International Journal of Infectious Diseases 131 (2023) 166–172

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Risk factors for late-onset *Pneumocystis jirovecii* pneumonia in liver transplant recipients

Eun-Ki Min [1,2], Juhan Lee [1,2], Su Jin Jeong [3], Deok-Gie Kim [1,2], Seung Hyuk Yim [1,2], Mun Chae Choi [1,2], Dong Jin Joo [1,2], Myoung Soo Kim [1,2], Jae Geun Lee [1,2,*]

[1] *Department of Surgery, Yonsei University College of Medicine, Seoul, Republic of Korea*
[2] *The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul, Republic of Korea*
[3] *Division of Infectious Diseases, Department of Internal Medicine and AIDS Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea*

ARTICLE INFO

Article history:
Received 8 November 2022
Revised 5 April 2023
Accepted 5 April 2023

Keywords:
Pneumocystis jirovecii pneumonia
Risk factor
Liver transplantation

ABSTRACT

Objectives: The risk factors for late-onset *Pneumocystis jirovecii* pneumonia (PCP) after liver transplantation (LT) have not been well studied. We aimed to analyze the clinical features preceding PCP in LT recipients that would guide individualized prophylaxis.

Methods: Among 742 patients who underwent LT and routine PCP prophylaxis from January 2009 through December 2019 at Severance Hospital, 27 patients developed PCP. We conducted a retrospective case-control study matching each patient with four controls and analyzed the risk factors for late-onset PCP.

Results: After 6 months, post-transplant PCP cases increased steadily with an overall incidence of 6.36 cases per 1000 patient-year. The PCP-related mortality was 37.0%. In the multivariate analyses, age at LT ≥65 years (odds ratio [OR], 13.305; 95% confidence interval [CI], 2.507-70.618; $P = 0.002$), cytomegalovirus infection (OR, 5.390; 95% CI, 1.602-18.132; $P = 0.006$), steroid pulse therapy (OR, 6.564; 95% CI, 1.984-21.719; $P = 0.002$), hepatocellular carcinoma recurrence (OR, 18.180; 95% CI, 3.420-96.636; $P = 0.001$), and lymphocytopenia (OR, 3.758; 95% CI, 1.176-12.013; $P = 0.026$) were independently associated with PCP.

Conclusion: Late-onset PCP after routine prophylaxis after LT remains a lethal infection and is associated with age ≥65 years at LT, cytomegalovirus infection, steroid pulse therapy, hepatocellular carcinoma recurrence, and lymphocytopenia. Targeted prophylaxis considering these risk factors could improve the prevention of this potentially lethal complication.

© 2023 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Introduction

Pneumocystis jirovecii pneumonia (PCP) is a potentially life-threatening pulmonary infection that primarily affects immunocompromised patients, including solid organ transplant (SOT) recipients. Before the era of routine prophylaxis, PCP occurred in 5-15% of SOT cases [1]. Although prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX) has reduced the incidence of PCP in SOT recipients by 91%, it remains a lethal disease, with a mortality rate of at least 25%, even with modern treatment [2].

Antipneumocystis prophylaxis is currently recommended for all SOT recipients for at least 6-12 months after transplantation [3]. However, the occurrence of late-onset PCP in SOT recipients despite routine prophylaxis underscores the importance of identifying risk factors that could inform the duration and reinstitution of prophylaxis. Although the incidence of PCP in liver transplantation (LT) recipients is low, ranging from 2.19 to 2.6 cases per 1000 person-years in recent European cohorts [1,4], the high mortality rate associated with the infection highlights the need for individualized prophylactic strategies rather than a fixed period of prophylaxis for all LT recipients.

Previous case-control studies have identified cytomegalovirus (CMV) viremia, treatment for acute rejection, older age, and lymphocytopenia as risk factors for late-onset PCP in SOT recipients

* Corresponding author: Tel: +82-2-2228-2131; fax: +82-2-313-8289.
E-mail address: drjg1@yuhs.ac (J.G. Lee).

https://doi.org/10.1016/j.ijid.2023.04.387
1201-9712/© 2023 The Author(s). Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

[2,4–6]. However, the limited number of PCP cases in LT recipients included in these studies (2-5 cases) restricts their applicability to this patient population [1,2,4–6]. In addition, few studies have exclusively focused on PCP risk factors in LT recipients, and those that have were limited by small sample sizes [7–9]. These studies also did not investigate the impact of operation-related factors, such as cold ischemic time and transfusions, which may affect immunity and increase the risk of PCP in LT recipients.

Moreover, unlike other SOT recipients, approximately 50% of recipients undergo LT for malignancy in Asian countries. After hepatocellular carcinoma (HCC)-related LT, immunosuppressant use is often modified to reduce HCC recurrence, and the treatments for recurrence can affect the post-transplant immune status. These factors warrant consideration, along with previously reported PCP risk factors. Currently, no guidelines exist for PCP prophylaxis exclusively in LT recipients. Accordingly, we conducted a retrospective case-control study in LT recipients analyzing the clinical parameters preceding PCP development, including reported risk factors, as well as oncologic features, to identify high-risk patients who may benefit from prolonged or reinitiated PCP prophylaxis.

