American Journal of Transplantation 24 (2024) 2092–2107

Contents lists available at ScienceDirect

American Journal of Transplantation

journal homepage: www.amjtransplant.org

Original Article

Invasive aspergillosis in liver transplant recipients in the current era

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Abbreviations: aHR, adjusted hazard ratio; aOR, adjusted odds ratio; BALF, bronchoalveolar lavage fluid; CI, confidence interval; CMV, cytomegalovirus; date-IA/matched, IA diagnosis date for IA cases or the matched date after transplant for controls; EORTC-MSG, European Organization for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium; GM, galactomannan; IA, invasive aspergillosis; ICU, intensive care unit; LiTRs, liver transplant recipients.

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https://doi.org/10.1016/j.ajt.2024.05.016
Received 11 December 2023; Received in revised form 13 April 2024; Accepted 22 May 2024
Available online 25 May 2024

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ARTICLE INFO

Keywords:
invasive aspergillosis
liver transplantation
targeted antifungal prophylaxis

ABSTRACT

Invasive aspergillosis (IA) is a rare but fatal disease among liver transplant recipients (LiTRs). We performed a multicenter 1:2 case-control study comparing LiTRs diagnosed with proven/probable IA and controls with no invasive fungal infection. We included 62 IA cases and 124 matched controls. Disseminated infection occurred only in 8 cases (13%). Twelve-week all-cause mortality of IA was 37%. In multivariate analyses, systemic antibiotic usage (adjusted odds ratio [aOR], 4.74; $P = .03$) and history of pneumonia (aOR, 48.7; $P = .01$) were identified as independent risk factors associated with the occurrence of IA. Moreover, reoperation (aOR, 5.99; $P = .01$), systemic antibiotic usage (aOR, 5.03; $P = .04$), and antimold prophylaxis (aOR, 11.9; $P = .02$) were identified as independent risk factors associated with the occurrence of early IA. Among IA cases, *Aspergillus* colonization (adjusted hazard ratio [aHR], 86.9; $P < .001$), intensive care unit stay (aHR, 3.67; $P = .02$), disseminated IA (aHR, 8.98; $P < .001$), and dialysis (aHR, 2.93; $P = .001$) were identified as independent risk factors associated with 12-week all-cause mortality, while recent receipt of tacrolimus (aHR, 0.11; $P = .001$) was protective. Mortality among LiTRs with IA remains high in the current era. The identified risk factors and protective factors may be useful for establishing robust targeted antimold prophylactic and appropriate treatment strategies against IA.

1. Introduction

Invasive aspergillosis (IA) is the second most common invasive fungal infection and is associated with substantial mortality in solid organ transplant recipients.[1,2] IA occurs in 1.8% of liver transplant recipients (LiTRs) and mortality rates have been reported approximately between 60% and 90% in LiTRs who developed IA.[1,3-8] In a recent study, the mortality rate of IA in LiTRs (85.7%) was significantly higher than that of other solid organ transplant recipients (15.9%).[7] The higher mortality was attributable to the higher incidence of disseminated IA in LiTRs than in other solid organ transplant recipients.[4,7] Therefore, prophylactic strategies for IA have been investigated in LiTRs who have risk factors for IA. Providing targeted prophylaxis for LiTRs who have risk factors for IA may be more efficient than providing universal antifungal prophylaxis due to the low incidence of IA in this population. In earlier studies, previous liver transplantation, dialysis after transplantation, renal failure, transplantation for fulminant hepatitis, and cytomegalovirus (CMV) infection were identified as significant risk factors for IA in LiTRs.[9-11] However, these risk factors were noted in small studies performed more than a decade ago. Since the publication of these studies, surgical techniques for liver transplantation, prophylactic strategies, diagnostic strategies, international diagnostic criteria, and treatment strategies for IA have evolved.[11-13] Targeted antifungal prophylaxis has now been recommended for LiTRs who have risk factors for invasive fungal infections, including invasive candidiasis and IA.[11,14-16] In fact, many antimold agents, such as voriconazole, echinocandins (micafungin, anidulafungin, and caspofungin), and liposomal amphotericin B have recently been administered as targeted antifungal prophylaxis for high-risk LiTRs.[15,17-20] The aforementioned changes in practices, including antimold targeted prophylaxis, may impact the epidemiology of IA, including survival outcomes in LiTRs. The aim of this multicenter retrospective study was to evaluate the clinical characteristics, risk factors, therapeutic strategies, and outcomes of IA in LiTRs in the current era.

2. Methods

2.1. Study design

This multicenter matched case-control study utilized retrospective data obtained from 14 transplant centers in Canada,

Thailand, the USA, Spain, Switzerland, Italy, Slovenia, and Saudi Arabia. Adult LiTRs (aged ≥18 years) who were diagnosed with IA between January 01, 2014, and December 31, 2018, were included.

All patients with proven or probable IA were identified as cases and matched with controls at a 1:2 ratio. Controls had no possible, probable, or proven invasive fungal infection at any time post liver transplantation and were matched according to the date of transplantation (± 90 days) and institution. Controls must have had a follow-up of at least the same duration as the interval between the date of transplant and IA diagnosis in the case. Each institution selected the 2 controls that were transplanted closest to the IA cases and satisfied the above criteria. If more than 2 controls were identified that satisfied the above criteria, the 2 whose dates of transplant were closest to the index case were selected. An example control selection is shown in the Supplementary Appendix. The matched date after transplant was defined as the same relative time after transplant (ie, if IA occurred 14 days after transplant, the index date for the 2 matched controls was also day 14.). Combined organ and multivisceral transplants were excluded. This study was approved by the institutional ethics committee of each center.

2.2. Definitions

The diagnosis of proven and probable IA was based on the criteria of the European Organization for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium (EORTC-MSG) which was published in 2008.[12] Collection of data in this study was completed prior to publication of the EORTC-MSG 2020.[21] The cut-off values of serum and bronchoalveolar lavage fluid (BALF) galactomannan (GM) antigen level were ≥0.5 and ≥1.0, respectively. One or more times of serum GM antigen level ≥0.5 was considered positive. The IA diagnosis date was the date when the first positive diagnostic microbiological test was performed. Early IA and late IA were defined as IA diagnosed ≤90 days and >90 days after the day of transplantation, respectively.[3,11]

Intensive care unit (ICU) stay was defined as ≥48 hours of ICU admission until transplantation; mechanical ventilation was defined as ≥7 days usage of mechanical ventilation until transplantation; reoperation was defined as history of reoperation within 3 days after transplantation; recent history of pneumonia was defined as history of pneumonia within the 2 weeks prior to the IA diagnosis date for IA cases or the matched date after transplant for controls (date-IA/matched); recent tacrolimus administration was defined as tacrolimus usage within 2 weeks before date-IA/matched; recent use of systemic antibiotics was defined as more than 3 days of treatment with systemic antibiotic usage within 2 weeks before date-IA/Matched (prophylactic antibiotics [eg, pneumocystis prophylaxis] were excluded); recent history of dialysis was defined as history of dialysis within 1 week before date-IA/matched; recent receipt of antimold prophylaxis was defined as history of receiving any antifungal agent with antimold activity (voriconazole, itraconazole, posaconazole, isavuconazole, liposomal amphotericin B or echinocandins) in the 7 days prior to date-IA/matched[22]; fungal colonization was defined as the isolation of a fungal organism 1 or more times within the 3 months prior to the date of transplantation without evidence of infection; CMV disease and/or CMV DNAemia were defined as the occurrence of them within 2 weeks before date-IA/matched[23]; breakthrough IA was defined as IA occurrence while receiving an antimold antifungal agent[19]; and rejection was defined as history of rejection requiring treatment within 3 months before date-IA/matched.

