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CD4$^+$ T cell lymphopenia predicts mortality from *Pneumocystis* pneumonia in kidney transplant patients

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Abstract

Background: *Pneumocystis jirovecii* pneumonia (PcP) remains a life-threatening opportunistic infection after solid organ transplantation, even in the era of Pneumocystis prophylaxis. The association between risk of developing PcP and low CD4$^+$ T cell counts has been well established. However, it is unknown whether lymphopenia in the context of post-renal transplant PcP increases the risk of mortality.

Methods: We carried out a retrospective analysis of a cohort of kidney transplant patients with PcP (n = 49) to determine the risk factors for mortality associated with PcP. We correlated clinical and demographic data with the outcome of the disease. For CD4$^+$ T cell counts, we used the Wilcoxon rank sum test for in-hospital mortality and a Cox proportional-hazards regression model for 60-day mortality.

Results: In univariate analyses, high CRP, high neutrophils, CD4$^+$ T cell lymphopenia, mechanical ventilation, and high acute kidney injury network stage were associated with in-hospital mortality following presentation with PcP. In a receiver-operator characteristic (ROC) analysis, an optimum cutoff of ≤200 CD4$^+$ T cells/μL predicted in-hospital mortality, CD4$^+$ T cell lymphopenia remained a risk factor in a Cox regression model.

Conclusions: Low CD4$^+$ T cell count in kidney transplant recipients is a biomarker for disease severity and a risk factor for in-hospital mortality following presentation with PcP.

KEYWORDS

clinical immunology, immunosuppression, infection, lymphocytes, mortality risk, pneumocystis, renal transplantation, risk factors, survival, transplantation

1 | INTRODUCTION

Kidney transplantation is the treatment of choice for most patients with end-stage renal disease, improving both mortality and quality of life in comparison with dialysis.[1,2] Improvements in short-term allograft loss in the first year post-transplantation, attributable to advances in modern immunosuppression,[3] have not been matched by long-term allograft outcomes, which remain poor.[4] Both cardiovascular mortality and death from infection remain among the top causes of long-term allograft loss.[5]

Pneumocystis jirovecii pneumonia (PcP) is a particularly life-threatening opportunistic infection after solid organ transplantation, even in the era of pneumocystis prophylaxis. PcP is caused by the fungus *Pneumocystis jirovecii* (*P jirovecii*) in humans. Prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX) is highly effective and can reduce the incidence of PcP in the post-transplant period.[6] Although routine prophylaxis has shifted the peak incidence of PcP to the second post-transplant year, mortality from PcP remains high.[7,8]

$CD4^+$ T cell counts play a pivotal role in the decision for prophylaxis of PcP.[9,10] The importance of $CD4^+$ T cells in the clearance of *P jirovecii* has clearly been experimentally demonstrated in the context of animal models of both primary (genetic) as well as acquired immunodeficiency.[11-13] Thus, loss of $CD4^+$ T cells in HIV disease causes susceptibility to PcP.[14] Several retrospective studies in recent years have shown that a low $CD4^+$ T cell count is also an important risk factor for the *development* of PcP in kidney transplant recipients (see Table S1) among other risk factors.[7,15,16]

In HIV patients with PcP, the degree of $CD4^+$ T cell lymphopenia is reciprocally associated with elevated risk of mortality.[17-20] However, it is not currently known whether outcomes from PcP infection, particularly mortality, in kidney transplant recipients are associated with $CD4^+$ T cell count. We carried out a retrospective observational study of PcP infection in kidney transplant recipients presenting with PcP over a 12-year period at our center in order to determine whether the $CD4^+$ T cell count has any associations with outcomes from PcP.

2 | MATERIALS AND METHODS

2.1 | Study design and population

We carried out a retrospective cohort study of renal transplant patients with PcP hospitalized between the years 2005 and 2016, inclusive, in our tertiary care center at University Hospital Frankfurt. Cases were identified based on the ICD-10 codes B59 ("Pneumocystosis") and Z94.0 ("Kidney transplant status") in the hospital information system. Simultaneous kidney-pancreas or liver-kidney transplant recipients were excluded to avoid raising the complexity of an already heterogeneous population of patients. The study was approved by an independent ethics committee (340/17) and was conducted in accordance with the Helsinki Declaration. A waiver of informed consent was approved.