Patients and methods

Study population

A total of 832 patients who received LT and routine prophylaxis for PCP at Severance Hospital, Yonsei University College of Medicine, Republic of Korea, between January 2009 and December 2019, were evaluated. Pediatric patients, retransplants, and combined transplants were excluded. Among the 742 remaining patients, 27 developed PCP (PCP cases), whereas 715 did not (controls). All PCP cases in this study were probable PCP infections that satisfied the triad criteria of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium consensus definition: host factor (SOT recipient) and clinical and radiologic criteria plus positive real-time polymerase chain reaction (PCR) for *P. jirovecii* in bronchoalveolar lavage fluid or sputum [10]. The real-time PCR assay was performed with the AmpliSens *Pneumocystis jirovecii*-FRT PCR kit (Moscow, Russia), a well-validated, widely used qualitative real-time PCR assay. Amplification was performed on the Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, USA). A positive test result was defined as a cycle threshold ≤35, according to the manufacturer's instructions. The clinical and radiologic findings of PCP cases are described in Supplementary Table 1.

We conducted a nested case-control study, with each case matched to four controls. To minimize the historical differences in immunosuppressant protocols, we first screened for controls among patients undergoing LT 1 month before or after the transplantation date of the index case. We then randomly selected those with ≥1 chest X-ray (CXR) or chest computed tomography (CT) scan without signs of pneumonia or a negative PCP PCR test performed close to the diagnosis date (D_P) of the matched case to ensure that the controls did not develop PCP and that the duration of exposure to immunosuppressant (time between LT and D_P) was controlled. For the control group, the date of the negative CXR, CT scan, or PCR test was regarded as D_P.

Immunosuppressive regimens

All patients received basiliximab for induction immunosuppression. Rituximab was used according to a desensitization protocol in ABO-incompatible recipients. Maintenance immunosuppression consisted of a calcineurin inhibitor, prednisolone, and mycophenolate mofetil or mammalian target of rapamycin inhibitor (mTORi). mTORi, mostly everolimus for patients with HCC, was usually initiated 4 weeks after LT, as described previously [11].

P. jirovecii pneumonia and CMV prophylaxis

Routine PCP prophylaxis consists of TMP/SMX (400/80 mg daily) for 6-12 months after LT. CMV prophylaxis is considered only in high-risk patients: seropositive donor/seronegative recipients. Preemptive ganciclovir treatment is administered when CMV infection (quantitative PCR >500 IU/ml) is detected. Plasma CMV PCR is routinely performed 7, 14, 21, and 28 days after LT and on an as-needed basis thereafter.

Clinical and laboratory assessments

Data from medical records were collected for each case and control, including age at LT and D_P, sex, donor type, donor age at LT, model for end-stage liver disease score, Child-Turcotte-Pugh score, original liver disease, ABO incompatibility, pretransplant laboratory values (including CMV serologic status of the donor/recipient [D/R]), operation details, and duration of PCP prophylaxis. We also collected data on immunosuppression regimen, as well as trough levels of immunosuppressants 180 days before and during the D_P. Episodes of CMV infection, rejection, steroid pulse therapy (methylprednisolone 500 mg/day for ≥2 days), and recurrent or *de novo* HCC within a look-back window of 1-5 years before D_P were recorded. Blood cell counts, liver function tests, international normalized ratio, and serum creatinine at D_P and during the 180 days before D_P were also collected. Monthly assessment of serum liver function tests, international normalized ratio, creatinine, and immunosuppressant levels were performed until 1 year after LT, then every 1 or 2 months during follow-up.

Statistical analyses

Continuous variables were presented as median with interquartile range (IQR) and analyzed using the Mann-Whitney U test. Categorical variables were reported as frequency with percentage and compared using the chi-square or Fisher's exact test. The Kaplan-Meier method was used to calculate cumulative incidences of PCP after LT. The independent predictors for PCP development were determined using a logistic regression model. Variables with *P*-values <0.05 were included in the multivariate analyses. The data analyses were performed using SPSS 26.0 software (SPSS, Chicago, IL, USA). *P*-values <0.05 were considered statistically significant.

Results

Frequency of P. jirovecii pneumonia occurrence and baseline patient characteristics

During a median follow-up of 71.0 months (IQR, 38.0-99.0), 27 PCP cases occurred among 742 patients. The median follow-up duration after D_P was 36.4 months (IQR, 13.3-59.5). The incidence of PCP after LT was 6.36 cases per 1000 patient-years over a total of 4247 patient-years. The median time to PCP diagnosis was 25.6 months from LT (IQR, 12.9-53.9). The PCP incidence after 6 months post-transplant steadily increased at approximately 2.2 cases per year. Cumulative probabilities of PCP were 0.6%, 1.0%, 2.9%, and 6.2% at 1, 2, 5, and 10 years (Figure 1).

The baseline characteristics of the 27 PCP cases and 108 controls are described in Table 1. Median age at LT was significantly higher in PCP cases than in controls (57.0 years vs 54.0 years; $P = 0.023$) and remained significant when stratified using an age cut-off of 65 years (18.5% [5/27] vs 5.6% [6/108], $P = 0.043$). There

