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Original Article

# Cryptococcosis in wait-listed liver transplant candidates: Prevalence, manifestations, and risk factors

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**KEYWORDS**
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**Abstract** *Background:* Liver cirrhosis compromises immunity against cryptococcosis, and liver transplant recipients tend to develop the disease earlier after transplantation, possibly due to unrecognized pretransplant infection. We assessed the prevalence and characteristics of cryptococcosis among liver transplant candidates and whether pre-transplant cryptococcal antigen (CrAg) can detect the disease before transplantation.
*Methods:* We retrospectively included liver transplant candidates in a tertiary hospital during 2017—2022. Serum CrAg and pulmonary computed tomography were incorporated in routine transplant evaluation. Other investigations were done if indicated. Cryptococcosis was diagnosed by positive culture or CrAg. Risk factors for cryptococcosis were also assessed.
*Results:* Of the 377 candidates with a median MELD-Na score of 18, 84.4% had hepatitis B virus (HBV) infection. Cryptococcosis was diagnosed in 10 (2.6%) candidates, by CrAg in 6, culture in 2, or both in 2. Only 3 had fever, and 3 were asymptomatic; 7 had pulmonary cryptococcosis. Of the 10 candidates with cryptococcosis, one underwent transplantation after 143-day antifungals. Of the 87 candidates undergoing liver transplantation, one (1.2%) recipient developed cryptococcosis 14 days post-transplant with negative CrAg three weeks before transplantation. HBsAg-positive chronic HBV infection with HBV DNA loads $<$2000 IU/mL was significantly associated with cryptococcosis (odds ratio 4.4, 95% confidence interval 1.2—16.5, p = 0.03) after the adjustment of MELD-Na score.

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*Conclusions:* The prevalence of cryptococcosis was 2.6% among our liver transplant candidates and CrAg detected 80% of the cases. Disease presentation was mild and pulmonary disease predominated. HBsAg-positive chronic HBV infection with HBV DNA loads <2000 IU/mL was significantly associated with cryptococcosis.


**Abbreviations**

| | |
|---|---|
| ACLF | acute on chronic liver failure |
| AIDS | acquired immunodeficiency syndrome |
| APASL | Asian Pacific Association for the Study of the Liver |
| CAD | coronary artery disease |
| CKD | chronic kidney disease |
| CMV | cytomegalovirus |
| CrAg | cryptococcal antigen |
| CSF | cerebrospinal fluid |
| CT | computed tomography |
| DNA | deoxyribonucleic acid |
| DM | diabetes mellitus |
| HBV | hepatitis B virus |
| HCC | hepatocellular carcinoma |
| HCV | hepatitis C virus |
| HFpEF | heart failure with preserved ejection fraction |
| HIV | human immunodeficiency virus |
| irAE | immune-related adverse events |
| IRIS | immune reconstitution inflammatory syndrome |
| LAT | latex agglutination test |
| LFA | lateral flow assay |
| LR | logistic regression |
| MELD-Na score | model for end-stage liver disease -Na score |
| NSTEMI | non-ST elevation myocardial ischemia |
| NTUH | National Taiwan University Hospital |
| P-H assumption | proportional-hazards assumption |
| PWH | people with HIV |
| SLL | small lymphocytic lymphoma |
| SOT | solid organ transplantation |
| TLR | toll-like receptor |

## Introduction

Cryptococcosis is the third most common invasive fungal infection among solid organ transplantation (SOT) recipients with reported incidences ranging from 0.14% to 0.37%,[1–3] and a mortality rate of 10–27% within 12 months.[1–3] Cryptococcosis typically occurs 16–21 months after transplantation. However, 5–10% of cases occur within 30 days post-transplant (very early-onset cryptococcosis) in previous studies.[2,4] Unrecognized pretransplant infection is thought to explain very early-onset cryptococcosis with a higher frequency among lung and liver transplant recipients.[2,4]

Decompensated liver cirrhosis is a recognized risk factor for cryptococcosis among non-HIV infected patients,[5,6] which manifests as disseminated infection[7] and causes a 4.33-fold higher risk of in-hospital mortality.[1,6] In liver transplant recipients, cryptococcosis tends to occur earlier, typically within the initial 12 months post-transplant,[2,4,8] and carries a six-fold higher risk of disseminated infection than in other types of SOT recipients.[9]

Previous studies have primarily focused on post-transplant cryptococcosis in transplant recipients while limited attention is paid to liver transplant candidates with decompensated liver cirrhosis.[10] Since the incidence of cryptococcal meningitis is second highest in the Asia and Pacific region,[11] and people with HIV infection (PWH), SOT recipients, and cirrhosis group have the top three disease-specific incidence rates of cryptococcosis in our nationwide, population-based study,[1] we aimed to investigate the prevalence and manifestations of cryptococcosis in liver transplant candidates through routine pretransplant serum cryptococcal antigen (CrAg) and pulmonary computed tomography (CT) scans during transplant evaluation. Risk factors of cryptococcosis in transplant candidates and waitlist outcomes were also assessed.