In cases of suspected PcP, a chest computed tomography (CT) scan and a bronchoscopy with bronchoalveolar lavage (BAL) were performed on admission. The BAL was always evaluated with a microbiological culture, Grocott stain, and CMV-PCR. Upon clinical suspicion, we additionally screened for other Herpesviridae. Diagnosis of PcP was based on clinical plus radiographic signs and a positive Grocott stain in the BAL. Additionally, a *P jirovecii* polymerase chain reaction (PCR) from the BAL was frequently noted. It is important to note that the Grocott stain is the most sensitive/specific assay for *P jirovecii* infection and is considered the gold standard.[21,22] Therefore, Grocott stain positive, PCR negative, patients with clinical suspicion of PcP were diagnosed as having PcP. Blood lymphocyte subsets ($CD4^+$ and $CD8^+$ T cells) and CMV-PCR were routinely measured in all patients that presented with infection on admission. Lymphocyte subsets were repeatedly measured during the course of the hospitalization to detect immune system decline or recovery. All patients were treated for PcP with high dose TMP/SMX. The dose was adjusted to the estimated glomerular filtration rate in mL/min/1.73 m^2 according to the Modification of Diet in Renal Disease (MDRD) formula.[23]

All patients received anti-CD25 antibody basiliximab (BAS) for non-sensitized patients and anti-thymocyte globulin (ATG) for sensitized patients as induction immunosuppression together with triple therapy with a calcineurin inhibitor (CNI), mycophenolate mofetil (MMF), and glucocorticosteroids (GC) for maintenance. The protocol of induction with rATG (Fresenius) was as follows: 2-3 mg/kg body weight on day 0 (6 hours post-transplantation). The dose of ATG was repeated during the first 7-10 days if $CD4^+$ T cell count was greater than 40/μL. The choice between tacrolimus (TAC) and ciclosporin A (CSA) was based on individual patient risk profiles. Sirolimus (SRL) was also used as an alternative maintenance agent in some patients. During infectious episodes, we paused or reduced the immunosuppression based on clinical appraisal. Our center has used PcP prophylaxis routinely with TMP/SMX for 6 months after kidney transplantation since 2007. In the years 2004-2006, PcP prophylaxis was not yet routinely used. This strategy was implemented following updated guidelines in 2007. After that period, TMP/SMX prophylaxis was only continued or reinitiated if an episode of acute rejection was treated and/or immunosuppression was increased. Early-onset disease refers to the onset within 12 months, and late onset refers to the onset after 12 months of transplantation.[24]

CMV prophylaxis with oral valganciclovir was given for 3 months to patients with a moderate risk (D^+/R^+ and $D^-/R+$), but for 6 months in CMV high-risk patients (D^+/R^-). The prophylaxis for patients with moderate risk was established in the year 2010. In the subgroup of patients with moderate risk treated with rATG, this was already established before. In low-risk patients (D^-/R^-), we employed a preemptive strategy. Post-transplantation, some patients received adjunct steroids at the discretion of the responsible physician. Adjunctive steroid therapy was defined as 20-100 mg prednisolone (or equivalent) per day for at least five consecutive days. Steroids were not prescribed by protocol at the time and were dependent on clinical response and individual clinician's decision-making.

2.2 | Clinical and epidemiological data

The day of admission to hospital for PcP was defined as D0. The baseline patient characteristics and clinical data were collected at D0. Additional clinical parameters were collected during hospital stay. Rejection episodes were noted between transplantation and D0. Rejection episodes were usually diagnosed by biopsy. Occasionally, a clinical diagnosis of acute rejection was reached in the setting of rising creatinine when biopsy was not possible or contraindicated and the patient responded to empirical steroid therapy.

Total lymphocytes, $CD4^+/CD8^+$ T cell counts, and neutrophil counts were noted at their lowest in-hospital level. $CD4^+/CD8^+$ T cell counts were measured by flow cytometry analysis in our routine clinical laboratory using BD Simultest CD4/CD8 kit (CD4-FITC, CD8-PE) in erythrocyte-lysed whole blood.

Bacterial or fungal infection was assumed if pathogens were detected in cultured specimens independent of the amount of pathogen. Sites of infection were urinary, bloodstream, bronchoalveolar, and others (stool, sputum, lymphocele puncture, and tissue specimens). Infected central venous lines were evaluated as bloodstream infections. BAL was additionally evaluated for human Herpesviridae (CMV, EBV, HHV6, HSV, VZV) based on the level of clinical suspicion. CMV reactivation (including primary infection) was defined by a positive CMV real-time PCR (>200 international units per milliliter [IU/mL]) during hospital stay.

Closely resembling the revised definitions from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group, we slightly modified the definitions for invasive candidiasis, adapted to routine clinical care. In accordance with the updated definitions,[25] invasive candidiasis was defined as histopathologic examination of a specimen obtained from a normally sterile site or blood culture that yields yeast and additionally a positive culture from other sites that are accompanied by related infectious symptoms (see as well previous definitions Segal BH *et al*, Clin Infect Dis 2008[26]). Colonizations were excluded from further evaluation.

Multiple drug resistance (MDR) analyses were obtained from all mentioned sites. Screening smear results were not included. Multiple drug-resistant gram-negative bacteria with carbapenem resistance and multiple drug-resistant Staphylococcus aureus (MRSA) were reported.