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

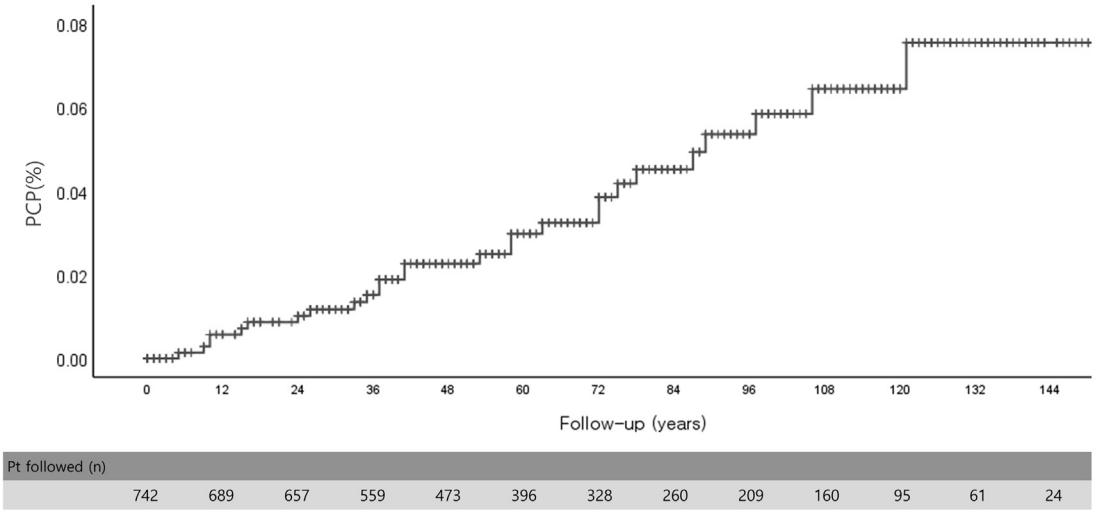

Figure 1. Cumulative probability of PCP after liver transplantation. The probability of PCP was 0.6%, 1.0%, 2.9%, and 6.2% at 1, 2, 5, and 10 years. Cumulative probabilities were calculated using the Kaplan-Meier method.
PCP, *Pneumocystis jirovecii* pneumonia; Pt, patient.

Table 1
Baseline characteristics of liver transplant recipients with or without PCP.

	PCP-positive (n = 27)	PCP-negative (n = 108)	*P*-value
Age at LT (year)	57.0 (55.0-63.0)	54.0 (48.3-59.8)	**0.023**
Age at LT ≥65 years, n (%)	5 (18.5)	6 (5.6)	**0.043**
Age at D_P (year)	60.9 (56.9-65.1)	57.0 (51.9-63.5)	0.050
Age at D_P ≥65 years, n (%)	7 (25.9)	15 (13.9)	0.149
Male sex, n (%)	76 (70.4)	20 (74.1)	0.815
Body mass index (kg/m^2)	23.4 (20.6-26.0)	23.5 (21.7-25.1)	0.795
Donor type, n (%)			**0.035**
Living donor	14 (51.9)	80 (74.1)	
Deceased donor	13 (48.1)	28 (25.9)	
Donor age at LT (years)	37.0 (31.0-53.0)	37.5 (26.0-47.0)	0.263
Donor male sex, n (%)	18 (66.7)	64 (59.3)	0.243
Hypertension at LT, n (%)	5 (18.5)	14 (13.0)	0.751
Diabetes mellitus at LT, n (%)	7 (25.9)	26 (24.1)	0.883
Original liver disease			
Hepatitis B virus, n (%)	15 (55.6)	61 (56.5)	0.931
Hepatitis C virus, n (%)	4 (14.8)	6 (5.6)	0.113
Alcoholic liver cirrhosis	4 (14.8)	27 (25.0)	0.260
Autoimmune hepatitis	2 (7.4)	4 (3.7)	0.345
Primary biliary cirrhosis or primary sclerosing cholangitis	2 (7.4)	2 (1.9)	0.178
Nonalcoholic fatty liver disease	1 (3.7)	2 (1.9)	0.491
Hepatocellular carcinoma, n (%)	16 (59.3)	58 (53.7)	0.669
ABO-incompatible LT, n (%)	4 (14.8)	18 (16.7)	0.248
PCP prophylaxis duration (days)	305 (160-382)	318 (198-348)	0.991
Time between LT and D_P (days)	770 (328-1614)	780 (388-1633)	0.800
Time from end of PCP prophylaxis to D_P (days)	480 (115-1258)	490 (100-1288)	0.873

Data are median (interquartile range), unless otherwise indicated.
D_P, date of PCP diagnosis in the case group and date of chest imaging without pneumonia or negative PCP PCR in the control group; LT, liver transplantation; PCP, *Pneumocystis jirovecii* pneumonia.

were no differences between groups for median age at D_P and proportion of male sex, original liver disease, or HCC cases. The PCP cases had a higher proportion of deceased donors than controls (48.1% vs 25.9%, $P = 0.035$), but there was no difference in donor age or sex at LT between groups. The median PCP prophylaxis duration, time from LT to D_P, and time from the end of PCP prophylaxis to D_P were similar between groups because of routine prophylaxis and uniform exposure duration in our study design.

Preoperative biologic and operation-related factors

At LT, then blood cell counts, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, creatinine, estimated glomerular filtration rate, model for end-stage liver disease score, and Child-Turcotte-Pugh score were similar between the PCP cases and controls. Likewise, operation-related factors, such as total operation time, anhepatic time, cold ischemic time, warm ischemic time, blood loss, and transfusions, showed no significant differences between groups (Supplementary Table 2).

Immunosuppressive regimens

Everolimus use was more frequent in PCP cases than in controls (59.3% vs 27.8%, $P = 0.003$). Everolimus trough level at D_P was higher in PCP cases, whereas the average everolimus trough level during the 180 days before D_P did not differ between groups. Tacrolimus trough levels at D_P and the average tacrolimus trough level during the 180 days before D_P did not differ between groups (Supplementary Table 3).

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

Table 2
CMV and rejection-related features associated with PCP after liver transplantation.