2.3. Statistical analysis

Data were expressed as the median and interquartile range for continuous variables and as numbers and percentages for categorical variables. As previously mentioned, each case was matched to 2 controls according to the date of transplantation and institution. We used conditional logistic regression (controlling for case-control triplets) in univariate analysis to identify risk factors and calculate odds ratios associated with the occurrence of IA. Multivariable conditional logistic regression was performed using the Stata clogit command to determine independent risk factors for the occurrence of IA. Construction of the model was based on the inclusion of clinically plausible factors in addition to factors with $P < .20$ in the univariate analysis. Variables were assessed for confounding and collinearity. The final model was adjusted for well-known risk factors from previously published studies, and goodness-of-fit was examined using likelihood-ratio tests.

Univariate survival analysis was performed using the Kaplan-Meier product-limit method and log-rank statistic to test the null hypothesis of no difference between survival curves. Cox proportional hazards models were used to analyze the relationship between survival and clinically plausible factors or factors with $P < .20$ identified in the univariate analysis. The final model was adjusted for well-known risk factors from previously published studies. The assumption of proportionality was graphically examined using log-log (cumulative hazard) plots and evaluated scaled Schoenfeld residuals and globally in the final regression models. No important violations of the proportionality assumption were identified. The criterion for statistical significance was set a priori at $\alpha = 0.05$, with all tests of significance being 2-tailed. All data were analyzed using StataMP 12 (StataCorp LP, College Station, Texas).

3. Results

During the study period, 62 IA cases and 124 matched controls were identified from 14 centers (center number, [number of IA cases/number of matched controls] = center 1 [16/32]; center 2 [1/2]; center 3 [4/8]; center 4 [2/4]; center 5 [2/4]; center 6 [1/2]; center 7 [1/2]; center 8 [2/4]; center 9 [2/4]; center 10 [10/20]; center 11 [5/10]; center 12 [7/14]; center 13 [7/14]; center 14 [2/4]). Detailed information about each center is shown in Supplementary Table S1.

3.1. Clinical characteristics of LiTRs with IA

During the study period, 62 LiTRs with acquired IA were identified. Forty-nine out of 62 (79%) were classified as probable

Table 1
The clinical characteristics of 62 liver transplant recipients with IA.

Factors	IA cases N = 62 (%)	Early IA N = 41 (%)	Late IA N = 21 (%)	*P* value
Proven IA	13 (21)	8 (20)	5 (24)	.75
Probable IA	49 (79)	33 (80)	16 (76)	.75
Median days to IA from the day of transplant (IQR)	37 d (10-129)	11 d (7-35)	198 d (129-475)	<.001
Donor type				
Deceased	53 (85)	37 (90)	16 (76)	.25
Living	5 (8.1)	0	5 (24)	.003
After cardiac death	4 (5.6)	4 (9.8)	0	.29
None disseminated infection	54 (87)	36 (88)	18 (86)	1.0
Lung	48 (77)	34 (83)	14 (67)	.20
Liver	2 (3.2)	1 (2.4)	1 (4.8)	1.0
Sinus	2 (3.2)	0	2 (9.5)	.11
Perivertebral abscess	1 (1.6)	0	1 (4.8)	.34
Abdominal abscess	1 (1.6)	1 (2.4)	0	1.0
Disseminated infection	8 (13)	5 (12)	3 (14)	1.0
Lung + brain	5 (8.1)	4 (9.8)	1 (4.8)	.65
Lung + brain + skin	1 (1.6)	0	1 (4.8)	.34
Lung + liver	1 (1.6)	0	1 (4.8)	.34
Lung + heart	1 (1.6)	1 (2.4)	0	1.0
Culture-positive case	N = 44	N = 25	N = 19	.02
Aspergillus fumigatus	28 (64)	15 (60)	13 (68)	.75
A. fumigatus + *A. terreus*	1 (2.3)	1 (4.0)	0	1.0
A. flavus	5 (11)	4 (16)	1 (5.3)	.37
A. niger	5 (11)	4 (16)	1 (5.3)	.37
A. lentulus	1 (2.3)	0	1 (5.3)	.43
Unknown *Aspergillus* spp.	4 (5.6)	1 (4.0)	3 (16)	.30
Chest imaging at the onset of IA	N = 56	N = 39	N = 17	
Nodules	18 (32)	10 (26)	7 (41)	.34
Ground glass opacity	17 (30)	13 (33)	4 (24)	.54
Halo signs	6 (11)	3 (7.7)	3 (18)	.35
Consolidation	39 (70)	30 (77)	9 (53)	.11
Cavity	8 (14)	6 (15)	3 (18)	1.0
Pleural effusion	22 (39)	19 (49)	3 (18)	.04
A new infiltrate + obstruction of the main bronchus	1 (1.8)	0	1 (5.9)	.3
Breakthrough IA	10 (16)	10 (24)	0	.01
Voriconazole breakthrough IA	1 (1.8)	1(2.4)	0	1.0
Liposomal amphotericin B breakthrough IA	4 (6.3)	4 (9.8)	0	.29
Micafungin breakthrough IA	2 (3.2)	2 (4.9)	0	.55
Anidulafungin breakthrough IA	3 (4.8)	3 (7.3)	0	.55
Initial antifungal regimens	N = 62			

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Table 1 (*continued*)

Factors	IA cases N = 62 (%)	Early IA N = 41 (%)	Late IA N = 21 (%)	*P* value
Voriconazole	25 (40.3)	14 (34)	11 (50)	.18
Posaconazole	2 (3.2)	1 (2.4)	1 (4.8)	1.0
Isavuconazole	1 (1.6)	0	1 (4.8)	.34
Liposomal amphotericin B	13 (21.0)	11 (27)	2 (9.5)	.19
Caspofungin	6 (9.7)	6 (15)	0	.09
Micafungin	6 (9.7)	4 (9.8)	2 (9.5)	1.0
Any combination antifungal regimens	3 (4.8)	2 (4.9)	1 (4.8)	1.0
No antifungal treatment	6 (9.7)	3 (7.3)	3 (14)	.4
All-cause mortality				
Death within 6 weeks after diagnosis of IA	22 (35.5)	16 (39.0)	6 (28.6)	.43
Death within 12 weeks after diagnosis of IA	23 (37.1)	16 (39.0)	7 (33.3)	.62
Death within 12 months after diagnosis of IA	25 (40.4)	17 (41.5)	8 (38.1)	.71