In this study, we defined acute kidney injury (AKI) according to the Acute Kidney Injury Network (AKIN) criteria based on changes in serum creatinine level.[27]

In our picture archiving and communication system (PACS), 41 of 49 CT scans were available. The other 8 patients had CT scans in a secondary care center before referral to our tertiary care hospital. In such circumstances at times, only a written report of the CT scan was transmitted. All 41 of the available scans were viewed at standard lung windows and analyzed by two radiologists with expertise in the field of chest diagnosis, who were blinded to clinical information, such as therapy or primary disease. In case of discrepancy, decisions were reached by consensus. CTs were reported according to pre-established criteria. "Classical PcP" (type 1) was characterized by bilateral ground-glass opacities sparing peripheral and subpleural zones.[28]

2.3 | Literature review

Literature review (Table S1) was conducted on July 29, 2019, by searching for "pneumocystis AND (lymphocytopenia OR lymphopenia OR lymphocytes OR CD4) AND (kidney transplantation OR solid organ)" in the MEDLINE database via PubMed. We excluded studies where risk factors were not separately itemized for solid organ transplant recipients in the analysis.

2.4 | Statistical analyses

Data were analyzed using R statistical language and Python package statsmodels.[29,30] Aggregated data were reported as median and interquartile range (IQR) [25%; 75%] for non-parametric data and mean ± SD for parametric data. For non-parametric data, we used Mann-Whitney U test for a two-group comparison by interval scaled data and Fisher's exact test for a multi-group comparison by nominal or ordinal scaled data. For parametric data, we used t test for two-group comparisons of interval scaled data. For a comparison of two nominal scaled variables, we used chi-square test or Fisher's exact test. CD4+ T cell counts were correlated with the in-hospital mortality by using the Wilcoxon rank sum test. For all variables, the test used is indicated in the result tables. The effect size Cohen's d $\left(\frac{\text{mean difference}}{\text{standard deivation}}\right)$, a measure of the magnitude of the effect of a variable, was additionally calculated for the CD4+ T cell count.[31]

Sensitivity/specificity receiver operating characteristic (ROC) curves were used to evaluate the performance of the significant results of the univariate analysis to predict in-hospital mortality during PcP. We calculated Youden's J statistic (Youden's index; J = sensitivity + specificity − 1) for every point on each ROC curve and selected the points with the maximum Youden's index as the optimal cutoff thresholds for predicting outcome.[32] Additionally, Cox proportional-hazards regression to model the association of $CD4^+$ T cell counts with increased 60-day mortality was used. $CD4^+$ T cell count was included at its lowest and binarized according to the results of the ROC analysis. A survival plot to visualize the results was drawn.

A P-value < .05 was considered statistically significant throughout this study.

3 | RESULTS

3.1 | Baseline patient characteristics

A total of 49 kidney transplant patients with a diagnosis of PcP were identified between 2005 and 2016, in whom overall in-hospital mortality was 18% (n = 9). All had a positive Grocott BAL stain, the gold-standard test for *P jirovecii*. There were no significant differences

TABLE 1 Baseline patient characteristics and their association with outcome

	Outcome			
	Total	**Not deceased**	**Deceased**	***P*-value**
Total, n	49	40	9	
Sex, male (%)	36 (74)	28 (70)	8 (89)	.458[a]
Age, y[b]	56 (± 14.4)	54 (± 14.9)	64 (± 8.8)	.067[c]
Race, Caucasian (%)	48 (98)	39 (98)	9 (100)	1.000[a]
Weight in kg[d]	77.4 [66.2, 84.0]	77.4 [67.4, 82.1]	81.3 [66.4, 98.0]	.697[e]
Diabetes (%)	14 (29)	11 (28)	3 (33)	.702[f]
COPD (%)	3 (6)	3 (8)	0 (0)	1.000[f]
Coronary heart disease (%)	12 (24.5)	9 (22.5)	3 (33.3)	.669[f]
Heart failure (%)	3 (6.1)	3 (7.5)	0 (0.0)	1.000[f]
CMV episodes pre-admission (%)	11 (22.4)	10 (25.0)	1 (11.1)	.662[f]
Acute rejection episodes (%)	17 (35)	14 (35)	3 (33)	1.000[f]
Time from acute rejection to Dx, d[d]	152 [107, 321]	144 [68, 261]	366 [259, 376]	.115[e]
Induction therapy (%)				
BAS	34 (89.5)	28 (73.7)	6 (15.8)	1.000[f]
ATG	4 (10.5)	3 (7.9)	1 (2.6)	
Lowest lymphocyte (x1000/μL) pre-admission[d,g]	5.6 [4.4, 6.7]	5.5 [4.3, 6.6]	6.46 [5.3, 7.0]	.479[e]
CSA through level ng/mL pre-admission[d,g,h]	286 [174, 343]	212 [173, 315]	835 [566, 1105]	.154[e]
TAC through level ng/mL pre-admission[d,g,h]	9.5 [8.1, 12.0]	10.4 [8.6, 12.3]	7.2 [6.8, 7.7]	.085[e]
Immunosuppression (%)				
GC, CSA	1 (2)	1 (3)	0 (0)	.561[f]
GC, MMF	5 (10)	4 (10)	1 (11)	
GC, MMF, CSA	23 (47)	19 (48)	4 (44)	
GC, MMF, TAC	18 (37)	15 (38)	3 (33)	
GC, MMF, TAC, SRL	1 (2)	1 (3)	0 (0)	
GC, TAC	1 (2)	0 (0)	1 (11)	