	PCP-positive (n=27)	PCP-negative (n=108)	*p* value
Pre-LT cytomegalovirus serologic status, n (%)			0.698
D(+)/R(+)	94 (87.0)	22 (81.5)	
D(+)/R(-)	0 (0)	0 (0)	
D(-)/R(+)	7 (6.5)	3 (11.1)	
D(-)/R(-)	0 (0)	0 (0)	
Not determined	3 (11.1)	7 (6.5)	
Patients with cytomegalovirus prophylaxis, n (%)	0	0	1.000
Patients with cytomegalovirus infection			
Between LT and D_P	15 (55.6)	31 (28.7)	**0.012**
During 1 year before D_P, n (%)	11 (40.7)	12 (11.1)	**0.001**
Patients with rejection episode(s) before D_P, n (%)	11 (40.7)	32 (29.6)	0.355
Patients with biopsy-proven rejection, n (%)	5 (18.5)	10 (9.3)	0.180
Treatment of rejection, n (%)			
Steroid pulse therapy[†]	11 (40.7)	32 (29.6)	0.355
Anti-thymocyte globulin	0	1 (0.9)	1.000
Plasma exchange	1 (3.7)	2 (1.9)	0.491
Patients with steroid pulse therapy before D_P, n (%)	14 (51.9)	32 (29.6)	**0.041**
Patients with rejection episode(s) during 2 yr before D_P, n (%)	10 (37.0)	14 (13.0)	**0.009**
Patients with biopsy-proven rejection, n (%)	5 (18.5)	4 (3.7)	**0.016**
Patients with steroid pulse therapy during 2 yr before D_P, n (%)	13 (48.1)	14 (13.0)	**<0.001**

Data are median [interquartile range], unless otherwise indicated.
PCP, *Pneumocystis jirovecii* pneumonia; LT, liver transplantation; D, donor; R, recipient; D_P, date of PCP diagnosis in the case group and date of chest imaging without pneumonia or negative PCP PCR test in the control group; BPR, biopsy-proven
[†] Methylprednisolone 500 mg/day for ≥2 days.

CMV and rejection-related parameters

Parameters related to CMV and rejection after LT are presented in Table 2. Distributions of D/R serologic CMV status before LT were similar between groups, with D(+)/R(+) being the most frequent status in both groups (PCP cases, 87.0%; controls, 81.5%). D(+)/R(−) was not observed in either group, and no patient received CMV prophylaxis. The rate of CMV infection during the total time between LT and D_P, as well as annually up to 5 years before D_P, were significantly higher in PCP cases than in controls (Supplementary Table 4). In the year before D_P, the difference was most significant (40.7% vs 11.1%, $P = 0.001$).

During the total time between LT and D_P, the proportion of patients with rejection episode(s) (total or biopsy-proven) and the treatments for these episodes did not differ between groups. However, steroid pulse therapy was more frequently performed in PCP cases than in controls (51.9% vs 29.6%, $P = 0.041$) during the same period. The proportion of patients receiving steroid pulse therapy during the look-back periods annually up to 5 years before D_P are summarized in Supplementary Table 4.

In the 2 years before D_P, the proportion of patients with rejection episodes and the rate of steroid pulse therapy were both higher in PCP cases than in controls (37.0% vs 13.0%, $P = 0.009$; 48.1% vs 13.0%, $P < 0.001$). In three cases among PCP recipients, steroid pulse therapy was administered under suspicion of rejection, but the final pathological diagnosis did not confirm the rejection.

Oncologic and biologic features in LT recipients with or without P. jirovecii pneumonia

Because more than 50% of patients in both the PCP and control groups had HCC at LT, the data regarding HCC recurrence between LT and D_P with look-back windows annually up to 5 years before D_P were recorded (Table 3, Supplementary Table 4). The rate of HCC recurrence was significantly higher in the PCP group than in the control group during the 3 years preceding D_P (25.9% vs 3.5%, $P = 0.001$).

Average blood cell counts during the 180-day look-back window before D_P revealed significantly lower lymphocyte counts in PCP cases than in controls: 600.0 cells/mm^3 (IQR, 373.9-820.0) versus 1011.7 (IQR, 734.1-1320.1) cells/mm^3 ($P < 0.001$). The average lymphocyte count was <750 cells/mm^3 in the 180 days before D_P in 70.4% of PCP cases, whereas only 27.8% of 108 controls ($P < 0.001$). During the same period, the average AST and ALT levels were higher in PCP cases than in controls; although, the median AST and ALT values in both groups were below the upper limit of normal (46 IU/l at our institution) (Table 3).

Multivariate analyses

In multivariate analyses, including covariates with $P < 0.05$ in the univariate analyses, age at LT ≥65 years (odds ratio [OR], 10.564; 95% confidence interval [CI], 2.107-52.969; $P = 0.004$), CMV infection in the year before D_P (OR, 5.566; 95% CI 1.676-18.486; $P = 0.005$), steroid pulse therapy in the 2 years before D_P (OR, 5.420; 95% CI, 1.717-17.106; $P = 0.004$), HCC recurrence in the 3 years before D_P (OR, 16.900; 95% CI, 3.247-87.956; $P = 0.001$), and average lymphocyte count <750 cells/mm^3 in the 180 days before D_P (OR, 3.888; 95% CI, 1.230-12.288; $P = 0.021$) were independently associated with PCP (Table 4).

