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*Original Article*

# Comparison of caspofungin and trimethoprim-sulfamethoxazole combination therapy with standard monotherapy in patients with *Pneumocystis jiroveci* pneumonia following kidney transplantation: a retrospective analysis of 22 cases

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**Abstract:** Pneumocystis jiroveci pneumonia (PCP) is one of the most common and fatal opportunistic infections of renal transplant recipients. The objective of this retrospective study was to compare the therapeutic effect and safety of caspofungin and trimethoprim-sulfamethoxazole (TMP-SMZ) combination therapy with standard TMP-SMZ monotherapy in 22 patients with severe PCP following kidney transplantation. The presence of P. jiroveci was determined by direct fluorescence staining, PCR analysis, detection of beta-1, 3-glucan and serum lactate dehydrogenase levels. Thirteen patients received combination therapy, and nine patients received standard TMP-SMZ monotherapy for PCP. There were no significant differences in the baseline demographics and clinical characteristics were detected. Patients in the combination therapy group experienced better overall outcomes characterized by reduced duration of increased body temperature ($P < 0.001$), respiratory intensive care unit stay ($P < 0.001$), less requirement for mechanical ventilation ($P = 0.005$) and shorter high dosage TMP-SMZ treatment course ($P < 0.01$). 44.4% of patients receiving standard TMP-SMZ progressed to acute respiratory distress syndrome (ARDS), none of the patients in the combination therapy group experienced ARDS ($P = 0.017$). In addition, patients in the combination treatment group had greater $PaO_2/FiO_2$ ($P = 0.007$), and experienced less neutropenia. Two patients in the TMP-SMZ monotherapy group died, while no deaths were observed in patients receiving combination therapy. In conclusions, PCP patients receiving caspofungin and TMP-SMZ co-treatment may experience better clinical outcomes with fewer side effects. Large-scale, prospective studies are needed to confirm that the improved clinical outcomes were due to the combination therapy.

**Keywords:** Kidney transplantation, immunosuppression, opportunistic infection, respiratory failure, beta-1, 3-glucan

## Introduction

Occurring in as little as 3-6 months after transplantation [1, 2], *Pneumocystis jiroveci* pneumonia (PCP) was associated with a mortality rate as high as 50% [3, 4]. Symptoms of PCP include non-productive cough, low- or high-grade fever, severe dyspnea and hypoxemia. Although airborne transmission is thought to be the route by which PCP is transmitted, patient-to-patient transmission has been reported [5, 6], resulting in the recommendation for prophylactic therapy and immunosuppressant dose reduction [6, 7]. Given the high mortality rates associated with PCP, new treatment modalities to quickly reduce the PCP burden in transplant patients is crucial.

The standard first-line therapy for PCP includes large doses of sulfa drugs. Although this regimen is effective for some patients, the disease progresses to severe respiratory failure and even death in a considerable proportion of patients, with even worse prognosis in patient on immunosuppressive treatment such as transplant recipients, than that in HIV patients

[8]. In addition, significant side-effects have been associated with prolonged large dosage trimethoprim and sulfamethoxazole (TMP-SMZ) therapy. Second-line treatments for PCP, including clindamycin and primaquine phosphate, pentamidine inhalation, trimetrexate and atovaquone, and intravenous pentamidine, can be used for severe cases. However, poor tolerance and extensive side-effects have also been noted with these second-line therapies; therefore, they are not ideal for clinical practice.

In the mammalian lung, P. jiroveci has a biphasic life cycle that includes asexual (trophic form) and sexual (cyst form) cycles. TMP-SMZ is active against the trophic form but much less effective to clear the cyst that is encapsulated and protected by a thick layer containing mostly beta-1, 3-glucan; therefore, addition of a second drug that targets the cyst form may more fully inhibit the life cycle of the organism. Caspofungin, a new class of antifungals belonging to the echinomycin class, inhibits beta-1, 3-glucan synthesis, thereby targeting the principal component of the *P. jiroveci* cyst wall and reducing its integrity [9]. Moreover, because beta-1, 3-glucan itself can induce inflammation, inhibition of its synthesis further reduces the pathogenicity of *P. jiroveci* [10].

