1, 51]. This recommendation was based on a study in SOT recipients done by Vail et al., as serologic signs of previous infection and imaging findings did not appear to increase the risk of developing infection [51]. There are currently no recommendations to screen donors for histoplasmosis.

9. Discussion

We present two cases of unique presentations of *Histoplasma* infection in OLT recipients and add to the limited published data (Table 1). Case 1 described a subtle presentation of presumed

gastrointestinal histoplasmosis occurring 12 years after OLT. The differential diagnosis for this case included malignancy, autoimmune disease, MMF-related colitis, cytomegalovirus infection, or other bacterial causes of colitis. A presumed diagnosis of *Histoplasma capsulatum* was ultimately made by broad range PCR performed on colonic tissue. Due to difficulties tolerating itraconazole, treatment consisted of two weeks of liposomal amphotericin B with resolution of the patient's diarrhea and colonic ulcers.

Case 2 illustrated severe pulmonary histoplasmosis with dissemination in an OLT recipient one year after transplantation, presenting as weeks of fever and cough. The differential diagnosis for this case included respiratory viruses, pneumocystis pneumonia, bacterial pneumonia, invasive fungal pneumonias with aspergillosis, and coccidioidomycosis. The diagnosis was made through observation of intracellular yeast forms on BAL and culture that grew *Histoplasma capsulatum*, which was confirmed by DNA probe. Treatment was complicated by the development of refractory shock and ARDS, requiring adjunctive corticosteroids.

In summarizing a review of the literature on histoplasmosis in OLT recipients, we focused on the following areas: epidemiology, clinical manifestations, diagnosis, treatment, and prevention. The primary weakness of our review was a lack of available large case series focusing on histoplasmosis, specifically in OLT patients, as many of the larger case series included a blended SOT population. Because of this, there remain several unanswered questions regarding optimal diagnosis and treatment of histoplasmosis in OLT recipients. New advances in fungal diagnostics are allowing for more precise and rapid diagnosis of histoplasmosis, but their availability and validation in the transplant population are not yet standardized. The rarity of histoplasmosis in OLT recipients has limited attempts at performing randomized controlled trials to provide evidence-based recommendations on treatment. At this time, azoles and amphotericin B remain the only antifungals available for the treatment of histoplasmosis. The newer azoles are available for treatment and prophylaxis, but further studies are needed to validate their use in OLT recipients. As in other fungal infections, the development of new antifungal drug classes for histoplasmosis is needed to provide additional treatment options in cases of intolerance, treatment failure, and severe drug interactions. Lastly, additional studies to provide better understanding of the role of the host immune system, iatrogenic immunosuppression, immune modulation, by histoplasmosis, and immune clearance of infection are needed to advance our knowledge and possibly provide immunotherapeutic treatment options. With an ever-increasing transplant patient population and an expanding endemic region, more *Histoplasma* infections are likely to occur in both donors and OLT recipients, necessitating the need for further studies in each of the fields discussed above.

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Author Contributions

All authors contributed to this manuscript equally.

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Competing Interests

The authors have declared that no competing interests exist.

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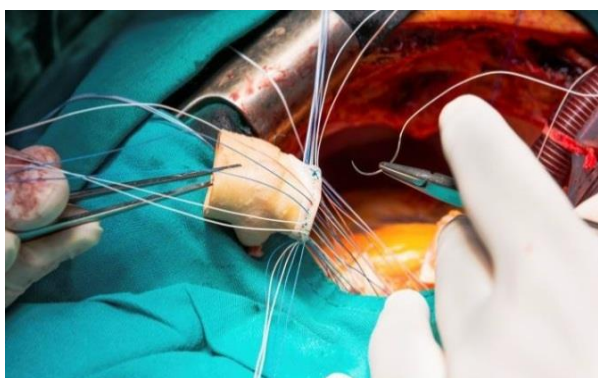
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