Discussion

With universal PCP prophylaxis recommended for SOT recipients in the current guidelines [3], late-onset PCP after the postprophylaxis period has become an area of interest, highlighting the necessity for more focused interventions. This case-control study aimed to investigate the risk factors for late-onset PCP in 27 LT recipients who developed the condition to identify the characteristics of recipients at higher risk of PCP and enable more individualized prophylaxis. We identified age ≥65 years at LT, CMV infection, steroid pulse therapy, lymphocytopenia (average count <750 cells/mm^3 over 6 months), and HCC recurrence as significant independent predictors of late-onset PCP after LT. This study also analyzed these factors based on specific look-back windows, which identified the high-risk period for each factor, providing useful information to guide the duration of prophylaxis.

The incidence of PCP was found to be 6.36 cases/1000 patient-years during the 4247 patient-years of follow-up, with no break-

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

Table 3
Oncologic and biologic features associated with PCP infection after LT.

	PCP-positive (n = 27)	PCP-negative (n=108)	*P*-value
Hepatocellular carcinoma at LT, n (%)	16 (59.3)	58 (53.7)	0.669
Hepatocellular carcinoma recurrence during 3 years before D_P, n (%)	7 (25.9)	4 (3.7)	**0.001**
Average cell count during 180 days before D_P (cells/mm^3)			
White blood cells	6918 (4652-9151)	5928 (4835-6862)	0.099
Neutrophils	4961 (3463-6200)	4239 (2834-5552)	0.204
Monocytes	357.3 (280.0-546.6)	417.1 (325.5-546.0)	0.551
Lymphocytes	600.0 (373.9-820.0)	1011.7 (734.1-1320.1)	**<0.001**
Patients with average lymphocyte count <750 cells/mm^3 during 180 days before D_P, n (%)	19 (70.4)	30 (27.8)	**<0.001**
Average laboratory values during 180 days before D_P			
Aspartate aminotransferase (IU/l)	32.1 (20.0-79.3)	23.0 (19.2-30.0)	**0.021**
Alanine aminotransferase (IU/l)	31.8 (20.1-55.7)	20.1 (13.5-32.2)	**0.007**
Total bilirubin (mg/dl)	0.89 (0.63-1.15)	0.82 (0.63-1.15)	0.367
Creatinine (mg/dl)	1.09 (0.69-1.64)	0.98 (0.81-1.14)	0.274
International normalized ratio	0.99 (0.91-1.21)	0.97 (0.92 -1.01)	0.436

Data are median (interquartile), unless otherwise indicated.
D_P, date of PCP diagnosis in the case group and date of chest imaging without pneumonia or negative PCP PCR test in the control group; LT, liver transplantation; PCP, *Pneumocystis jirovecii* pneumonia.

Table 4
Multivariate analysis of risk factors for developing late-onset PCP after LT.

	Adjusted odds ratio	95% confidence interval	*P*-value
Age at LT ≥65 years	13.305	2.507-70.618	**0.002**
Cytomegalovirus infection during 1 year before D_P	5.390	1.602-18.132	**0.006**
Steroid pulse therapy during 2 years before D_P	6.564	1.984-21.719	**0.002**
Hepatocellular carcinoma recurrence during 3 years before D_P	18.180	3.420-96.636	**0.001**
Average lymphocyte count <750 cells/mm^3 during 180 days before D_P	3.758	1.176-12.013	**0.026**

D_P, date of PCP diagnosis in the case group and date of chest imaging without pneumonia or negative PCP PCR test in the control group; LT, liver transplantation; PCP, *Pneumocystis jirovecii* pneumonia.

through infections during the PCP prophylaxis. Unlike previous reports of higher incidence between 12 and 24 months after transplantation in kidney transplant recipients [1,4,12], PCP in LT recipients increased steadily at a rate of approximately 2.2 cases per year after 6 months post-LT. In our study of only LT recipients, the post-transplant duration itself was not a risk factor; PCP occurrence was influenced by the presence of other identified risk factors. Despite prophylaxis, PCP remained a lethal disease in our cohort, with a mortality rate of 37% (Supplementary Table 1).

As reported in previous studies [1,4,12], our analyses revealed age as an independent risk factor for PCP, with a threshold of 65 years at LT. Consistent with previous findings, CMV infection was also identified as a significant risk factor for PCP [1,2,4,5,13–15]. In both groups, most CMV serologic combinations were D+/R+, indicating that the detected CMV infections were likely due to CMV reactivation or superinfection with donor-transmitted CMV. Particularly in CMV-seropositive recipients with pre-existing CMV-specific immunity, CMV infection may reflect impaired CMV-specific immunity resulting from a high degree of immunosuppression. Although lymphocytopenia and the resulting depletion of CMV-specific T cells increase the likelihood of CMV infection [16], our study identified lymphocytopenia and CMV infection as independent risk factors for PCP. This suggests that the immunomodulatory effects of CMV contribute to the development of PCP, similar to the previously reported association between CMV and opportunistic infections [17,18].

Although the percentage of patients with CMV infection was higher in the PCP group throughout the LT D_P period, it is noteworthy that this association was most obvious in the year before D_P, with 40.7% of patients with PCP having a CMV infection during that period. Iriart *et al.* also identified CMV infection in the preceding year as an independent risk factor for PCP in SOT recipients, with 51.5% of PCP patients having a CMV infection during that period [4]. Considering that current guidelines recommend prolonged PCP prophylaxis for patients with chronic CMV infection [3], our study indirectly suggests that patients with CMV infection may have an increased risk of PCP for at least 1 year after the infection, highlighting the need for clinicians to reinitiate PCP prophylaxis during the treatment and follow-up of CMV infection.