## Methods

### Study setting and population

From July 2017 to October 2022, liver transplant candidates undergoing transplant evaluation at the National Taiwan University Hospital (NTUH), a tertiary university hospital with liver transplantation since 1989, were included to assess their prevalence and manifestations of cryptococcosis. Patients were retrospectively included upon consenting to transplant evaluation for their hepatocellular carcinoma (HCC), end-stage liver diseases, or impending hepatic failure. The study flow is shown in Fig. 1. Serum CrAg and pulmonary CT scans with and without contrast were performed once as part of the transplant protocol routinely and would be repeated at discretion of the primary care physicians if indicated. For candidates who were evaluated multiple times, only the last evaluation was studied. Candidates were excluded if either serum CrAg or pulmonary CT scans were missing. Those with relevant symptoms or abnormal radiologic findings underwent diagnostic tests such as bronchoscopy or CT-guided biopsy. Cultures or repeated CrAg from different sites were obtained at the discretion of the primary physicians. Antifungals were prescribed after the candidates were diagnosed of cryptococcosis by positive CrAg or cultures

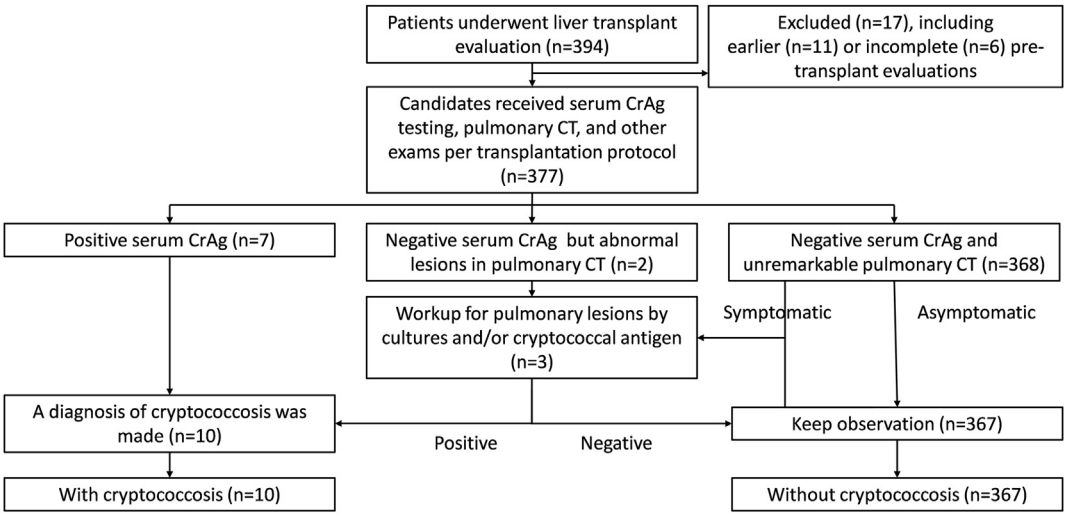


**Figure 1.** The study flow of pretransplant screening. Seventeen candidates were excluded. Ten patients were diagnosed of cryptococcosis after screening or workup and 367 patients were not diagnosed of the disease.

even in patients with low-titer CrAg levels. Candidates were followed until loss to follow-up, death, or end of the study in 2023, whichever came first. We chose the cohort inception years to allow a minimum follow-up of six months. This retrospective study was approved by the Research Ethics Committee or Institutional Review Boards of NTUH (202302115RIND).