The efficacy of caspofungin has been shown using *in vivo* models of PCP [11, 12]. For example, Powles et al. [12] observed a 90% reduction in PCP cysts 4 days after caspofungin monotherapy and a 66% reduction in trophozoites 21 days after treatment. Therefore, caspofungin primarily targets the cyst, which is responsible for continuously producing large amounts of trophozoites in PCP. Furthermore, a recent *in vivo* study in mice showed that the efficacy of caspofungin and TMP-SMX combination therapy was greater than that observed for either drug alone [13]. However, the few case studies that have assessed the application of caspofungin for the treatment of PCP in patients have reported varying outcomes, which may be due to the limited number of patients analyzed [14, 15]. Moreover, analysis of its safety and effectiveness in the treatment of transplant recipients is even rarer [16, 17]. Therefore, this retrospective study examined the hypothesis that caspofungin and TMP-SMX combination therapy would offer superior efficacy over standard TMP-SMX treatment. This hypothesis was tested in 22 renal transplant recipients with severe PCP following kidney transplantation to investigate the outcome and safety of combined application of caspofungin and TMP-SMZ as compared with conventional TMP-SMZ monotherapy.

## Materials and methods

### *Subjects*

Renal transplant recipients, who were diagnosed with severe PCP in the First Affiliated Hospital of PLA's General Hospital between September 2004 and October 2009, were included in the current study. These patients have had received kidney transplants either in our hospital or in other transplant centers. Patients that received additional transplantations beyond the kidney transplantation, had an additional infection of other etiologies, or previously had severe chronic pulmonary diseases were excluded from the present study. Severe pneumonia was diagnosed according to the diagnosis and treatment guidelines as recommended by the Respiratory Disease Branch of the Chinese Medical Association, which are based on the Infectious Diseases Society of America (IDSA) guidelines for the management of community-acquired pneumonia [18] and hospital-acquired pneumonia [19]. All patients in this study have pursued medical help within 2 days of the initial symptom onset. This study was approved by the institutional review board (IRB) of the First Affiliated Hospital of PLA's General Hospital. Informed written consent was obtained from each patient enrolled in the study.

### *Pathogen detection and diagnosis*

In addition to chest X-ray (CXR) and/or CT analysis, pathogen detection was carried out for patients with fever and suspected lung infection after admission. Bacterial and fungal cultures and drug sensitivity tests together with acid-fast staining of blood, sputum, throat swab and midstream urine specimens were performed routinely. For patients without sputum, secretions were obtained by sputum induction or fiber optic bronchoscopy or via the artificial airway (BAL, broncho alveolar lavage). Direct fluorescence staining of *P. jiroveci* cysts and polymerase chain reaction (PCR) analysis for

the gene encoding the large subunit ribosomal RNA in the mitochondrion of *P. jiroveci* were also performed. In addition, because the diagnostic accuracy of determining serum beta-1, 3-glucan for PCP is high [20], beta-1, 3-glucan levels were determined to assess the degree of infection using a specific antiserum as previously described [21]. Furthermore, serum lactate dehydrogenase (LDH) levels were also determined to indirectly evaluate PCP as in Vogel et al. [22]. Cytomegalovirus (CMV) DNA was identified by routine PCR amplification of the HindII-X fragment region or CMV-specific IgM detection.

*Treatment*

Because all patients were immunosuppressed, delayed treatment may have resulted in serious consequences. Therefore, cases with positive cyst/trophozoite staining and PCR alone or in combination were diagnosed clinically with PCP in combination with the clinical symptoms and imaging findings; corresponding treatments were immediately initiated. The mean interval between admission and initiation of treatment was 2 days, ranging from 1 to 3 days.