Abbreviations: COPD, chronic obstructive pulmonary disease; CSA, ciclosporin A; Dx, days of PcP diagnosis; GC, glucocorticoids; MMF, mycophenolate mofetil; SRL, sirolimus; TAC, tacrolimus.

[a]Chi-square test.

[b]Mean (SD).

[c]Paired *t* test.

[d]Median interquartile range [25%; 75%].

[e]Wilcoxon rank sum test.

[f]Fisher's exact test.

[g]Pre-admission values were limited to 3 months before admission.

[h]Last value before admission.

in the baseline characteristics of the deceased *versus* non-deceased patients (Table 1), confirming homogeneity between the two groups. We did, however, note a tendency to older age among the deceased patients ($P = .067$). Immunosuppressive regimens consisted mainly of a combination of glucocorticoids (GC), mycophenolate mofetil (MMF), and a calcineurin inhibitor (CNI) in 41 of 49 patients (84%). Of those, 23 patients had ciclosporin A (CSA), and 18 patients had tacrolimus (TAC). One patient was on immunosuppression with GC and CSA, one on GC and TAC, and one was taking GC and sirolimus (SRL). Five patients were taking GC and MMF. The immunosuppressive regimen on admission was not significantly associated with the outcome.

In 2 of 20 reported rejection episodes, the treatment was initiated by clinical judgment and not biopsy-proven. The first case had clinical signs of rejection and an immediate initiation of a prednisone pulse and confirmation of rejection by biopsy after 3 weeks. The second case was judged to have humoral rejection clinically on the 5th post-op day and had initiation of plasmapheresis. A biopsy was judged as too risky because of thrombocytopenia. The other 18 of 20 patients had biopsy-proven rejection.

TABLE 2 Clinical parameters and their association with outcome

	Outcome			
	Overall	Not deceased	Deceased	p-value
Total, n	49	40	9	
Fever = 1 (%)	28 (58)	23 (58)	5 (63)	1.000[a]
Arterial pO2, mm Hg[b]	64 [54, 76]	66 [57, 78]	50 [48, 64]	.054[c]
Arterial pCO2, mm Hg[b]	31 [28, 33]	31 [29, 33]	27 [26, 31]	.120[c]
CRP, mg/dL[b]	16.0 [7.5, 24.3]	13.4 [6.7, 21.3]	30.1 [18.2, 39.7]	**.016**[c]
LDH, U/L[b]	355 [264, 450]	310 [262, 431]	494 [269, 560]	.124[c]
Hyperkalemia (%)[d]	24 (49.0)	18 (45.0)	6 (66.7)	.289[e]
Lymphocytes, (×1000/μL)[b]	0.50 [0.30, 0.70]	0.54 (0.34)	0.48 (0.32)	.604[c]
Neutrophils (×1000/μL)[b]	2.9 [1.3, 4.0]	2.1 [1.3, 3.3]	5.8 [4.5, 8.2]	**<.001**[c]
CD4/μL[b]	218 [127, 331]	243 [157, 359]	115 [72, 191]	**.009**[c]
CD8/μL[b]	181 [94, 270]	199 [115, 303]	107 [26, 151]	.054[c]
Time from KTx to D0, days[b]	200 [121, 776]	187 [120, 708]	400 [128, 776]	.502[c]
Hospitalization, d[b]	27 [22, 36]	28 [23, 34]	18 [13, 42]	.359[c]
Duration of TMP/SMX, d[b]	21 [20, 25]	22 [21, 26]	15 [9, 19]	**.001**[c]
Initial TMP/SMX dose, g/d[b]	5.8 [4.3, 5.8]	5.8 [4.3, 5.8]	4.3 [4.3, 5.8]	.703[c]
Adjunctive steroids (%)	24 (57)	19 (56)	5 (63)	1.000[a]
Mechanical ventilation (%)	12 (25)	4 (10)	8 (89)	**<.001**[a]
Creatinine baseline, mg/dL[b]	2.10 [1.60, 2.55]	2.00 [1.60, 2.70]	2.10 [1.73, 2.18]	.845[c]
AKI (%)	44 (90)	36 (90)	8 (89)	1.000[a]
AKIN stage (%)				
0	5 (10)	4 (10)	1 (11)	**.030**[c]
1	25 (51)	24 (60)	1 (11)	
2	0 (0)	0 (0)	0 (0)	
3	19 (39)	12 (30)	7 (78)	

Note: Bold indicates a *P*-value <.05.