## Data collections and disease definitions

Data including underlying systemic diseases, clinical manifestations of cryptococcosis, and laboratory investigations were retrospectively collected from electronic medical records. Acute on chronic liver failure was defined based on the consensus recommendations of 2019 Asian Pacific Association for the Study of the Liver (APASL).[12] Generally, patients with inactive chronic hepatitis B have HBV DNA $<$2000 IU/mL.[13] Candidates with hepatitis B virus (HBV) infection were defined as follows; those with HBsAg-positive chronic HBV infection with HBV DNA loads $<$2000 IU/mL; those with functional HBV cure (negative HBsAg); HBV flare as HBV DNA viral loads $\geq$2000 IU/mL and acute on chronic liver failure.[13] Patients with cryptococcosis were treated with standard antifungal treatment as the 2016 guidelines for invasive fungal diseases in Taiwan.[14] In patients with meningoencephalitis, a 2- to 4-week induction of amphotericin B-based combination therapy was initiated, followed by consolidation and maintenance therapy with fluconazole. For cases with non-meningeal cryptococcosis, Fluconazole was administered at a dosage of 400 mg daily. Amphotericin-B based therapy was administered to salvage severe infection.

## Outcome measures

The primary outcomes of this study included the prevalence and presentations of cryptococcosis in liver transplant candidates. The secondary outcomes were the factors associated with cryptococcosis in liver transplant candidates and 3-month survival after the admission for transplant evaluation.

## Detection of cryptococcal antigen and cultures

Until March 15, 2019, CrAg was detected using the latex agglutination test (LAT) employing the Latex-Cryptococcus Antigen Detection System (IMMY, Norman, OK, USA). Subsequently, the lateral flow assay (LFA) utilizing the CrAg® LFA cryptococcal antigen (IMMY, Norman, OK, USA) was adopted. There were no standard conversion criteria between the two methods. Fungi were identified by MALDI Biotyper® sirius one IVD System.

## Statistical analyses

All statistical analyses were conducted using the Stata software package, version 17.0 (StataCorp). Categorical variables were assessed using Chi-squared test or Fisher's exact test while continuous variables were compared using the Student's t-test or Wilcoxon-Mann—Whitney test. Both the Child-Pugh and MELD-Na scores were calculated using the original scoring system and stratified groups.[15] Univariable and multivariable logistic regression models were employed to determine the risks associated with cryptococcosis and 3-month survival. Variables with a P-value $\leq$0.1 in univariable analyses or factors with known clinical significance associated with cryptococcosis specifically MELD-Na scores,[16] were included in the multivariable analyses. Survival analysis with log rank test between candidates with and without cryptococcosis was depicted as Kaplan-Meier curve, and marked with a composite end point combining transplantation or death. All statistical tests were two-sided, and variables with a P-value $<$0.05 were considered statistically significant.

## Results

During the study period, a total of 394 liver transplant candidates underwent transplant evaluation, but 17 were excluded due to earlier or incomplete screening (Fig. 1). The median age of the included 377 candidates was 59 years old, and 69.0% were men (Table 1). The reasons for transplantation were cirrhosis without acute on chronic liver failure or HCC in 57.8%, acute on chronic liver failure in 32.1%, and HCC with compensated cirrhosis (Child-Pugh A) in 10.1%. The most common underlying diseases were HBV infection (84.4%) followed by alcoholic liver cirrhosis (22.8%) and cryptogenic hepatitis (13.3%). The median Child-Pugh score was 9 (interquartile range [IQR] 7—10), and MELD-Na score was 18 (IQR 11—26). Except for one candidate with HCC and immunotherapy-induced pneumonitis treated by corticosteroids and another candidate with newly-diagnosed HIV infection with acquired immunodeficiency syndrome during his transplant evaluation for acute on chronic liver failure, no other known immunocompromised diseases or use of chemotherapy were noted at the diagnosis of cryptococcosis. After a median follow-up duration of 194 days (IQR 49—519) from admission for transplant evaluation to the end of study in April 2023, 20.4% (77/377) were lost to follow-up, 32.4% died, and 23.1% underwent transplantation. The comparisons of demographics between liver candidates with and without loss to follow-up are presented in Supplement table 1.

Cryptococcosis was diagnosed by CrAg (60%), culture (20%), and both (20%) in the 10 liver transplant candidates leading to the overall prevalence of 2.6% (10/377) (Table 2). Two patients had negative serum CrAg by LFA but positive cultures of lung biopsy or pleural effusion, respectively. *Cryptococcus neoformans*_var_*grubii* was isolated from all the four cases with culture-proven cryptococcosis.