Early IA and late IA were defined as IA which diagnosed ≤90 days and >90 days after the day of transplantation, respectively.
Information regarding 12-week and 12-month all-cause mortality was lacking in 1 out of the 62 liver transplant recipients because the recipient lost follow-up before these dates.
The regimens that the 3 recipients received as an initial therapy were a combination of voriconazole and liposomal amphotericin B, a combination of voriconazole and caspofungin, and a combination of voriconazole, liposomal amphotericin B, and caspofungin. No antifungal treatment was defined as a recipient who did not receive any antifungal agents within 2 weeks after the onset of IA or before a patient died. Among the 6 recipients who did not receive any antifungal treatment, 4 died within 5 days after the onset of IA, 1 recipient received voriconazole >2 weeks after the onset of IA, and 1 recipient received anidulafungin after >2 weeks after the onset of IA.
IA, invasive aspergillosis; IQR, interquartile range.

IA (Table 1). The median duration of IA diagnosis after liver transplant was 37 days (interquartile range: 10-129 days) after liver transplantation. None of the 62 LiTRs had neutropenia (absolute neutrophil count ≤500/μL) at the onset of IA. Hepatitis C was the most common underlying liver disease (Table 2). A deceased donor was the most common (85%) donor source. There was no IA nor *Aspergillus* colonization among the donors. However, 2 IA cases had *Aspergillus* colonization (positive sputum culture) of the respiratory tract prior to transplantation. Fifty-six patients (90%) had pulmonary IA and 8 patents were diagnosed with disseminated IA. *Aspergillus* was cultured in 44 cases, with *A. fumigatus* being the most common causative species (64%). In our IA cohort of 44 culture-positive cases, GM levels in BALF were evaluated in 21 instances. Remarkably, in each of these cases, the GM level in BALF was found to be ≥1.0. In 18 culture-negative IA cases, 17 cases were diagnosed with IA based on a combination of GM level in BALF ≥1.0 and radiological findings. One case was classified as probable IA based on the combination of a serum GM antigen level of 1.5, and the radiological finding. Thus, all 62 IA cases fulfilled the EORTC/MSG 2020 criteria as well. In addition, 41 cases were early IA (66%) and the remaining 21 cases (34%) were late IA. Post-transplant, 19 patients were administered antimold antifungal agents as a prophylactic measure. Specifically, 11 patients received antimold prophylaxis (liposomal amphotericin B [n = 4], micafungin [n = 3], and anidulafungin [n = 3], and voriconazole [n = 1]) around the onset of IA (11 cases fulfilled the definition of recent receipt of antimold prophylaxis.). Of the 11 cases, 10 were considered as breakthrough IA (Supplementary Table S2); the remaining 1 had IA 2 days following the cessation of a 38-day anidulafungin prophylactic regimen. Ten cases of breakthrough IA (24%) occurred in the early IA group and no breakthrough IA occurred in the late IA group ($P = .01$). The percentage of disseminated IA (12% [early IA] vs 14% [late IA], $P = 1.00$) was not significantly different between the 2 groups (Table 1). The culture-positive IA cases were significantly less common in the early IA group (25/41) than in the late IA group (19/21) ($P = .02$).

Six-week, 12-week, and 12-month all-cause mortality rates after IA diagnosis were 35.5%, 37.1%, and 40.4%, respectively. These mortality rates were not significantly different between the early IA and the late IA group (Table 1). The characteristics of IA cases are summarized in Tables 1 and 2.

3.2. Factors associated with the occurrence of IA in LiTRs

In univariate analysis, 62 IA cases were compared with 124 matched controls (Table 2). In this analysis, 112/124 (90%) of case-control pairs underwent transplantation within 30 days of each other. Recent receipt of systemic antibiotics was significantly more common in IA (67%) than in controls (20%) ($P < .001$). The antibiotic regimens of both the IA group and controls are shown in Supplementary Table S3. Recent history of pneumonia was significantly more common in the IA group than in the matched controls (20/62 [31%] vs 1/124 [0.8%], $P < .001$). In addition, the causative pathogens in 16 out of 20 cases of pneumonia (80%) in the IA group were gram-negative rods. No pathogens could be identified in the remaining 4 cases. The results of the univariate analysis are shown in Table 2.

Table 2
Univariate analysis for comparing 62 IA cases and 124 matched controls in liver transplant settings.

Factors	IA cases (N = 62)	Matched controls (N = 124)	Odds ratio (95% CI)	*P* values
	Number (%)	Number (%)		
Male/female	47 (76)/15 (24)	83 (67)/41 (33)	1.60 (0.78-3.29)	.20
Age (IQR), years	58.5 (49.3-61.8)	57 (50-64)	0.99 (0.96-1.02)	.42
Ethnicity				
Caucasian	46 (74)	95 (77)	Reference	Reference
Black	2 (3.2)	2 (1.6)	2.00 (0.28-14.3)	.49
Asian	8 (13)	8 (6.5)	2.93 (0.69-12.5)	.15
Hispanic	4 (6.5)	9 (7.3)	1.01 (0.21-4.80)	.99
Unknown	2 (3.2)	10 (8.1)	0.35 (0.06-2.03)	.24
Underlying LDs				
HBV hepatitis	4 (6.5)	5 (4.0)	1.71 (0.41-7.05)	.46
HCV hepatitis	18 (29)	27 (22)	1.43 (0.73-2.79)	.30
Nonalcoholic LD	7 (11)	25 (20)	0.49 (0.19-1.25)	.13
Alcoholic LD	14 (23)	35 (28)	0.73 (0.35-1.52)	.40
Autoimmune hepatitis	5 (8.1)	5 (4.0)	2.00 (0.58-6.91)	.27
Hepatocellular carcinoma	15 (24)	27 (22)	1.14 (0.56-2.30)	.72
Primary biliary cirrhosis	4 (6.5)	4 (3.2)	2.00 (0.50-8.00)	.33
Primary sclerosing cholangitis	2 (3.2)	3 (2.4)	1.33 (0.22-7.98)	.75
Other LDs	11 (18)	22 (18)	1.00 (0.47-2.14)	1.00
Condition of LDs				
Fulminant hepatitis	11 (18)	3 (2.4)	10.3 (2.27-46.7)	.003
MELD score $>$ 30	16 (28)	10 (8.1)	4.00 (1.63-9.84)	.003
Transplant factor				
Donor type				
Deceased	53 (85)	105 (85)	Reference	Reference
Living	5 (8.1)	12 (9.7)	0.81 (0.26-2.58)	.73
After cardiac death	4 (6.5)	7 (5.6)	1.10 (0.32-3.82)	.88
Anastomosis				
Duct to duct	47 (82)	104 (91)	Reference	Reference
Roux-en-Y	10 (18)	10 (8.8)	2.09 (0.84-5.18)	.11
Cold ischemic time (h) (IQR)	6.36 (5.35-7.94)	7 (5-8.25)	0.92 (0.76-1.12)	.41
Unit of intraoperative transfusion $\geq$ 10U	17 (30)	18 (16)	2.80 (1.15-6.77)	.02
Induction				
Basiliximab	25 (42)	38 (32)	2.17 (0.89-5.30)	.09
Antithymocyte globulin	6 (10)	10 (8.3)	1.82 (0.28-12.0)	.53
Alemtuzumab	1 (1.7)	0	NA	NA
Steroid	25 (42)	58 (48)	0.30 (0.56-1.56)	.15
No induction	5 (8.5)	21 (18)	0.39 (0.13-1.13)	.08