On hospitalization, patients received continuous oxygen therapy or ventilator-assisted breathing according to their ABG results or their hypoxic complaints. Immunosuppressive agents were withdrawn or reduced, and methylprednisolone (40-160 mg/d) was administered after being diagnosed with PCP. Broad-spectrum antibiotics were also administered to prevent secondary bacterial infection. After anti-fungal drugs and valganciclovir hydrochloride were also withdrawn, a large dose of TMP-SMZ (320/1600 mg over 3-5 days) was administered. For 13 patients diagnosed with PCP after 2009, caspofungin was co-administered with a large dose of TMP-SMZ. The first dose of caspofungin was 70 mg, and the maintenance dose was 50 mg per day as previously described [17]. One patient with a history of sulfa allergy was initially treated with caspofungin followed by a large dose of TMP-SMZ after successful sulfa desensitization. However, this patient was not included in the comparison between the groups. The sputum or BAL pathogen spectrum was monitored for all patients, and the antibiotic regimen was adjusted according to the clinical outcomes. The doses of antibiotics and methylprednisolone were reduced step by step after symptom relief after which immunosuppressive agents were gradually re-administered.

*Outcome analysis*

Routine blood tests and blood biochemistry analyses were evaluated every day to monitor the complete blood count and kidney function. In addition, arterial blood gases and oxygenation indices ($PaO_2/FiO_2$) were measured daily to detect improvements in the oxygenation index. CXR or chest CT was taken every 2-3 days to observe the range and nature of inflammation. Outcomes were evaluated by a combined of variables, including body temperature, pulmonary signs, and oxygenation index. In-hospital mortality was determined at discharge.

*Safety analysis*

The safety of the medication was determined by analysis of the adverse drug reactions, including chills, fever, skin rash, nausea, vomiting, and diarrhea, as described in the usage instruction for caspofungin.

*Statistical analysis*

Continuous variables were presented as medians and inter-quartile ranges (IQR, the range between the 25th and 75th percentile) due to the small sample size; comparisons between the two treatment groups were undertaken using the Mann-Whitney U test. Categorical variables were expressed by counts and percentages, and compared using the Fisher's exact test. Comparisons of continuous variables between time of measurements (i.e., before and after treatment) were performed by Wilcoxon signed-rank test. SAS software, version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. All statistic assessments were evaluated at a two-sided *P*-value of 0.05.

## Results

*Patient demographics*

A total 22 renal transplant patients were enrolled in the study from September 2004 to October 2009. These patients included 19

**Table 1.** Baseline demographics and clinical characteristics of patients receiving TMP-SMZ or combination therapy

| | TMP-SMZ (n = 9) | Combination (n = 13) | *P*-value |
|---|---|---|---|
| Age (y)* | 39.0 (32.0, 53.0) | 42.0 (38.0, 51.0) | 0.525 |
| Gender (male)† | 8 (88.9) | 11 (84.6) | 1.000 |
| Immunosuppressive therapy† | | | 1.000 |
| FK506+ MMF + prednisone | 9 (100.0) | 12 (92.3) | |
| CyA+ MMF + Prednisone | 0 (0.0) | 1 (7.7) | |
| FK506 (ng/mL)* | 8.9 (7.5, 9.8) | 8.3 (7.6, 9.1) | 0.593 |
| Onset time from transplant (months)* | 3.2 (2.5, 4.6) | 2.6 (1.9, 3.2) | 0.217 |
| Temperature (°C)* | 38.8 (38.3, 39.2) | 38.8 (38.5, 39.2) | 0.788 |
| Heart rate (beats per minute) | 109.0 (103.0, 122.0) | 115.0 (109.0, 123.5) | 0.431 |
| Blood pressure (mmHg) | 138.0 (125.0, 142.5) | 135.0 (126.0, 140.0) | 0.845 |
| Time from symptom presentation to admission | 1.0 (1.0, 1.0) | 1.0 (1.0, 2.0) | 0.171 |
| Time from admission to therapy initiation (days)* | 2.0 (1.0, 2.0) | 2.0 (1.5, 2.0) | 0.647 |
| Presence of hypoxia† | 6 (66.7) | 10 (76.9) | 0.655 |
| Presence of shock† | 1 (11.1) | 2 (15.4) | 1.000 |
| Pneumothorax† | 0 (0.0) | 0 (0.0) | - |
| Supplemental oxygen† | 7 (77.8) | 4 (30.8) | 0.08 |
| Respiratory rate* | 40.0 (32.5, 44.0) | 40.0 (37.5, 45.0) | 0.209 |
| Altered mental status† | 1 (11.1) | 2 (15.4) | 1.000 |
| $PaO_2$, mmHg* | 62.4 (54.7, 73.3) | 64.0 (58.8, 71.9) | 0.794 |
| $PaO_2/FiO_2$, mmHg* | 312.0 (273.5, 366.5) | 320.0 (294.0, 359.5) | 0.794 |
| LDH, U/L* | 427.0 (284.0, 578.0) | 276.0 (220.0, 305.0) | 0.082 |
| HLA mismatch | 3 (2, 3.5) | 3 (2, 3) | 0.948 |
| Acute rejection | 22.2% | 30.8% | 0.658 |
| Presence of delayed graft function | 11.1% | 7.7% | 0.784 |
| Cold ischemia time, minutes | 90.0 (60.0, 165.0) | 120.0 (60.0, 165.0) | 0.865 |
| Warm ischemic time, minutes | 2.0 (1.0, 3.0) | 3.0 (2.0, 3.5) | 0.370 |