Abbreviations: AKI, acute kidney injury defined by the AKIN criteria; AKIN, Acute Kidney Injury Network; D0, admission date; KTx, kidney transplantation.

[a]Chi-square test.

[b]Median interquartile range [25%; 75%].

[c]Wilcoxon rank sum test.

[d]Serum K greater than 5.5 mmol/L.

[e]Fisher's exact test.

3.2 | Clinical parameters and their association with outcome

The median time from transplantation to diagnosis of PcP was 200 days (median IQR [121, 776] days). Twenty-two of 49 patients (45%) were diagnosed with PcP less than 180 days after transplantation. In the years before 2007, there were 25 (93%) early-onset (within 12 months post-transplant) and 2 (7%) late-onset (after at least 12 months post-transplant) patients, whereas in the years 2007 and after there were 8 (36%) early-onset and 14 (64%) late-onset patients. The mortality within the late-onset group was higher than in the early-onset group (late onset: 5 patients [31% of all late onset cases]; early onset: 4 patients [12% of all early-onset cases]). All patients with PcP were not receiving PcP prophylaxis at the time of diagnosis. In addition, mortality in those diagnosed with PcP prior to the institution of routine PcP prophylaxis (pre-2007; n = 27) was not higher than in those diagnosed with PcP afterward (post-2007; n = 22). All patients with PcP were started on treatment with TMP/SMX, and all deceased patients were still on TMP/SMX therapy on their last day of life.

There were differences between the clinical parameters of deceased and non-deceased patients with PcP in univariate analyses (Table 2). The arterial pO2 on admission before initiation of O2 supplementation was lower in the group of deceased patients than in the group of not deceased patients, although this did not reach statistical significance. The arterial pCO2 was overall low (median 31 mm Hg) indicating respiratory failure type I. Fever was a common symptom but not significantly different between the two groups.

TABLE 3 Categorization of the computed tomography scan results on admission and their association with outcome

	Outcome			
	Overall	Not deceased	Deceased	*P*-value
Total, n	41	35	6	
Category				
Classical PcP (type 1)	16 (39.0)	14 (40.0)	2 (33.3)	.267
Fibrosing PcP (type 2)	2 (4.9)	2 (5.7)	0 (0.0)	
Classical PcP with irregular sparing (type 3)	5 (12.2)	5 (14.3)	0 (0.0)	
Fibrosing and classical PcP with irregular sparing (Type 2 + 3)	1 (2.4)	1 (2.9)	0 (0.0)	
Edema/overhydration (type 4)	7 (17.1)	4 (11.4)	3 (50.0)	
Atypical (type 5)	7 (17.1)	7 (20.0)	0 (0.0)	
Classical PcP in combination with other disease (type 6)	3 (7.3)	2 (5.7)	1 (16.7)	

Note: P-value calculated with Fisher's exact test.

Abbreviation: PcP, Pneumocystis pneumonia.

A higher CRP was significantly associated with a worse outcome (median 13.4 vs 30.1 mg/dL, $P = .016$) but the serum LDH was only non-significantly higher ($P = .124$). The application of adjunctive steroids in addition to maintenance immunosuppression did not affect the outcome. The necessity for mechanical and higher AKIN stages was, as expected, associated with a significantly higher in-hospital mortality rate. Almost all patients with PcP, irrespective of outcome, had acute kidney injury. The use of mechanical ventilation was significantly higher in the deceased group, reflecting critical illness in those patients.

Patients that died had a substantially lower $CD4^+$ T cell count, approximately half, compared to those that survived (median 243 vs 115 cells/μL, $P = .009$). Cohen's *d* for $CD4^+$ T cells and mortality was $d = 0.80$, indicating a large effect. Importantly, the total number of lymphocytes in the peripheral blood, although lower in the deceased group, did not reach statistical significance.

The radiographic findings were not significantly different between the two groups (Table 3).

3.3 | Co-infections and their association with the outcome

Bacterial or fungal co-infection and viral reactivation were frequent events and evident in 32 patients (65% overall) (Table 4). The urinary tract was the commonest site of bacterial co-infection. Multidrug-resistant (MDR) gram-negative bacteria, namely carbapenem resistance (n = 1) and MRSA (n = 0 vs n = 2), were only detected in deceased patients. The MDR gram-negative bacteria with CR in one patient was *Pseudomonas aeruginosa* and was detected at multiple sites. MRSA was detected in a lymphocele and in the BAL of another patient. There were no occurrences of Nocardia, Listeria, or Toxoplasma in the observed cohort. Fungal infections (all *Aspergillus* sp) were rare, and none were present in the bloodstream.