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Table 2 (*continued*)

Factors	IA cases (N = 62)	Matched controls (N = 124)	Odds ratio (95% CI)	*P* values
	Number (%)	Number (%)		
Pretransplant factors				
Comorbidity and underlying status				
Diabetes mellitus	15 (24)	38 (31)	0.69 (0.33-1.45)	.33
Creatinine (mg/dL) (IQR)	1.1 (0.8-1.68)	0.93 (0.74-1.45)	1.10 (0.83-1.46)	.49
Previous liver transplantation	12 (19)	4 (3.2)	7.53 (2.11-26.8)	.002
Dialysis	10 (16)	5 (4.0)	4.66 (1.45-15.0)	.01
Aspergillus colonization	2 (3.2)	0	NA	NA
Bacteremia	10 (16)	1 (0.8)	20.0 (2.56-156)	.004
Pneumonia	2 (3.2)	4 (3.2)	1.00 (0.18-5.46)	1.00
Systemic antibiotics	14 (23)	17 (15)	1.96 (0.84-4.57)	.12
ICU stay	15 (26)	10 (8.8)	3.81 (1.53-9.49)	.004
Mechanical ventilation	8 (14)	8 (7.0)	2.00 (0.75-5.33)	.17
Pretransplant immunosuppressive agents				
Systemic steroid	15 (26)	14 (12)	2.80 (1.15-6.77)	.02
Immunosuppressive agents other than steroid	9 (16)	7 (6.1)	3.67 (1.09-12.3)	.04
Cyclosporin	0	1 (0.9)	NA	NA
Tacrolimus	6 (11)	4 (3.5)	3.56 (0.88-14.5)	.08
Mycophenolate	1 (1.8)	2 (1.8)	1.00 (0.91-11.0)	1.00
Azathioprine	2 (3.5)	1 (0.9)	4.00 (0.36-44.1)	.26
mTOR inhibitor	1 (1.8)	1 (0.9)	2.00 (0.13-32.0)	.62
Posttransplant factor				
Antifungal prophylaxis				
Recent receipt of nystatin	9 (15)	25 (20)	0.41 (0.12-1.44)	.163
Recent receipt of antimold prophylaxis	11 (18)	2 (1.6)	11.0 (2.44-49.6)	.002
Posttransplant status				
Creatinine (mg/dL)	1.38 (1-2.13)	1.1 (0.89-1.52)	1.17 (0.90-1.53)	.25
Recent history of dialysis	20 (35)	11 (9.6)	6.33 (2.34-17.1)	<.001
Reoperation	21 (34)	12 (9.7)	5.62 (2.24-14.1)	<.001
Recent history of bacteremia	16 (26)	7 (5.6)	5.11 (1.99-13.1)	.001
Recent history of pneumonia	20 (31)	1 (0.8)	40.0 (5.37-298)	<.001
Recent receipt of systemic antibiotics	38 (67)	23 (20)	16.8 (5.12-55.4)	<.001
CMV DNAemia and/or diseases	10 (16)	8 (6.5)	3.68 (1.12-12.1)	.03
Rejection	6 (11)	6 (4.8)	2.00 (0.65-6.20)	.23
Recent receipt of immunosuppressive agents				
Systemic steroid	53 (85)	97 (78)	3.21 (0.83-12.3)	.09
Immunosuppressive agents other than steroid	59 (92)	119 (96)	0.78 (0.15-4.20)	.77
Cyclosporin	3 (4.8)	2 (1.6)	3.00 (0.50-18.0)	.23
Tacrolimus	50 (81)	113 (91)	0.36 (0.14-0.94)	.04

(*continued on next page*)

Table 2 (*continued*)

Factors	IA cases (N = 62)	Matched controls (N = 124)	Odds ratio (95% CI)	*P* values
	Number (%)	Number (%)		
Mycophenolate	30 (48)	54 (44)	1.39 (0.63-3.07)	.42
mTOR inhibitor	2 (3.2)	4 (3.2)	1.00 (0.16-6.42)	1.00

Alemtuzumab, *Aspergillus* colonization, and pretransplant cyclosporin could not be evaluated by conditional logistic regression analysis because the number of these variables of either late IA cases or matched controls was 0.
CI, confidence interval; CMV, cytomegalovirus; IA, invasive aspergillosis; ICU, intensive care unit; IQR, interquartile range; LD, liver disease; mTOR, mammalian target of rapamycin; NA, not available.

In the multivariate analysis, recent receipt of systemic antibiotics (adjusted odds ratio [aOR], 4.74; 95% confidence interval [CI], 1.16-19.4; $P = .03$) and recent history of pneumonia (aOR, 48.7; 95% CI, 2.79-849; $P = .01$) were identified as significant independent risk factors associated with IA (Table 3).

Factors associated with the occurrence of early IA and late IA are detailed in Supplementary Tables S4 and S5, respectively. Recent receipt of antimold prophylaxis was significantly more common in early IA (27%) than in matched controls (2.4%) ($P = .002$). In addition, of the 11 patients who received antimold prophylaxis recently, 10 (91%) had ≥1 traditional risk factor for early IA (reoperation, fulminant hepatitis, dialysis, MELD score >30, and previous liver transplantation). Rejection tended to be more common in late IA than in the matched controls (5/21 [26%] vs 2/42 [4.8%] $P = .05$). No other factors had a significant association (*P* value was <.05) and tendency (*P* value was between .05 and .10) with late IA (Supplementary Table S5).

In the multivariate analysis of risk factors for early IA (Table 4), reoperation (aOR, 5.99; 95% CI, 1.45-24.7; $P = .01$), recent receipt of systemic antibiotics (aOR, 5.03; 95% CI, 1.09-23.2; $P = .04$), and recent receipt of antimold prophylaxis (aOR, 11.9; 95% CI, 1.45-97.6; $P = .02$) were identified as independent risk factors.