Previous studies have reported graft rejection [2,5,8,14,15] and steroid pulse therapies [12] as potential risk factors for PCP after SOT, primarily in kidney transplant recipients. Our study also found an association between these factors and PCP; although, only steroid pulse therapy was identified as an independent risk factor in the multivariate analyses. Notably, we included all instances of steroid pulse therapy, including those given for suspected rejection that was later ruled out. In LT recipients, steroid pulse therapy may be initiated before or without biopsy confirmation of rejection due to the risk of bleeding from depressed liver function. The latter cases were found only in the PCP group, which may explain why steroid pulse therapy was a more important risk factor than rejection episodes or allograft dysfunction itself. This finding suggests that PCP prophylaxis should be used in LT recipients whenever steroid pulse therapy is administered, irrespective of rejection diagnosis. Our analyses also revealed that the risk of PCP from steroid pulse therapy can persist as long as 2 years, derived by the multivariate analyses including look-back periods up to 5 years.

We found lymphocytopenia to be an independent risk factor for PCP, as reported in several previous studies [4,6,12,19]. Corticosteroid-induced immunosuppression is known to cause depletion of pulmonary T helper cells, which is a mechanism underlying the development of PCP [20]. Moreover, global depletion of clusters of differentiation (CD)4+ and CD8+ T lymphocytes in the years before PCP diagnosis has been observed in kidney transplant recipients [12]. To eliminate the effects of PCP infection on lymphocyte counts, we evaluated the average blood cell counts in the 180 days before PCP rather than at D_P. The average total blood lymphocyte count in the 180 days preceding PCP was 600 cells/mm^3 in the PCP group, which is consistent with the finding of 615 cells/mm^3 in the 50 days before PCP reported by Iriat *et al.*

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

[4]. These results indicate that lymphocyte counts can be a predictive marker of PCP and used to guide the selection of higher risk patients who require reinitiation of PCP prophylaxis.

That high-dose steroid therapy and lymphocytopenia contribute to late-onset PCP may also explain the emerging reports of PCP arising in patients with COVID-19. In accordance with the findings in this study, extended glucocorticoid courses used to treat COVID-19 and severe COVID-19-related lymphocytopenia have been suggested as factors that increase susceptibility to PCP after COVID-19 infection [21]. Although there were no preceding or combined COVID-19 infections in this study because only six PCP cases occurred during the pandemic, severe COVID-19 infection should also be taken into account as a condition for PCP prophylaxis in the context of immunomodulation during the treatment course.

In our study, everolimus use and higher trough level at D_P were associated with late-onset PCP, although not significantly in multivariate analysis. Recent studies reported mTORi administration as a risk factor for PCP after SOT [12,22]. Everolimus has mostly replaced mycophenolate mofetil in our institution's maintenance immunosuppressant combination therapy for preventing HCC recurrence after HCC-related LT [11,23]. Because HCC recurrence was a strong risk factor for PCP in the final multivariate analyses, the effects of everolimus may have been attenuated. Further investigation is needed to explore the association between mTORi and PCP, given the widespread use of mTORi in LT recipients with HCC.

This is the first study to focus on the malignant status of post-LT recipients in the context of PCP development. LT recipients differ from other SOT recipients in that >30% of LT recipients have cancer. Currently, approximately 30% of LT recipients in the United States have HCC [24], and nearly 50% have HCC in Asian countries, mostly arising from chronic hepatitis B virus infection. Although LT is considered the best treatment for localized HCC, recurrence occurs in 6-18% of recipients [25]. In our study, 16 (59.3%) of our 27 patients with PCP had HCC at the time of LT, and seven (43.8%) of these 16 patients experienced HCC recurrence in the 3 years before PCP diagnosis.

After HCC-related LT, treatments for HCC recurrence (*e.g.*, radiotherapy, chemotherapy) can also have systemic immune effects. Although radiotherapy can upregulate the immune response against tumor, it can also lead to depletion of $CD4^+$ T cells [26,27], which increases susceptibility to opportunistic infections. In a previous study of PCP in patients with solid tumors and lymphomas, radiotherapy preceded the development of PCP in 22 (85%) patients [28]. In another study of patients with solid tumors, 10 (71%) of 14 patients with PCP who did not receive prolonged moderate-to-high corticosteroids were noted to have undergone chemotherapy within 90 days before PCP diagnosis [29]. Recent guidelines recommend lifelong PCP prophylaxis for patients with solid tumors receiving persistent immunosuppression [30]. Confounded by immunosuppressant use and treatments for recurrence, LT recipients with HCC recurrence are at high-risk for PCP development, which should be considered an indication for reinitiating TMP/SMX. In multivariate analyses including HCC recurrence with various look-back periods up to 5 years, the 3-year period remained as the independent risk factor, suggesting that PCP prophylaxis should be reinitiated for at least 3 years after HCC recurrence.

This study has limitations that warrant consideration. One is its retrospective, case-control design, which was necessary due to the rarity of PCP in LT recipients, similar to most studies on PCP after SOT. Next, all PCP cases in this study were probable PCP, not proven PCP based on the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium definition. Immunofluorescent staining has not been conducted in our center for PCP diagnosis, and conventional microscopy was performed in cytologic specimens for only six of 27 PCP cases (bronchoalveolar lavage in five and sputum in one). In these cases, *Pneumocystis* was not detected, probably due to suboptimal sensitivity of conventional microscopy. Routine cytologic studies and adaption of immunofluorescent staining, as well as quantitative PCR, would enable more accurate diagnosis in the future studies. Finally, despite identifying the risk factors for PCP in LT recipients, the rarity of the condition and the low number of patients number ultimately prevented us from establishing composite criteria for PCP prophylaxis based on patient stratification according to the combination(s) of the identified risk factors.