We noted 18 cases (37%) overall of CMV reactivation during hospital stay. This was not significantly different between deceased and non-deceased patients. Five patients were on CMV prophylaxis while CMV reactivation occurred. Detected viruses in the BAL were CMV (five patients), HSV (two patients), HHV6 (one patient), VZV (one patient), and EBV (one patient). No patient had more than one virus in their BAL.

3.4 | Lymphocyte subsets and their association with outcome

To investigate the predictors of in-hospital mortality, ROC curves were constructed for CRP, $CD4^+$, and AKIN, the variables that were identified in our univariate analyses as being significantly different between deceased and non-deceased subjects with PcP (Figure 1). We did not construct ROC curves for duration of TMP/SMX, because therapy was limited due to mortality—no deceased patient had the TMP/SMX therapy stopped before the final day of life. The area under the curve (AUC) for $CD4^+$ T cell counts was 0.783 [95% CI: 0.643-0.924; $P = .009$], indicating that $CD4^+$ T cell count is a significant predictor of in-hospital mortality. The area under the curves for CRP [0.761, 95% CI: 0.541-0.981; $P = .016$] and AKIN [0.711, 95% CI: 0.511-0.912; $P = .009$] were slightly lower (Figure 1).

We calculated the Youden's index for every point on the ROC curves to determine the optimal cutoff thresholds for predicting outcome. For CRP, the optimal cutoff was determined at 30 mg/dL with a specificity/sensitivity of 0.97/0.56 and an AUC of 0.762. The optimal $CD4^+$ T cell count cutoff was determined at 200 cells/μL with a specificity/sensitivity of 0.65/0.89 and an AUC of 0.783. The

TABLE 4 Co-infections and their association with outcome. Colonizations without clinical evidence of infection were not included

	Outcome			
	Overall	Not deceased	Deceased	*P*-value
Total, n	49	40	9	
Bacterial co-infection (%)	21 (43)	17 (43))	4 (44)	1.000
Bloodstream (%)	7 (14)	3 (8)	4 (44)	
Urinary tract (%)	14 (29)	12 (30)	2 (22)	
Bronchoalveolar (%)	6 (12)	3 (8)	3 (33)	
Other site (%)	7 (14)	5 (13)	2 (22)	
Fungal co-infection (%)	4 (8)	2 (5)	2 (22)	.149
Bloodstream (%)	0 (0)	0 (0)	0 (0)	
Urinary tract (%)	0 (0)	0 (0)	0 (0)	
Bronchoalveolar (%)	2 (4)	1 (3)	1 (11)	
Other site (%)	1 (2)	0 (0)	1 (11)	
Viral reactivation (%)	20 (41)	16 (40)	4 (44)	1.000
Bloodstream [CMV] (%)	18 (37)	14 (35)	4 (44)	
Bronchoalveolar (%) [a]	8 (16)	5 (13)	3 (33)	

Note: p-values calculated with Fisher's exact test. Bold indicates a *P*-value <.05.

Abbreviation: CMV, Cytomegalovirus.

[a]Referring to Herpesviridae (EBV, HHV6, HSV, VZV).

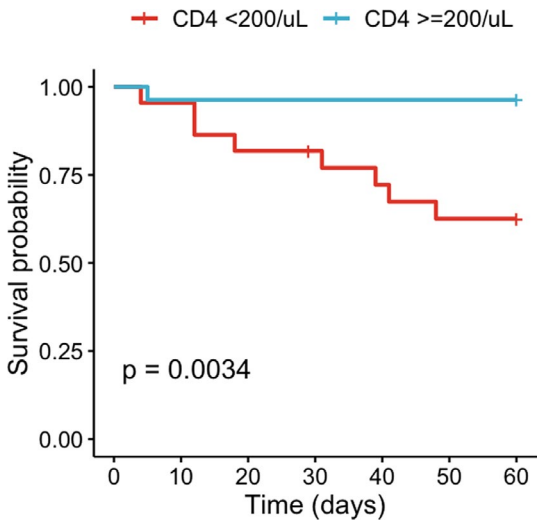

FIGURE 2 Survival curve for the Cox proportional-hazards regression model of the association between CD4+ T cell count and 60-day mortality. Survival has been stratified by $CD4^+$ T cell counts <200/μL (red) and ≥200/μL (blue). *P*-value calculated by logrank test

optimal AKIN stage cutoff was determined at stage 2 with a specificity/sensitivity of 0.70/0.78 and an AUC of 0.711.