In contrast, no independent risk factors associated with late IA could be identified in the multivariate analysis because of the limited number of patients in this population.

3.3. Impact of IA on mortality of LiTRs

The 100-day, 180-day, and 12-month all-cause mortality after IA/matched date was higher than in controls (0.8%, 0.8%, and 1.6%, vs 37.1%, 38.8%, and 40.4%, respectively; $P < .001$) (Fig. 1). Furthermore, IA was still identified as 1 of the significant

Table 3
The multivariate analysis for risk factors associated with the occurrence of IA.

Factors	Odds ratio	95% confidence interval	*P* value
Recent receipt of systemic antibiotics	4.74	1.16-19.4	.03
Recent history of pneumonia	48.7	2.79-8.49	.01
Recent history of dialysis	4.06	0.73-22.5	.11
Fulminant hepatitis	7.17	0.49-105	.15
MELD score >30	1.96	0.45-8.54	.37
Previous liver transplantation	2.06	0.06-75.1	.69
Reoperation	3.06	0.69-13.5	.14

IA, invasive aspergillosis.
In the multivariate analysis for risk factors associated with the occurrence of IA, recent history of dialysis, recent receipt of systemic antibiotics, and recent history of pneumonia were identified as independent significant factors by the statistical software. Thereafter, they were adjusted by using well-known risk factors (fulminant hepatitis, MELD score >30, previous liver transplantation, and reoperation) which were already excluded once by the statistical software. The final model is presented in Table 3.

Table 4
The multivariate analysis for risk factors associated with the occurrence of early IA.

Factors	Odds ratio	95% confidence interval	*P* value
Reoperation	5.99	1.45-24.7	.01
Recent receipt of systemic antibiotics	5.03	1.09-23.2	.04
Recent receipt of antimold prophylaxis	11.9	1.45-97.6	.02
Fulminant hepatitis	1.44	0.08-26.4	.81
Recent history of dialysis	0.94	0.19-4.74	.95
MELD score > 30	4.63	0.65-33.1	.13
Previous liver transplantation	1.12	0.10-14.3	.89

IA, invasive aspergillosis.
In the multivariate analysis for risk factors associated with the occurrence of early IA, reoperation, recent receipt of systemic antibiotics, and recent receipt of antimold prophylaxis were identified as independent significant factors by the statistical software. Thereafter, they were adjusted by using well-known risk factors (fulminant hepatitis, recent history of dialysis, MELD score >30, and previous liver transplantation) which were already excluded once by the statistical software. The final model is presented in Table 4.

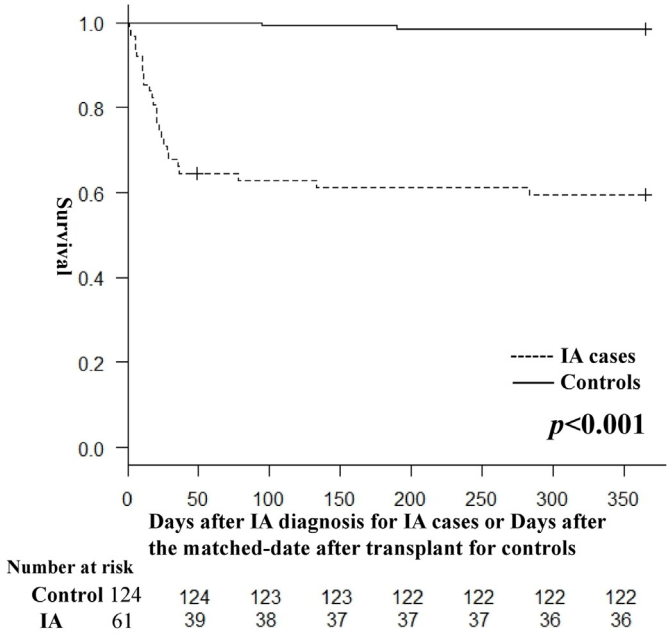

Figure 1. The analysis of 12-month all-cause mortality after the diagnosis of invasive aspergillosis (IA) or after the matched date after transplant.

independent risk factors for 12-month all-cause mortality (adjusted hazard ratio [aHR], 18.1; 95% CI, 4.02-81.9; $P < .001$) in multivariate analysis (Table 5). The results of the univariate analysis are shown in Supplementary Table S6.

3.4. Factors associated with 12-week all-cause mortality in LiTRs with IA

In the univariate analysis of the 62 IA cases, some important factors associated with 12-week all-cause mortality were identified (Table 6). In particular, mortality among patients with disseminated IA (87.5%) was higher than among patients with localized IA (29.6%) ($P < .001$) (Fig. 2A). In addition, IA cases who received an antimold azole (voriconazole, posaconazole, or isavuconazole) empirically at IA diagnosis tended to have lower 12-week all-cause mortality compared with IA cases who did not receive it at that time (20.0 % vs 42.6 %, $P = .09$) (Table 6). The 12-week all-cause mortality was not significantly different between early IA (39.0 %) and late IA (33.3 %) ($P = .62$) (Fig. 2B).

In multivariate analysis, *Aspergillus* colonization (aHR, 86.9; 95% CI, 14.1-534; $P < .001$), ICU stay (aHR, 3.67; 95% CI, 1.27-10.6; $P = .02$), disseminated IA (aHR, 8.98; 95% CI, 3.44-23.4; $P < .001$), and recent history of dialysis (aHR, 2.93; 95% CI, 1.10-7.80; $P = .001$) were significant risk factors for 12-week all-cause mortality whereas recent receipt of tacrolimus (aHR, 0.11; 95% CI, 0.03-0.41; $P = .001$) was protective (Table 6).

4. Discussion

To the best of our knowledge, this study is the largest contemporary case-control study focusing on IA in LiTRs. All 62 IA cases were diagnosed based on EORTC/MSG 2008 and also fulfilled the recently revised EORTC/MSG 2020 criteria.[21]

This study has yielded 3 noteworthy findings. First, it has shed light on the contemporary clinical characteristics of IA, elucidating aspects such as the IA mortality rate and the prevalence of disseminated IA. Second, the study has uncovered previously unrecognized, yet significant, independent risk factors associated with IA occurrence, such as the recent utilization of systemic antibiotics, a history of pneumonia, and utilization of antimold prophylaxis. Lastly, the study has shown that the recent administration of tacrolimus was associated with a reduction in 12-week all-cause mortality in IA cases.