Among the 10 candidates with cryptococcosis, 70% exhibited lower respiratory tract symptoms, only 30% of cases were febrile, and 30% were asymptomatic (Table 2). The prevalence of asymptomatic cryptococcosis was 0.8% (3/377) with an antigen titer of 1:256 in one patient, negative antigen but positive culture in one, and a titer of 1:2 in one, respectively (Patient 7, 8, 10 in Supplement table 2). Pulmonary cryptococcosis was most common (70%), including 1 case with concurrent positive pleural and peritoneal CrAg, respectively, and 1 case with concomitant positive pleural effusion culture. Two patients had cryptococcal antigenemia only (20%), and one patient had cryptococcemia (10%). Only one patient had neurological symptoms, and three underwent lumbar punctures for a high titer of 1:512 in one, altered consciousness in another, and for unknown reason in the third. No cases of cryptococcal meningoencephalitis were diagnosed. One out of the two patients receiving paracentesis had positive peritoneal CrAg. The detailed clinical presentations of the 10 candidates are presented in Supplement table 2.

Most (80%) patients were treated with fluconazole for non-meningeal cryptococcosis, and one (10%) patient received isavuconazole due to severe hyperbilirubinemia. One (10%) candidate with acute on chronic liver failure and newly-diagnosed HIV infection did not receive antifungals due to rapid progression of hepatorenal syndrome and his family's decision for palliative care. Only one patient developed skin rashes under fluconazole. Another patient developed acute kidney failure after 1-week liposomal amphotericin B and flucytosine. Although drug-related nephrotoxicity might cause the kidney event, progression of hepatorenal syndrome could also be the etiology.

Among the six deceased patients with cryptococcosis, except for the one with newly-diagnosed HIV infection, the remaining 5 had no clinical or laboratory progression of cryptococcosis under antifungals before their death caused by progressive multiorgan failure weeks after cryptococcosis. Another 3 alive patients completed 3—12 months of fluconazole. One patient with CrAg seroconversion 1 week before transplantation, underwent surgery successfully after 143-days of fluconazole. However, she was lost to follow-up three months post-transplantation (Patient 7 in Supplement Table 2).

Compared with patients without cryptococcosis, those with pre-transplant cryptococcosis were more likely to have HBsAg-positive cirrhosis with HBV viral loads $<$2000 IU/mL and without flare up (60.0% vs. 28.6%, $p = 0.032$) (Table 1). In multivariable analysis for risk factors associated with cryptococcosis, these candidates also had a higher risk of cryptococcosis (odds ratio [OR] 4.4, 95% CI 1.2—16.5, $p = 0.03$) after the adjustment for MELD-Na score (Table 3, Supplement Table 3). Survival analysis revealed no significant survival difference between candidates with and without cryptococcosis (log rank $p = 0.54$) (Fig. 2).

Among the 87 included candidates undergoing transplantation, one patient (1.2%, 1/87) developed cryptococcosis on the 14th day post-transplant. The liver graft was from a living donor with negative serum CrAg. Although the recipient had negative serum CrAg and normal chest radiography three weeks before the transplantation, he subsequently developed fever, productive cough, and dyspnea and was positive for serum cryptococcal antigen (1:320). *C. neoformans*_var_*grubii* was isolated from his blood and pleural effusion. He improved under fluconazole.

## Discussion

The overall prevalence of cryptococcosis among our 377 liver transplant candidates was 2.6% (10/377). Of the 10 cases with cryptococcosis, their symptoms were mild, if any, and non-specific. Serum CrAg detected 80% of cases. Most (70%) presented with pulmonary disease. Cryptococcosis was significantly associated with chronic HBsAg-positive cirrhosis with HBV viral loads $<$2000 IU/mL and without flare up (OR 4.4).

In a retrospective study in Korea with 294 hospitalized cirrhotic patients,[17] no cases were detected with positive CrAg compared with our prevalence of 2.6%. The prevalence might be higher if we included those with obvious infection signs, which were initially excluded from transplant evaluation. The difference in prevalence might be attributed to differences in diagnostic tools. LFA exhibits superior sensitivity of 97.6—99.3% and specificity of 93—98.1%, respectively, than LAT.[18—20] The Korean study employed LAT while we used LFA mainly and also pulmonary