*Continuous data were presented as median (IQR) and compared using the Mann-Whitney U test. †Categorical variables were expressed by counts and percentages and compared using the Fisher's exact test. TMP-SMZ, trimethoprim-sulfamethoxazole; MMF, Mycophenolate mofetil; CyA: cyclosporine A; LDH, lactate dehydrogenase.

(86.4%) males and three (13.6%) females with a mean age of 43.0 ± 12.0 years old. Among these patients, nine (40.9%), who received a definite diagnosis of PCP before 2008, had received TMP-SMZ treatment; 13 (59.1%), who were diagnosed with PCP after 2008, had received combination treatment (**Table 1**). No differences in demographic and clinical characteristics at admission, including $PaO_2/FiO_2$, were observed between the two treatment groups ($P > 0.05$; **Table 1**).

*Clinical symptoms*

**Table 2** shows the clinical presentation and diagnostic evidence of PCP for each patient included in the study. The main clinical symptom was fever, which started as low-grade fever in the afternoon and later presented as a remittent fever or a continued fever without cough and expectoration. The median (IQR) body temperature at diagnosis was 38.8°C (38.3, 39.2). In most patients, the duration of fever was prolonged, and the body temperature increased with the disease progression before and after being hospitalized. The outcome of solely antibiotic administration was poor with the patients describing chest tightness and shortness of breath.

In many patients, no obvious abnormal lung sounds or only wet and dry rales could be heard in the bilateral lower lung fields/full lung, and CXR revealed only increased bronchovascular markings at the onset. At a later stage, ground-glass-like, patchy or cottony white shadows dis-

**Table 2.** Clinical presentation and diagnostic evidence of *Pneumocystis jiroveci* pneumonia for each patient (n = 22)