In the present study, the all-cause mortality rates at both the 12-week and 12-month period for patients with IA were notably lower, standing at 37.1 % and 40.4 %, respectively, in contrast to previous findings, which reported rates ranging approximately between 60% and 90%.[1,3-8] Additionally, the incidence of disseminated IA in our study, at 13%, was markedly lower compared to previous reports that had documented rates between 48% and 62% in LiTRs.[3,7-9] Significantly, our investigation

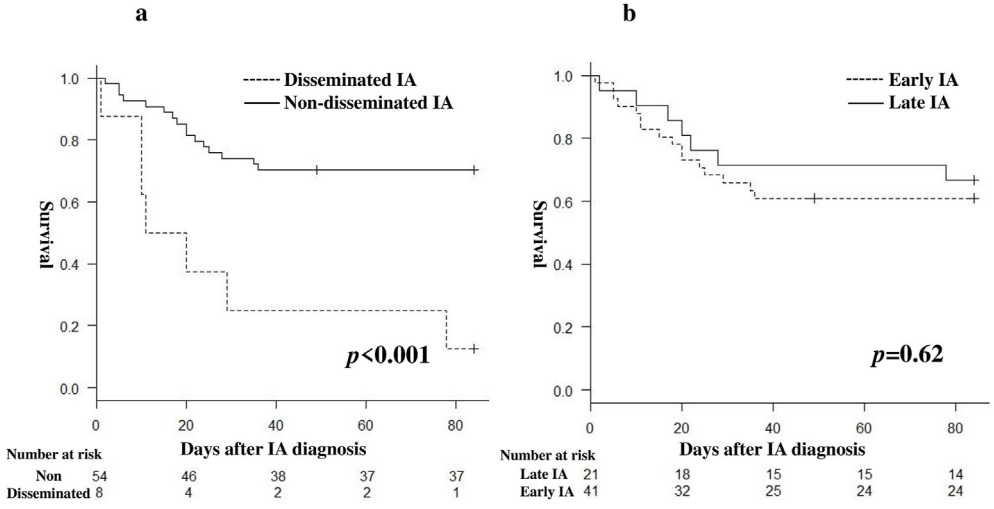

Figure 2. The 12-week all-cause mortality after the diagnosis of invasive aspergillosis (IA) in each group.
Abbreviation. IA, invasive aspergillosis.

Table 5
The multivariate analysis to identify factors associated with 12-month all-cause mortality after the diagnosis of IA or matched date after transplant in the cohort of 62 IA cases and 124 matched controls.

Factors	Adjusted hazard ratio	95% confidence interval	*P* value
IA	18.1	4.02-81.9	<.001
Recent history of dialysis	3.15	1.18-8.44	.02
Recent history of tacrolimus	0.32	0.12-0.84	.02
Induction with antithymocyte globulin	0.34	0.05-2.37	.28
MELD score >30	0.92	0.34-.45	.86

IA, invasive aspergillosis.
In the multivariate analysis, IA, recent history of dialysis, and recent receipt of tacrolimus were identified as independent significant factors by the statistical software. Thereafter, they were adjusted by using well-known risk factors (induction with antithymocyte globulin and MELD score >30) which were already excluded once. The final model is presented in Table 5.

identified disseminated IA as an independent and substantial risk factor associated with 12-week all-cause mortality among the 62 IA cases examined in this study (Table 6). The reduced occurrence of disseminated IA observed here may be attributed, in part, to changes in antifungal management practices. A recent guideline has recommended targeted antimold antifungal prophylaxis for high-risk LiTRs,[11] a strategy that might account for the observed decline in both mortality and the prevalence of disseminated IA to some extent. In fact, this strategy was applied to 6 out of 14 centers in our study (Supplementary Table S1). In addition, improvement of diagnostic tools for IA, such as BALF GM antigen testing and earlier recognition of IA, might decrease both mortality and disseminated IA.

However, it is noteworthy that the mortality of IA among LiTRs in our study remains higher when compared to recent studies involving lung transplant (ranging from 4.2% to 14.8%) and heart transplant (ranging from 26.7% to 31.2%) recipients.[5,7] Furthermore, even after adjusting for other factors linked to mortality (as depicted in Table 5), IA still exerts a negative impact on the mortality of the LiTRs in our case-control cohort. In light of these findings, the need to identify practical risk factors associated with the occurrence of IA persists, in order to optimize targeted prophylactic measures and early initiation of anti-*Aspergillus* treatment more effectively.

In the present study, reoperation was identified as an independent risk factor associated with the occurrence of early IA. Thus, reoperation remains relevant even in the current era.[6,11] In contrast, systemic antibiotic usage was identified as a new independent risk factor associated with the occurrence of both IA and early IA. In addition, recent history of pneumonia was identified as a new independent risk factor of IA. Significant collinearity and correlation between antibiotic usage and history of pneumonia were not identified (Data are not shown.). The biological plausibility of systemic antibiotic usage as a risk factor for IA and early IA is uncertain. Perhaps, systemic antibiotic usage represented critical illness after transplantation warranting antimicrobial therapy, that may facilitate the colonization of airways with mold. Regarding the history of pneumonia, 16 out of 20 LiTRs with pneumonia prior to IA had pneumonia caused by gram-negative bacteria. The combination of airway damage caused by pathogens other than *Aspergillus* spp., including gram-negative bacteria and severe immunocompromised status following liver transplant might be associated with the occurrence of IA. In general, targeted prophylaxis has been recommended for LiTRs with any 1 or more of risk factors which are associated with IA to prevent the occurrence of IA.[11] The recent history of pneumonia in these patients may warrant a closer look at the probability of fungal etiology of pneumonia. In addition, receipt of antimold prophylaxis was identified as a novel independent risk factor of early IA although antimold prophylaxis has anti-*Aspergillus* activity. It might be identified as the sum of traditional risk factors of early IA. In fact, among 11 early IA cases who received antimold prophylaxis, 10 had ≥1 traditional risk factor of early IA (reoperation, fulminant hepatitis, dialysis, MELD score >30, and previous liver transplantation[11]). This result may indicate that the traditional risk factors are relevant to the occurrence of early IA even in the current era and LiTRs receiving targeted antimold prophylaxis should still be considered to be at a high risk of developing IA. This understanding may be important for early recognition and initiation of treatment of breakthrough IA for this population.

In this study, no independent risk factor of late IA could be identified in multivariate analysis because of the limited number of late IA cases. Only rejection tended to be more common in late IA than in matched control ($P = .05$) (Supplementary Table S5). Rejection usually requires high-intensity immunosuppressive therapy.[24] Hence, rejection might be a true risk factor of late IA because the occurrence of IA is often associated with immunosuppression.[13] Further investigation is needed in the future to confirm if rejection is a true risk factor for late IA.

In this study, for the first time, we report the recent administration of tacrolimus as an independent significant protective factor against IA mortality among LiTRs, as shown in Table 6. The discontinuation of tacrolimus in sicker LiTRs, possibly due to renal injury or severe infections, might confound its association with other risk factors. Nonetheless, the antifungal activity of tacrolimus and its analogs against *Aspergillus* strains, demonstrated in vitro, along with reports of calcineurin inhibitors including tacrolimus offering protection against cryptococcosis in solid organ transplant recipients, lends biological credibility to our findings.[25-27] Despite tacrolimus' immunosuppressive properties, these data suggest a potential protective role against IA in our study.