To further gain insight into the interdependence of $CD4^+$ T cells as a determinant of 60-day mortality, we performed a Cox proportional-hazards regression analysis. $CD4^+$ T cell counts binarized as greater than or less than or equal to 200/μL were included as the covariate. One event was censored because of loss to follow-up after discharge. Low $CD4^+$ T cell count was significantly associated with high mortality (coefficient 2.457; HR 11.673, 95% CI 1.457-93.54, $P = .021$). The overall global significance of the model was confirmed by likelihood ratio testing ($P = .003$), Wald test ($P = .02$), and the logrank test ($P = .003$). The survival probability curve is shown in Figure 2.

4 | DISCUSSION

In the present study, we identified primarily high neutrophils, high CRP, low $CD4^+$ T cell count, mechanical ventilation, and AKIN stage

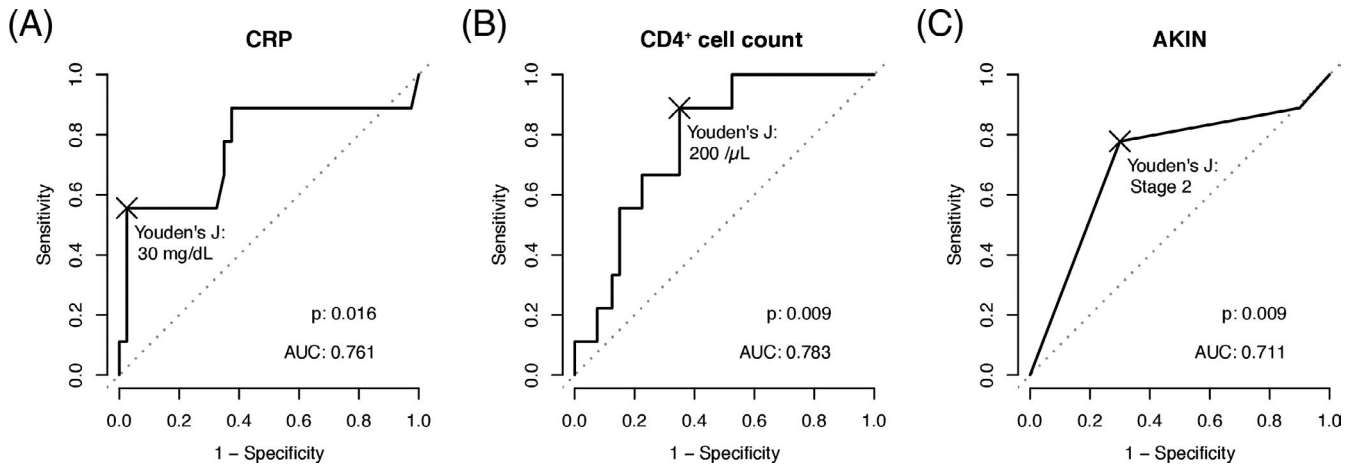

FIGURE 1 ROC analyses for in-hospital mortality for CRP, $CD4^+$ T cell count, and AKIN. Shown are ROC curves for CRP (A), $CD4^+$ T cell count (B), and AKIN (C) in predicting in-hospital mortality from PcP. Area under the curve (AUC) and associated p-values are shown for each curve. Indicated on each curve are the points with the maximum Youden's index (indicated by *X*) and the corresponding optimum cutoff threshold value of the parameter. CRP, C-reactive protein; AKIN, Acute Kidney Injury Network

as the clinical factors associated with mortality in PcP in kidney transplant patients. To our knowledge, ours is the first study investigating the determinants of mortality in kidney transplant patients with PcP, even though it has long been established that outcomes from PcP in patients without HIV are substantially worse than those with HIV infection.[33] The observed mortality in our cohort was high with 18%. In the same hospital over the same time frame, the overall mortality from pneumonia in kidney transplant patients, excluding PcP,[34] was about half of that at 7.3%, highlighting the life-threatening nature of PcP. In the literature, the PcP mortality in kidney transplant recipients is currently reported as 13%-20%, which is similar to that observed.[35,36]

In the present study, we focused on the association between low $CD4^+$ T cell counts and mortality. Low $CD4^+$ T cell counts were identified as a significant risk factor for higher in-hospital mortality in kidney transplant patients with PcP, and a cutoff of 200 $CD4^+$ T cells/μL was defined as the key threshold to predict mortality by ROC analysis. Furthermore, in a Cox proportional-hazards regression model $CD4^+$ T cell count <200 was significantly associated with 60-day mortality.