Nevertheless, this study had several strengths. This study included the highest number of PCP cases in an LT recipient cohort that received the same 6-12 months of PCP prophylaxis [1,2,4,6–9]. The homogeneity of our cohort was also refined by including only LT recipients. Furthermore, we checked all radiologic and laboratory data (CXR, chest CT, PCP PCR) available near the D_P to select controls without pneumonia, and the designation of four controls for each case improved the statistical power.

In conclusion, PCP is a life-threatening complication that can occur even after routine PCP prophylaxis in LT recipients. Targeted prophylaxis, considering the risk factors identified in this study, could help guide more effective prevention of this potentially lethal infection in LT recipients.

Funding

This study was supported by a new faculty research seed money grant of Yonsei University College of Medicine for 2022 (2022-32-0063).

Ethical approval

The study was approved by institutional review board of Yonsei University College of Medicine (4-2021-0536). The current study was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Declaration of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Eun-Ki Min: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Juhan Lee:** Data curation, Formal analysis, Investigation. **Su Jin Jeong:** Formal analysis, Methodology. **Deok-Gie Kim:** Formal analysis, Methodology. **Seung Hyuk Yim:** Data curation. **Mun Chae Choi:** Data curation. **Dong Jin Joo:** Data curation. **Myoung Soo Kim:** Data curation. **Jae Geun Lee:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.387.

References

[1] Neofytos D, Hirzel C, Boely E, Lecompte T, Khanna N, Mueller NJ, et al. Pneumocystis jirovecii pneumonia in solid organ transplant recipients: a descriptive analysis for the Swiss Transplant Cohort. *Transpl Infect Dis* 2018;**20**:e12984. doi:10.1111/tid.12984.

[2] Hosseini-Moghaddam SM, Shokoohi M, Singh G, Dufresne SF, Boucher A, Jevnikar A, et al. A multicenter case-control study of the effect of acute rejection and cytomegalovirus infection on pneumocystis pneumonia in solid organ transplant recipients. *Clin Infect Dis* 2019;**68**:1320–6. doi:10.1093/cid/ciy682.

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.