In the multivariate analysis of mortality (Table 6), pretransplant *Aspergillus* colonization in the respiratory tract was identified as 1 of the independent risk factors. Pretransplant *Aspergillus* colonization could not be evaluated as a risk factor for developing IA because no matched controls had *Aspergillus* colonization (Table 2). However, *Aspergillus* colonization has been reported to

Table 6
The univariate and multivariate analyses to identify factors associated with 12-week all-cause mortality after the onset of IA in the 62 liver transplant recipients.

Factors	Yes/No	Number	Death within 12 weeks (%)	*P* value
Male	Yes	47	20 (42.6)	.11
	No	15	3 (20.0)	
Age $\geq$ 60 years	Yes	25	9 (36.0)	.99
	No	37	14 (37.8)	
Affiliations				
Center 1		16	4 (25.0)	.02
Center 2		1	1 (100)	
Center 3		4	1 (25.0)	
Center 4		2	1 (50.0)	
Center 5		2	1 (50.0)	
Center 6		1	1 (100)	
Center 7		1	0	
Center 8		2	1 (50.0)	
Center 9		2	2 (100)	
Center 10		10	3 (30.0)	
Center 11		5	3 (60.0)	
Center 12		7	3 (42.9)	
Center 13		7	2 (28.6)	
Center 14		2	2 (100)	
Ethnicity				
Caucasian		46	16 (34.8)	$<$.001
Black		2	2 (100)	
Asian		8	3 (37.5)	
Hispanic		4	2 (50.0)	
Unknown		2	0	
Underlying liver diseases				
HBV hepatitis	Yes	4	3 (75.0)	.17
	No	58	20 (34.5)	
HCV hepatitis	Yes	18	5 (27.8)	.30
	No	44	18 (41.0)	
Nonalcoholic liver disease	Yes	7	4 (57.1)	.20
	No	55	19 (34.5)	
Alcoholic liver disease	Yes	14	5 (35.7)	.91
	No	48	18 (37.5)	
Autoimmune hepatitis	Yes	5	1 (20.0)	.42
	No	57	22 (38.6)	
Hepatocellular carcinoma	Yes	15	3 (20.0)	.11
	No	47	20 (42.6)	

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Table 6 (*continued*)

Factors	Yes/No	Number	Death within 12 weeks (%)	*P* value
Primary biliary cirrhosis	Yes	4	1 (25.0)	.70
	No	58	22 (37.9)	
Primary sclerosing cholangitis	Yes	2	0	.33
	No	60	23 (38.3)	
Other liver diseases	Yes	11	6 (54.5)	.20
	No	51	17 (33.3)	
Condition of liver diseases				
Fulminant hepatitis	Yes	11	7 (63.6)	.04
	No	51	16 (31.4)	
MELD score $>$ 30	Yes	16	7 (43.8)	.46
	No	41	13 (31.7)	
Transplant factor				
Donor type				
Deceased	Yes	53	20 (37.7)	.63
Living	Yes	5	1 (20.0)	
After cardiac death	Yes	4	2 (50.0)	
Roux-en-Y	Yes	10	2 (20.0)	.29
	No	47	18 (38.3)	
Cold ischemic time $\geq$ 7 h	Yes	17	5 (29.4)	.55
	No	30	11 (36.7)	
Unit of intraoperative transfusion $\geq$ 10 U	Yes	17	9 (52.9)	.11
	No	40	11 (27.5)	
Induction				
Basiliximab	Yes	25	9 (36.0)	.98
	No	34	12 (35.3)	
Antithymocyte globulin	Yes	6	1 (16.7)	.33
	No	53	20 (37.7)	
Steroid	Yes	25	10 (40.0)	.47
	No	34	11 (32.4)	
Any induction	Yes	54	18 (33.3)	.34
	No	5	3 (60)	
Pretransplant factors				
Diabetes mellitus	Yes	15	5 (33.3)	.74
	No	47	18 (38.3)	
Previous liver transplantation	Yes	12	4 (33.3)	.74
	No	50	19 (38.0)	
Dialysis	Yes	10	6 (60.0)	.09
	No	52	17 (32.7)	
Aspergillus colonization	Yes	2	2 (100)	$<$.001
	No	60	21 (35.0)	

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Table 6 (*continued*)

Factors	Yes/No	Number	Death within 12 weeks (%)	*P* value
Bacteremia	Yes	10	6 (60.0)	.15
	No	52	17 (32.7)	
Pneumonia	Yes	2	1 (50.0)	.66
	No	60	22 (36.7)	
Systemic antibiotics	Yes	14	5 (35.7)	.80
	No	43	15 (34.9)	
ICU stay	Yes	15	8 (53.3)	.11
	No	42	12 (28.6)	
Mechanical ventilation	Yes	8	4 (50)	.81
	No	49	16 (32.7)	
Systemic steroid	Yes	15	4 (26.7)	.40
	No	42	16 (38.1)	
Immunosuppressive agents other than steroid	Yes	9	2 (22.2)	.42
	No	48	18 (37.5)	
Tacrolimus	Yes	6	1 (16.7)	.38
	No	51	19 (37.3)	
Mycophenolate	Yes	1	1 (100)	.01
	No	56	19 (33.9)	
Azathioprine	Yes	2	1 (50.0)	.69
	No	55	19 (34.5)	
mTOR inhibitor	Yes	1	0	.51
	No	56	20 (35.7)	
Posttransplant factor at the onset				
Serum creatinine $\geq$ 1.5 mg/dL	Yes	25	12 (48.0)	.06
	No	32	8 (25.0)	
Dialysis	Yes	20	10 (50.0)	.08
	No	37	10 (27.0)	
Reoperation	Yes	21	7 (33.3)	.71
	No	41	16 (39.0)	
Recent history of bacteremia	Yes	16	7 (43.7)	.46
	No	46	16 (34.8)	
Recent history of pneumonia	Yes	20	8 (40.0)	.55
	No	42	15 (35.7)	
CMV DNAemia or diseases	Yes	10	6 (60.0)	.15
	No	52	17 (32.7)	
Rejection	Yes	6	3 (50.0)	.35
	No	51	17 (33.3)	
Recent receipt of antimold prophylaxis	Yes	11	5 (45.5)	.54
	No	51	18 (35.3)	
Recent receipt of nystatin	Yes	9	2 (22.2)	.36
	No	53	21 (39.6)	

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Table 6 (*continued*)