A low $CD4^+$ cell count as a determinant of higher mortality in HIV patients has been demonstrated in many large datasets.[19,20] In five smaller datasets, one showed significant, two non-significant, and two no differences.[17,37-40] Such disagreement could potentially be explained by the already very low baseline $CD4^+$ cell counts (median values ranging between 19 and 40 cells/μL) in HIV patients with PCP in these studies. Low $CD4^+$ T cell counts as a risk factor for higher in-hospital mortality in our cohort raise the question of the etiology of the low counts. One possibility is immunosuppression-induced T cell suppression. Although this is a well-known phenomenon, $CD4^+$ lymphopenia is a highly individual phenomenon and tends to be more frequent in the first six months post-transplantation.[41,42] The median time of presentation with PcP post-transplantation for our patients was 200 days, with a longer time out from transplantation in the deceased cohort (median 400 vs 187 days), so we believe that immunosuppression alone is an unlikely reason for the low $CD4^+$ T cell count. Another possibility is greater redistribution of $CD4^+$ T cells to lung tissue of patients that died. Previous studies in HIV have not supported this mechanism[43-45] but none have performed this evaluation in the context of transplantation and we did not quantitatively evaluate pneumocystis fungal burden in the BAL. Finally, the concept of sepsis-induced apoptosis of immune cells, including $CD4^+$ T cells, is well established in non-kidney transplant patients.[46,47] Despite this, the relationship between sepsis outcomes and $CD4^+$ T cell counts has not always been clear, possibly due to complex confounders.[48,49] Bacterial co-infections and viral reactivations were a frequent event in our cohort with a trend for higher infection rates in deceased patients. $CD4^+$ T cell count could thus be lower in the deceased patients because of the more severe infection status. The inflammatory markers CRP and neutrophil counts were significantly higher in deceased patients in our cohort reflecting an inflammatory state in severe disease.

$CD4^+$ T cells can differentiate to a number of effector and regulatory lineages based on environmental cues, and the phenotype of T cell response is linked to their effectiveness against pathogens.[50] Further studies could address the type of $CD4^+$ T cell responses in PcP patients that have transplants and determine whether there exists a qualitative difference apart from the quantitative difference in those that have poorer outcomes.

None of our patients was on PcP prophylaxis at the time of diagnosis. This is also commonly seen in other centers, reflecting the effectiveness of TMP/SMX prophylaxis.[6,51] Our center introduced routine PcP prophylaxis for the first 6-12 months in the year 2007. After this time, no *P jirovecii* infection within the first 6 months after transplantation has occurred. So according to guidelines and center experience, we now use PcP prophylaxis with TMP/SMX for at least 6 months in all patients. The shift from early- to late-onset disease highlights well the effect of the initiation of routine PcP prophylaxis. Of note in our data, the mortality within the late-onset group was higher than in the early-onset group. TMP/SMX is associated with renal toxicity and hyperkalemia.[52] Although there was a higher rate of hyperkalemia in deceased patients, this did not reach statistical significance. Furthermore, only two patients had a serum K greater than 6.5 mmol/L, of whom one survived and the other died of unrelated causes.

Results and interpretations of this study are limited by the retrospective design. Despite the clinical and radiological criteria used, it cannot be excluded that some patients were only colonized with *P jirovecii*. Nevertheless, all patients had a positive Grocott stain in the BAL which is considered the gold standard in diagnosing PcP and it is more likely that over-estimation of PcP occurred in the cohort surviving hospital admission than those that died. The initial identification of cases was through ICD10 codes in the hospital information system. Cases can be missed by basing the identification on billing information. Any upcoming studies on this subject would be very welcome to gauge the effect.

We see an association between mortality and low $CD4^+$ T cell counts in patients with PcP. Thus, the low $CD4^+$ T cell count reflects the severity of the disease. However, we cannot ascribe causation based on a retrospective analysis such as the one we have carried out. Therefore, whether the low $CD4^+$ T cell count caused the more severe disease or is a by-product of more severe infection cannot be determined without a prospective clinical study and mechanistic experiments. We hope the present study encourages and informs such endeavors.

$CD4^+$ T cell counts are easily and rapidly measured, and our findings suggest that they may act as biomarkers for disease severity. Thus, we propose that measurement of $CD4^+$ counts could help clinical decision-making in the context of PcP in transplant recipients, including whether to alter immunosuppression dose, anti-microbial therapy, and intensity of care.

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CONFLICT OF INTEREST

Drs. Freiwald, Büttner, Cheru, Avaniadi, Martin, Stephan, Pliquett, Vollkopf, Schüttfort, Jacobi, Herrmann, Geiger, and Hauser have nothing to disclose.

AUTHOR CONTRIBUTIONS

TF: designed the research; acquired, analyzed, and interpreted the data; and drafted the paper. SB: designed the research; analyzed and interpreted the data; and drafted the paper. NTC: acquired the data and drafted the paper. DA, SSM, RUP, AAV, GS: acquired the data and critically revised the article. CS: interpreted the data and critically revised the article. VJ: analyzed and acquired the data and critically revised the article. EH: analyzed and interpreted the data and critically revised the article. HG: designed the research and critically revised the article. IAH: designed the research, analyzed and interpreted the data, and drafted the paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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