| Patient no. | Group | Presence of symptoms | CXR result | Direct fluorescence staining | PCR | Methylprednisolone Dosage (mg) | $PaO_2$ (mmHg)* | $PaO_2/FiO_2$ (mmHg)* |
|---|---|---|---|---|---|---|---|---|
| 1 | Combination | Yes | patchy white shadows, diffuse | Positive | Positive | 80 | 43.8 | 219 |
| 2 | Combination | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 80 | 66.0 | 330 |
| 3 | Combination | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 40 | 64.0 | 320 |
| 4 | Combination | Yes | patchy or cottony white shadows | Positive | Positive | 80 | 77.4 | 387 |
| 5 | Combination | Yes | ground-glass-like shadows, diffuse | Positive | Negative | 160 | 60.6 | 303 |
| 6 | Combination | Yes | ground-glass-like shadows, diffuse | Negative | Positive | 80 | 72.8 | 364 |
| 7 | Combination | Yes | ground-glass-like shadows, partial | Positive | Negative | 40 | 71.0 | 355 |
| 8 | Combination | Yes | patchy white shadows, partial | Positive | Negative | 160 | 62.0 | 310 |
| 9 | Combination | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 40 | 62.4 | 312 |
| 10 | Combination | Yes | ground-glass-like shadows, partial | Positive | Positive | 80 | 67.4 | 337 |
| 11 | Combination | Yes | cottony white shadows, diffuse | Positive | Negative | 60 | 57.0 | 285 |
| 12 | Combination | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 80 | 55.6 | 278 |
| 13 | Combination | Yes | ground-glass-like shadows, partial | Positive | Positive | 80 | 80.4 | 402 |
| 14 | TMP-SMZ | Yes | ground-glass-like shadows, diffuse | Positive | Negative | 160 | 57.4 | 287 |
| 15 | TMP-SMZ | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 80 | 76.8 | 384 |
| 16 | TMP-SMZ | Yes | patchy white shadows, diffuse | Positive | Positive | 40 | 57.6 | 288 |
| 17 | TMP-SMZ | Yes | ground-glass-like shadows, diffuse | Positive | Negative | 80 | 75.6 | 378 |
| 18 | TMP-SMZ | Yes | increased bronchovascular markings | Positive | Negative | 60 | 62.4 | 312 |
| 19 | TMP-SMZ | Yes | ground-glass-like shadows, diffuse | Negative | Positive | 80 | 52.0 | 260 |
| 20 | TMP-SMZ | Yes | cottony white shadows, diffuse | Positive | Negative | 40 | 71.0 | 355 |
| 21 | TMP-SMZ | Yes | ground-glass-like shadows, partial | Positive | Positive | 80 | 46.0 | 230 |
| 22 | TMP-SMZ | Yes | ground-glass-like shadows, diffuse | Positive | Positive | 80 | 68.6 | 343 |

*These values were determined at admission. TMP-SMZ, trimethoprim-sulfamethoxazole; CXR, chest X-ray; PCR, polymerase chain reaction.

tributed in local or whole lung fields were identified (**Table 2**). CT often showed only minor changes in the lung when there was no obvious abnormality in the CXR. At later stages, perihilar ground-glass-like shadows and strip-shaped shadows between the lobes and large, diffuse shadows, which covered the whole lung in some cases, were observed (**Table 2**). Moreover, the WBC count was less than $4.0\times10^9$/L in nine cases (40.9%) at the time of diagnosis, and all responded well to single dose GM-CSF treatment shortly after admission. The rest of patients showed normal WBC count. At diagnosis, the median $PaO_2/FiO_2$ was 312.0 (273.5, 366.5) and 320.0 (294.0, 359.5) mmHg in patients treated with TMP-SMZ monotherapy and combination therapy, respectively ($P$ = 0.794; **Table 1**).

*PCP diagnosis*

As shown in **Table 2**, *P. jiroveci* was detected by direct fluorescence staining in 20 patients (90.9%); and was detected by PCR in 14 out of 22 patients (63.6%). The results of both cysts/trophozoite staining and PCR were positive in 12 cases (54.6%). Upon PCP diagnosis, immunosuppressive agents were withdrawn or reduced, and methylprednisolone was administered in all patients; there was no difference in the range of methylprednisolone dosage between the two treatment groups ($P$ > 0.05; **Table 2**).