**Table 1** The demographics of 377 liver transplant candidates.

| Variables | Total | Without cryptococcosis | With cryptococcosis | p-value[a] |
|---|---|---|---|---|
| Patient number, n | 377 | 367 | 10 | |
| Age, median (IQR) | 59.0 (49.0—66.0) | 59.0 (49.0—66.0) | 60.0 (55.0—66.0) | 0.4393 |
| Men, n (%) | 260 (68.97) | 251 (68.39) | 9 (90.00) | 0.1456 |
| Reasons for transplantation, n (%) | | | | |
| Cirrhosis | 218 (57.82) | 211 (57.49) | 7 (70.00) | 0.4300 |
| ACLF | 121 (32.10) | 119 (32.43) | 2 (20.00) | 0.4069 |
| Early-stage HCC[b] | 38 (10.08) | 37 (10.08) | 1 (10.00) | 0.9932 |
| Underlying liver diseases, n (%) | | | | |
| Hepatitis B virus infection | 318 (84.35) | 308 (83.92) | 10 (100.00) | 0.1680 |
| Chronic HBV infection[c] | 111 (29.44) | 105 (28.61) | 6 (60.00) | 0.0319 |
| Functional HBV cure[d] | 132 (35.01) | 130 (35.42) | 2 (20.00) | 0.3137 |
| Hepatitis B flare[e] | 75 (19.89) | 73 (19.89) | 2 (20.00) | 0.9932 |
| Alcoholism | 86 (22.81) | 85 (23.16) | 1 (10.00) | 0.3284 |
| Cryptogenic hepatitis | 50 (13.26) | 50 (13.62) | 0 (0.00) | 0.2107 |
| Hepatitis C virus infection | 45 (11.94) | 43 (11.72) | 2 (20.00) | 0.4260 |
| HCV viremia | 9 (2.39) | 8 (2.18) | 1 (10.0) | 0.1104 |
| Positive anti-HCV without HCV viremia | 36 (9.5) | 35 (9.54) | 1 (10.00) | 0.9608 |
| Primary biliary cirrhosis | 16 (4.24) | 16 (4.36) | 0 (0.00) | 0.5004 |
| Drug-induced liver injury | 9 (2.39) | 9 (2.45) | 0 (0.00) | 0.6167 |
| Autoimmune hepatitis | 6 (1.59) | 6 (1.63) | 0 (0.00) | 0.6840 |
| Primary sclerosing cholangitis | 6 (1.59) | 6 (1.63) | 0 (0.00) | 0.6840 |
| Other causes of cirrhosis | 33 (8.75) | 33 (8.99) | 0 (0.00) | 0.3215 |
| Severity of cirrhosis | | | | |
| Child-Pugh classification, score, median (IQR) | 9 (7—10) | 9 (7—10) | 10 (8—11) | 0.2895 |
| Child-Pugh classification, class, n (%) | | | | 0.1813 |
| Class A | 59 (15.65) | 58 (15.80) | 1 (10.00) | |
| Class B | 175 (46.42) | 172 (46.87) | 3 (30.00) | |
| Class C | 143 (37.93) | 137 (37.33) | 6 (60.00) | |
| MELD-Na score, median (IQR) | 18 (11.0—26.0) | 18 (11.0—26.0) | 18.5 (10.0—32.0) | 0.8138 |
| MELD-Na score (by stratification), n (%) | | | | 0.5865 |
| 1-9 | 64 (16.98) | 62 (16.89) | 2 (20.00) | |
| 10-19 | 145 (38.46) | 142 (38.69) | 3 (30.00) | |
| 20-29 | 111 (29.44) | 109 (29.70) | 2 (20.00) | |
| 30-39 | 47 (12.47) | 45 (12.26) | 2 (20.00) | |
| 40 | 10 (2.65) | 9 (2.45) | 1 (10.00) | |
| Comorbid conditions, n (%) | | | | |
| Diabetes mellitus | 81 (21.49) | 79 (21.53) | 2 (20.00) | 0.9078 |
| Other autoimmune diseases | 16 (4.24) | 16 (4.36) | 0 (0.00) | 0.3215 |
| Corticosteroid use[f] | 7 (1.86) | 7 (1.91) | 0 (0.0) | 0.6597 |
| HIV infection | 1 (0.27) | 0 (0.00) | 1 (10.00) | <0.0001 |
| Median follow-up duration, median (IQR), days | 194.0 (49.0—519.0) | 199.0 (47.0—519.0) | 151.0 (63.0—421.0) | 0.4796 |
| Outcomes, n (%) | | | | |
| Transplantation | 87 (23.08) | 86 (23.43) | 1 (10.00) | 0.3275 |
| Overall mortality | 122 (32.36) | 116 (31.61) | 6 (60.00) | 0.0586 |
| 3-month survival | 283 (75.07) | 276 (75.20) | 7 (70.00) | 0.7078 |
| Loss to follow-up | 77 (20.4) | 76 (20.21) | 1 (10.00) | 0.4086 |

[a] Comparison between the groups with and without cryptococcosis.
[b] Barcelona Clinic Liver Cancer classification A.
[c] Chronic HBV infection: HBsAg-positive cirrhosis with HBV viral loads <2000 IU/mL and without flare up.
[d] Functional HBV cure: HBsAg-negative cirrhosis caused by HBV infection (positive Anti-HBc).
[e] Hepatitis B flare: Acute HBV flare with HBV viral loads ≥2000 IU/mL.
[f] The dose of prednisolone at 10 mg or greater per day.