Factors	Yes/No	Number	Death within 12 weeks (%)	*P* value
Receipt of systemic steroid	Yes	53	21 (39.6)	.34
	No	9	2 (22.2)	
Recent receipt of immunosuppressive agents	Yes	3	1 (33.3)	.97
	No	59	22 (37.3)	
Recent receipt of cyclosporin	Yes	3	2 (66.7)	.06
	No	59	21 (35.6)	
Recent receipt of tacrolimus	Yes	50	17 (34.0)	.14
	No	12	6 (50.0)	
Recent receipt of mycophenolate	Yes	30	12 (40.0)	.64
	No	32	11 (34.4)	
Recent receipt of mTOR inhibitor	Yes	2	1 (50.0)	.46
	No	60	22 (36.7)	
Factors associated with IA				
Early IA ($\leq$ 90 d after Tx)	Yes	41	16 (39.0)	.62
	No	21	7 (33.3)	
Pulmonary IA (not disseminated)	Yes	48	15 (31.3)	.09
	No	14	8 (57.1)	
Disseminated IA	Yes	8	7 (87.5)	$<$.001
	No	54	16 (29.6)	
Factors associated with IA treatment				
Antimold antifungal administration at the onset	Yes	26	11 (42.3)	.54
	No	36	12 (33.3)	
Antimold azole administration at the onset of IA	Yes	15	3 (20.0)	.09
	No	47	20 (42.6)	

Multivariate analysis

Factors	Adjusted hazard ratio	95% confidence interval	*P* value
Aspergillus colonization	86.9	14.1-534	$<$.001
ICU stay	3.67	1.27-10.6	.02
Disseminated IA	8.98	3.44-23.4	$<$.001
Recent history of dialysis	2.93	1.10-7.80	.03
Recent receipt of tacrolimus	0.11	0.03-0.41	.001

Affiliations. center 1, Ajmera Transplant Center, University Health Network, Toronto, Ontario, Canada; center 2, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand; center 3, University Medical Centre Ljubljana, Slovenia; center 4, Hospital Universitario Reina Sofia-IMIBIC-UCO, CIBERINFEC (CB21/13/00049), Córdoba, Spain; center 5, Hospital Universitario "12 de Octubre," Madrid, Spain; center 6, King Fahad Specialist Hospital, Dammam, Saudi Arabia; center 7, Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada; center 8, University of Pittsburgh, Pennsylvania; center 9, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IRCCS ISMETT), Palermo, Italy; center 10, IRCCS Azienda Ospedaliero Universitaria di Bologna, Bologna, Italy; center 11, Swiss Transplant Cohort, Switzerland; center 12, University of Queensland School of Medicine, Ochsner Clinical School, Louisiana; center 13, Miami Transplant Institute, Jackson Health System, Miami, Florida; center 14; Washington University in St. Louis, Missouri.

Among 26 recipients who received antimold antifungal administration for their IA at the date of diagnosis, 15 received azole-containing regimens and the remaining 11 received nonazole regimens (liposomal amphotericin B [8 recipients], micafungin [2 recipients], and caspofungin [1 recipient]). Of the 15 recipients who received antimold azoles, 11 received voriconazole monotherapy, 1 received posaconazole monotherapy, 1 received isavuconazole, 1 received a combination of voriconazole and caspofungin, and 1 received a combination of voriconazole and liposomal amphotericin B.

CMV, cytomegalovirus; IA, invasive aspergillosis; ICU, intensive care unit.

be a risk factor for IA in critically ill patients, including patients with acute leukemia, lung transplant recipients, and allogeneic hematopoietic stem cell recipients.[28] In this context, targeted prophylaxis for LiTRs who have pretransplant *Aspergillus* colonization may be reasonable to reduce the risk of death associated with IA.

This study had several inherent limitations. First, it was a multicenter retrospective case-control study. Conducting a prospective study or a multicenter cohort study with a specific focus on IA in LiTRs proved challenging due to the remarkably low incidence of IA, standing at just 1.8%, particularly in the recent era.[6] In addition, the identification of IA cases varied across centers due to medical and microbiological record systems. However, each center selected matched controls according to the same protocol (Supplementary Appendix).

Second, this study was unable to provide the incidence rate of IA in LiTRs due to the case-controlled design. Their fundamental structure does not allow for the estimation of incidence or prevalence because they do not sample participants based on exposure within a defined population. Instead, controls are specifically selected to compare with cases, which inherently limits the study's ability to provide incidence-based measurements. However, this study did succeed in including a sufficient number of patients with late IA because patient inclusion was based on the IA diagnosis date rather than the date of transplant. Including a sufficient number of late IA allowed for a detailed evaluation of the clinical characteristics of late IA. Although the late IA group has heterogeneity because the range of days to IA from the day of transplant is wide (from 101 days to 3773 days), data of late IA are rare and worthy of further evaluation.

Third, this study might have significant heterogeneity because it was an international multicenter study. Each center contributed a varying number of cases and did not have identical prophylactic strategies for invasive fungal infection (Supplementary Table S1). It indicates that real-world targeted antimold prophylaxis strategies vary a great deal. Thus, the insights obtained from this real-world study might be practical in current liver transplant settings globally.

In conclusion, the mortality rate of IA in the LiTRs remains high even in the current era although it is lower than previously. The novel factors associated with the occurrence of IA may be useful in establishing a more efficient prophylactic strategy and initiating prompt empirical therapy against IA, which may decrease the mortality of IA in LiTRs.

Acknowledgments

The authors thank Michela Di Chiara (IRCCS Azienda Ospedaliero Universitaria di Bologna, Bologna, Italy); Eric Martin (University of Miami Miller School of Medicine and Miami Transplant Institute, Florida); Julie Castex (Ochsner Health, Louisiana); Mateja Zalaznik, Maja Cvelbar Vozelj (University Medical Centre Ljubljana, Slovenia); Lourdes Ruiz, Manuel Causse, José Manuel Vaquero-Barrios (Hospital Universitario Reina Sofia-IMIBIC. Córdoba, Spain); Mario Fernaández-Ruiz (Hospital Universitario "12 de Octubre," Instituto de Investigación Sanitaria Hospital "12 de Octubre" [imas12], Madrid, Spain); and Cherie Hill (Washington University in St. Louis, Missouri) for assistance with the data entry and/or the data retrieval.

Funding

Swiss patients' data were retrieved from the Swiss Transplant Cohort Study. The Swiss Transplant Cohort Study is supported by the Swiss National Science Foundation (SNSF, http://www.snf.ch), Unimedsuisse (https://www.unimedsuisse.ch), and the Transplant Centers.

Declaration of competing interest

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. J. Hand reports research grant funding from Pfizer, Janssen, Scynexis, and GlaxoSmithKline. D. Neofytos has received research support from MSD and Pfizer and consulting fees from MSD, Pfizer, Basilea, and Gilead. N.J. Mueller is on the scientific advisory board of Takeda MSD, and Pfizer and has received travel support from Biotest. M. Luong is on the Scientific Advisory Board of Takeda and Merck. S. Husain reports grant funding from Merck, Astellas, ScynexisInc, Pulmocide, Ltd, and Gilead Sciences Inc, outside the submitted work. All other authors have no potential conflicts.

Data availability

Individual patient data after deidentification will be available upon request to the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2024.05.016.

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