[3] Fishman JA, Gans H. AST Infectious Diseases Community of Practice. Pneumocystis jiroveci in solid organ transplantation: guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant* 2019;**33**:e13587. doi:10.1111/ctr.13587.
[4] Iriart X, Challan Belval T, Fillaux J, Esposito L, Lavergne RA, Cardeau-Desangles I, et al. Risk factors of Pneumocystis pneumonia in solid organ recipients in the era of the common use of posttransplantation prophylaxis. *Am J Transplant* 2015;**15**:190–9. doi:10.1111/ajt.12947.
[5] Hosseini-Moghaddam SM, Shokoohi M, Singh G, Nagpal AD, Jevnikar AM. Six-month risk of Pneumocystis pneumonia following acute cellular rejection: a case-control study in solid organ transplant recipients. *Clin Transplant* 2021;**35**:e14322. doi:10.1111/ctr.14322.
[6] Werbel WA, Ison MG, Angarone MP, Yang A, Stosor V. Lymphopenia is associated with late onset Pneumocystis jirovecii pneumonia in solid organ transplantation. *Transpl Infect Dis* 2018;**20**:e12876. doi:10.1111/tid.12876.
[7] Rostved AA, Sassi M, Kurtzhals JA, Sørensen SS, Rasmussen A, Ross C, et al. Outbreak of pneumocystis pneumonia in renal and liver transplant patients caused by genotypically distinct strains of Pneumocystis jirovecii. *Transplantation* 2013;**96**:834–42. doi:10.1097/TP.0b013e3182a1618c.
[8] Choi YI, Hwang S, Park GC, Namgoong JM, Jung DH, Song GW, et al. Clinical outcomes of pneumocystis carinii pneumonia in adult liver transplant recipients. *Transplant Proc* 2013;**45**:3057–60. doi:10.1016/j.transproceed.2013.08.074.
[9] Miguel Montanes R, Elkrief L, Hajage D, Houssel P, Fantin B, Francoz C, et al. An outbreak of Pneumocytis jirovecii pneumonia among liver transplant recipients. *Transpl Infect Dis* 2018;**20**:e12956. doi:10.1111/tid.12956.
[10] Lagrou K, Chen S, Masur H, Viscoli C, Decker CF, Pagano L, et al. Pneumocystis jirovecii disease: basis for the revised EORTC/MSGERC invasive fungal disease definitions in individuals without human immunodeficiency virus. *Clin Infect Dis* 2021;**72**:S114–20. doi:10.1093/cid/ciaa1805.
[11] Kang I, Lee JG, Choi SH, Kim HJ, Han DH, Choi GH, et al. Impact of everolimus on survival after liver transplantation for hepatocellular carcinoma. *Clin Mol Hepatol* 2021;**27**:589–602. doi:10.3350/cmh.2021.0038.
[12] Kaminski H, Belliere J, Burguet L, Del Bello A, Taton B, Poirot-Mazères S, et al. Identification of predictive markers and outcomes of late-onset Pneumocystis jirovecii pneumonia in kidney transplant recipients. *Clin Infect Dis* 2021;**73**:e1456–63. doi:10.1093/cid/ciaa1611.
[13] Faure E, Lionet A, Kipnis E, Noël C, Hazzan M. Risk factors for Pneumocystis pneumonia after the first 6 months following renal transplantation. *Transpl Infect Dis* 2017;**19**:e12735. doi:10.1111/tid.12735.
[14] Arend SM, Westendorp RG, Kroon FP, van't Wout JW, Vandenbroucke JP, van Es LA, et al. Rejection treatment and cytomegalovirus infection as risk factors for pneumocystis carinii pneumonia in renal transplant recipients. *Clin Infect Dis* 1996;**22**:920–5. doi:10.1093/clinids/22.6.920.
[15] Lee SH, Huh KH, Joo DJ, Kim MS, Kim SI, Lee J, et al. Risk factors for Pneumocystis jirovecii pneumonia (PJP) in kidney transplantation recipients. *Sci Rep* 2017;**7**:1571. doi:10.1038/s41598-017-01818-w.
[16] Fernández-Ruiz M, Giménez E, Vinuesa V, Ruiz-Merlo T, Parra P, Amat P, et al. Regular monitoring of cytomegalovirus-specific cell-mediated immunity in intermediate-risk kidney transplant recipients: predictive value of the immediate post-transplant assessment. *Clin Microbiol Infect* 2019;**25**:381.e1–381.e10. doi:10.1016/j.cmi.2018.05.010.
[17] Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant* 2019;**33**:e13512. doi:10.1111/ctr.13512.
[18] Razonable RR. Cytomegalovirus infection after liver transplantation: current concepts and challenges. *World J Gastroenterol* 2008;**14**:4849–60. doi:10.3748/wjg.14.4849.
[19] Permpalung N, Kittipibul V, Mekraksakit P, Rattanawong P, Nematollahi S, Zhang SX, et al. A comprehensive evaluation of risk factors for Pneumocystis jirovecii pneumonia in adult solid organ transplant recipients: a systematic review and meta-analysis. *Transplantation* 2021;**105**:2291–306. doi:10.1097/TP.0000000000003576.
[20] Walzer PD, LaBine M, Redington TJ, Cushion MT. Lymphocyte changes during chronic administration of and withdrawal from corticosteroids: relation to pneumocystis carinii pneumonia. *J Immunol* 1984;**133**:2502–8. doi:10.4049/jimmunol.133.5.2502.
[21] Amstutz P, Bahr NC, Snyder K, Shoemaker DM. Pneumocystis jirovecii infections among COVID-19 patients: a case series and literature review. *Open Forum Infect Dis* 2023;**10** ofad043. doi:10.1093/ofid/ofad043.
[22] Ghadimi M, Mohammadpour Z, Dashti-Khavidaki S, Milajerdi A. m-TOR inhibitors and risk of Pneumocystis pneumonia after solid organ transplantation: a systematic review and meta-analysis. *Eur J Clin Pharmacol* 2019;**75**:1471–80. doi:10.1007/s00228-019-02730-0.
[23] Grigg SE, Sarri GL, Gow PJ, Yeomans ND. Systematic review with meta-analysis: sirolimus- or everolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2019;**49**:1260–73. doi:10.1111/apt.15253.
[24] Puigvehí M, Hashim D, Haber PK, Dinani A, Schiano TD, Asgharpour A, et al. Liver transplant for hepatocellular carcinoma in the United States: evolving trends over the last three decades. *Am J Transplant* 2020;**20**:220–30. doi:10.1111/ajt.15576.
[25] Berenguer M, Burra P, Ghobrial M, Hibi T, Metselaar H, Sapisochin G, et al. Posttransplant management of recipients undergoing liver transplantation for hepatocellular carcinoma. Working Group Report From the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020;**104**:1143–9. doi:10.1097/TP.0000000000003196.
[26] Ellsworth S, Balmanoukian A, Kos F, Nirschl CJ, Nirschl TR, Grossman SA, et al. Sustained CD4+ T cell-driven lymphopenia without a compensatory IL-7/IL-15 response among high-grade glioma patients treated with radiation and temozolomide. *Oncoimmunology* 2014;**3**:e27357. doi:10.4161/onci.27357.
[27] Grassberger C, Ellsworth SG, Wilks MQ, Keane FK, Loeffler JS. Assessing the interactions between radiotherapy and antitumour immunity. *Nat Rev Clin Oncol* 2019;**16**:729–45. doi:10.1038/s41571-019-0238-9.
[28] Barbounis V, Aperis G, Gambletsas E, Koumakis G, Demiris M, Vassilomanolakis M, et al. Pneumocystis carinii pneumonia in patients with solid tumors and lymphomas: predisposing factors and outcome. *Anticancer Res* 2005;**25**:651–5.
[29] Takeda K, Harada S, Hayama B, Hoashi K, Enokida T, Sasaki T, et al. Clinical characteristics and risk factors associated with Pneumocystis jirovecii infection in patients with solid tumors: study of thirteen-year medical records of a large cancer center. *BMC Cancer* 2021;**21**:987. doi:10.1186/s12885-021-08727-2.
[30] Cooley L, Dendle C, Wolf J, Teh BW, Chen SC, Boutlis C, et al. Consensus guidelines for diagnosis, prophylaxis and management of Pneumocystis jirovecii pneumonia in patients with haematological and solid malignancies, 2014. *Intern Med J* 2014;**44**:1350–63. doi:10.1111/imj.12599.

Downloaded for Anonymous User (n/a) at Yonsei University College of Medicine from ClinicalKey.com by Elsevier on July 07, 2023. For personal use only. No other uses without permission. Copyright ©2023. Elsevier Inc. All rights reserved.