Abbreviations: ACLF, acute on chronic liver failure; anti-HCV, anti-HCV antibody; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile; MELD-Na score, model for end-stage liver disease -Na score.

**Table 2** The clinical characteristics of the 10 liver transplant candidates with cryptococcosis.

| Variables | Data |
|---|---|
| Age, median (IQR), years | 60 (55–66) |
| Men, n (%) | 9 (90) |
| Clinical manifestations, n (%) | |
| Symptoms of the lower respiratory tract | 7 (70) |
| Dyspnea | 5 |
| Cough or productive cough | 3 |
| Hemoptysis | 2 |
| Fever | 3 (30) |
| Asymptomatic | 3 (30) |
| Loss of consciousness | 2 (20) |
| Abdominal pain | 0 (0) |
| Diagnosis of cryptococcosis, n (%) | |
| Cryptococcal antigen | 8 (80) |
| Blood | 8 |
| Bronchoalveolar lavage | 1 |
| Pleural effusion | 1 |
| Culture | 4 (40) |
| Lung | 2 |
| Blood | 1 |
| Pleural effusion | 1 |
| Presumptive infection sites, n (%) | |
| Pulmonary infection | 7 (70) |
| Lung lesions and positive serum antigen[a] | 4 |
| Positive lung tissue cultures[b] | 2 |
| Positive BAL antigen | 1 |
| No identified sites with only positive serum cryptococcal antigen | 2 (20) |
| Cryptococcocemia | 1 (10) |
| Central nervous system infection | 0 (0) |
| Initial treatment, n (%) | |
| Fluconazole[c] | 8 (80) |
| Isavuconazole | 1 (10) |
| No treatment | 1 (10) |
| Outcomes, n (%) | |
| Death before transplantation | 6 (60) |
| On waiting list for liver transplantation | 3 (30) |
| Undergoing liver transplantation | 1 (10) |

[a] One patient also had pleural effusion culture of *C. neoformans*.
[b] One patient also had positive pleural and peritoneal Cryptococcal antigen, respectively.
[c] The antifungal regimen of one patient was changed from fluconazole to liposomal amphotericin B and flucytosine.
Abbreviations: BAL, bronchial lavage.

**Table 3** Multivariable analysis for factors associated with cryptococcosis in liver transplant candidates.

| Variables | OR | 95% CI | P value |
|---|---|---|---|
| Chronic HBV infection[a] | 4.380 | 1.161–16.529 | 0.0293 |
| MELD-Na score (score) | 1.036 | 0.967–1.110 | 0.3118 |

[a] Chronic HBV infection: HBsAg-positive cirrhosis with HBV viral loads <2000 IU/mL and without flare up.
Abbreviations: CI, confidence intervals; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; MELD-Na score, model for end-stage liver disease score including sodium values; OR, odds ratio.

CT scans to increase diagnostic yield. The incidence of cryptococcosis is also higher in Taiwan with the age-standardized incidence of 18.3 cases per million person-years,[1] and 3.99 cases per million person-years in Korea.[21]

A multicenter cohort study included 112 cirrhotic patients with cryptococcosis, and 46 were liver transplant candidates.[22] In that study, 45% had fever and 74% was not alert. In contrast, we included transplant candidates with mild chronic cough or fatigue indistinguishable from symptoms of cirrhotic patients. Patients with newly-developed symptoms or abnormal pulmonary lesions in CT scan went straight for workup instead. Although our patients constituted a smaller population and were often neglected, if they received transplantation, very early-onset cryptococcosis might occur and cause significant morbidity or mortality. We aimed to detect the unrecognized cryptococcosis before transplantation, and this population was not well-characterized in prior studies.

The diagnostic yield of cryptococcal antigen in patients with isolated pulmonary cryptococcosis is poor in the era of LAT, and the false-negative rate was up to 39%–60% in some case series.[23,24] After the employment of LFA, localized pulmonary cryptococcosis without culture proof can be detected in non-HIV patients.[25] Furthermore, CrAg positivity precedes clinical symptoms by a median of 22 days in a study of HIV-infected persons.[26] Although the current guidelines by the American Society of Transplantation Infectious Diseases Community of Practice do not recommend routine cryptococcal screening of transplant recipients before transplantation,[9] the utilization of CrAg as part of pretransplant surveillance may be valuable in the area of high-incidence cryptococcosis, such as Asia and Pacific.[11]

Pretransplant serum CrAg screening might help prevent majority of posttransplant cryptococcosis and its associated morbidities and mortality since it detected 80% of the cases. However, two patients with negative pretransplant serum CrAg had pulmonary and pleural cryptococcosis diagnosed by positive cultures. Thus, pulmonary CT scans with serum CrAg are more appropriate as the screening tools as we presented in Fig. 1. Bronchoscopy or CT-guided biopsy are suggested as the workup for pulmonary lesions in pulmonary CT. Cultures or tissue pathology are needed to make definitive diagnosis as indicated by symptoms or imaging, regardless of the results of CrAg. As for the treatment after the diagnosis of cryptococcosis, we recommend the 2016 guidelines for invasive fungal diseases in Taiwan.[14]

In patients with chronic HBV infection, prolonged persistence of HBV antigens suppresses Toll-like receptor (TLR) responses[27,28] and functionally inactivates HBV-specific CD8+ T cells *in vivo*.[29] For example, HBV uses HBsAg to block phosphorylation of cAMP response element-binding protein (CREB) and to bind to the cAMP response element (CRE) site on the TLR9 promoter, causing TLR9 dysfunction.[30] TLR9 expression and TLR9-mediated B cell functions are suppressed in all peripheral B cell subsets exposed to HBV.[27,30] TLR9 is important in anti-cryptococcal immunity since cryptococcal antigen is a TLR9 ligand.[31,32] Binding of TLR9 increases early IL-12 secretion by myeloid

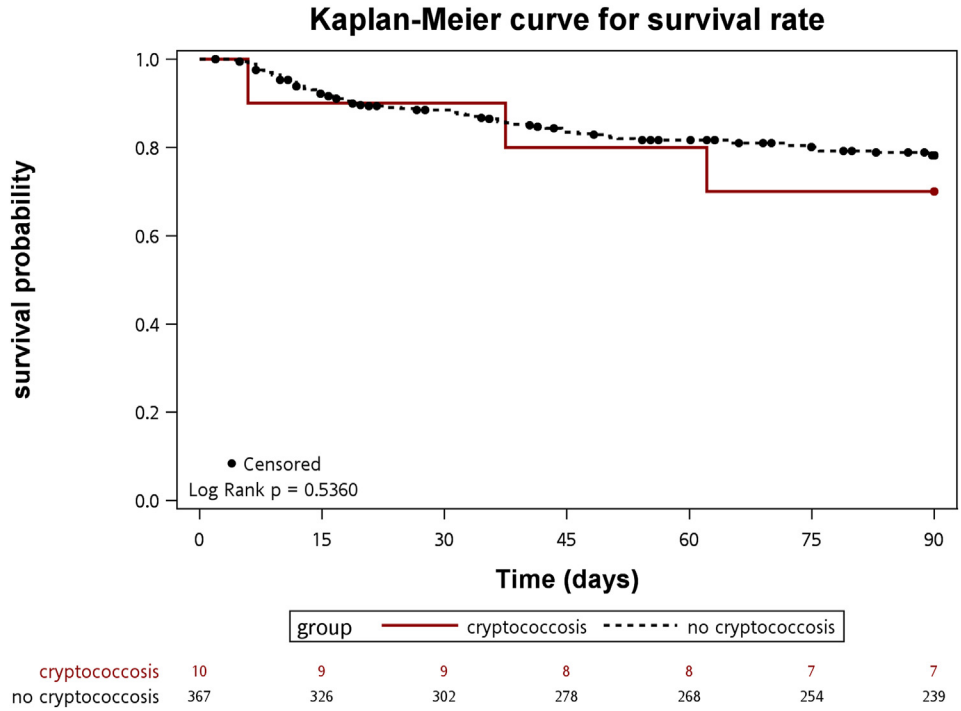


**Figure 2.** Kaplan-Meier curve for survival rate in 3 months. Statistical analysis showed the 10 patients with cryptococcosis and the 367 patients without had no differences in 3-month survival. The analysis was performed by log rank test, depicted as Kaplan-Meier curve, and marked with a composite end point combining transplantation or death.

cells, expresses protective Th1 (thus produces IFN-γ and IL-2), and ultimately improves fungal clearance.[33,34] HBsAg-induced TLR9 and other TLRs dysfunction may explain the increased risk of cryptococcosis among patients with circulating HBsAg. Although HCV and HBV inhibit similar TLRs (e.g., TLR2, 4, 7, 9) in dendritic cells, lower prevalence of HCV infection or clearance of HCV after direct-acting antiviral agents might contribute to insignificant relation to HCV infection in our study. The association with low HBV DNAemia <2000 IU/mL remained unclear.

It remains a concern for cirrhotic patients with pretransplant cryptococcosis to undergo transplantation since optimal management before transplantation is undefined. Cryptococcosis was also identified as a reason of transplantation delay in a multicenter study, and only 8 out of 39 alive candidates eventually received transplantation.[22] Of the 8 patients with pretransplant cryptococcosis that received transplantation in that study, the overall survival rate was 87.5%, and none died of uncontrolled cryptococcosis. One of our patients with cryptococcosis received transplantation. Under standard antifungals, her cryptococcosis did not worsen before she was lost to follow-up. This case also supports the conclusion of the multicenter's study that liver transplant candidates with well-controlled cryptococcosis still have good overall survival.[22]

We have limitations to be acknowledged. First, this is a retrospective study. There was no specific pre-defined protocol to survey pretransplant cryptococcosis except for routine serum CrAg and pulmonary CT scans, so other diagnostic tests were performed at the primary physicians' discretion. Only 3 of 10 patients with cryptococcosis underwent lumbar puncture, and 2 underwent paracentesis. Nevertheless, these candidates had mild or no relevant symptoms, so invasive diagnostic procedures were not performed routinely. Second, low (≤1:10) CrAg titers by LFA may be false-positive and leads to overdiagnosis. We had 5 patients with low CrAg titers in the study (one with 1:2, two with 1:5, two with 1:10, respectively). CrAg positivity precedes clinical symptoms.[26] In a case-control study with 2196 serum samples, there was no difference in the incidence of pulmonary or disseminated infection between low- and high-titer group.[35] Overdiagnosis and early initiation of treatment are preferable for the awaiting liver transplant candidates with positive serum CrAg. The interpretation of serum CrAg results in the clinical condition of liver candidates relied on the infection diseases specialists in charge.

Third, the immune status such as levels of the IgG, IgM, and CD4 count, were not checked because cirrhosis itself could explain the occurrence of cryptococcosis. Lastly, approximately 20% of the patients were lost to follow-up, and they were more likely to have alcoholism or HCV infection compared with those without. This did not impact our findings (Supplement Table 1).

## Conclusion

The prevalence of cryptococcosis among our liver transplant candidates was 2.6%, and the disease can present mildly or asymptomatically. Pretransplant surveillance by both serum CrAg and pulmonary CT helps identify the cryptococcosis in the liver transplant candidates, prompting timely treatment before transplantation. This non-

invasive screening might be considered in areas with high prevalence of cryptococcosis. HBsAg-positive chronic HBV infection with HBV DNA loads <2000 IU/mL was significantly associated with cryptococcosis in cirrhotic liver transplant candidates, but further studies are warranted to confirm our findings.

## Funding

There were no fundings for the study.

## Data availability

Deidentified participant-level will be available on publication of the study. Requests for data should be sent to hysun@ntu.edu.tw and, on review of the proposed protocol and signing of a data sharing agreement, the data will be made available. The protocol and consent form will also be available on email request.

## CRediT authorship contribution statement

**Wan-Ting Tsai:** Writing — original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Aristine Cheng:** Writing — review & editing, Writing — original draft, Conceptualization. **Yu-Chung Chuang:** Methodology, Formal analysis. **Cheng-Maw Ho:** Writing — review & editing, Supervision. **Yao-Ming Wu:** Writing — review & editing, Supervision. **Ming-Chih Ho:** Writing — review & editing, Supervision. **Hsin-Yun Sun:** Writing — review & editing, Supervision, Data curation, Conceptualization. **Ray-Hung Hu:** Writing — review & editing, Supervision. **Yee-Chun Chen:** Writing — review & editing, Supervision.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2024.08.001.