*Treatment outcomes in the combination therapy group*

For the 13 patients, who received the caspofungin in combination with TMP-SMZ (320/1600 mg, 3-5 times/day), the mean duration of caspofungin administration was 10.4 days (range, 6-15 days). None of the patients in this group required mechanical ventilation. A chest x-ray taken one week after treatment initiation showed a significant reduction in inflammation in patients receiving the combination therapy; the reduction was > 50% in seven patients. After withdrawal of caspofungin, patients continued to receive this therapeutic dosage TMP-SMZ for 2 or 3 more days, with the mean duration of high dosage TMP-SMZ treatment up to 12.6 days. After body temperature returned to

**Table 3.** Efficacy of combination therapy as compared to TMP-SMZ treatment for severe *Pneumocystis jiroveci* pneumonia following kidney transplantation

| | TMP-SMZ (n = 9) | Combination (n = 13) | *P*-value |
|---|---|---|---|
| Duration of temperature return to normal (days)* | 7.0 (6.0, 8.0) | 2.0 (2.0, 3.0) | < 0.001 |
| Mechanical ventilation | 5 (55.6%) | 0(0%) | 0.01 |
| ARDS† | 4 (44.4) | 0 (0.0) | 0.017 |
| Duration of RICU stay (days)* | 12.0 (8.0, 13.0) | 5.0 (4.0, 5.0) | < 0.001 |
| Death†,‡ | 2 (22.2) | 0 (0.0) | 0.156 |
| *One week after treatment* | | | |
| $PaO_2$, mmHg* | 56 (46.5-62.5) | 67.2 (61.74-75.5) | 0.004 |
| $PaO_2/FiO_2$, mmHg* | 200.0 (168.0, 288.0) | 320.0 (303.0, 355.0) | 0.007 |
| LDH, U/L* | 239.0 (179.0, 302.0) | 173.0 (162.0, 230.0) | 0.144 |

*Continuous data were presented as median (IQR) and compared using the Mann-Whitney U test. †Categorical variables were expressed by counts and percentages and compared using the Fisher's exact test. ‡In-hospital mortality was determined at discharge. TMP-SMZ, trimethoprim-sulfamethoxazole; ARDS, acute respiratory distress syndrome; RICU, respiratory intensive care unit; LDH, lactate dehydrogenase.

normal and chest X-ray revealed a normal lung field, oral intake of immunosuppressive agents was again initiated, and TMP-SMZ (320/1600 mg, once daily) was administered as a secondary prophylaxis for PCP. In one patient, who was diagnosed with PCP 12 days after renal transplantation, continuous renal replacement therapy (CRRT) was required due to impaired function of the transplanted kidney. The patient's kidney function returned to normal during the treatment. In the other patients, serum creatinine levels slightly decreased after the withdrawal of the immunosuppressants (and application of caspofungin. Although creatinine levels increased to varying degrees after re-administration of the immunosuppressive agents, it was within the normal range and comparable to the levels these patients had during their previous routine follow-ups.

Caspofungin, which was supplemented with antibiotic application (Piperacillin-tazobactam), was administered to another patient with a history of sulfa allergy after being diagnosed with PCP. However, the patient's fever, which started as remittent and progressed to continued high degree fever, peaking at 40.3°C. Five days after treatment, the oxygen saturation was reduced to < 50%, and an emergency tracheotomy was performed with mechanical ventilation. After sulfa desensitization, TMP-SMZ (320/1600 mg, 5 times/day) was administered via a nasal tube, which reduced his temperature to < 38°C after three days. However, the fever returned along with a large number of purulent tracheal secretions. Bacterial cultures revealed the presence of a *Pseudomonas aeruginosa* infection. After switching antibiotic to meropenem, the patient's body temperature gradually returned to normal, and the tracheal tube was removed 10 days after the tracheotomy. At this time, the dose of TMP-SMZ was also changed to 320/1600 mg 3 times/day following cessation of caspofungin, and the immunosuppressants were re-administered.

*Treatment outcomes in the TMP-SMZ group*

Among the nine patients, who received a large, oral dose of TMP-SMZ alone, one patient died of severe respiratory failure 4 days after treatment. Another patient, who had undergone kidney transplantation 5 months prior to diagnosis with PCP, had acute rejection one month before admission due to PCP. Although methylprednisolone pulse therapy and rATG therapy were carried out, the outcome was poor for this patient; kidney function was essentially lost, and hemodialysis was required at the time of admission. The patient died of respiratory failure and heart failure one week after treatment. Of the remaining seven patients, two underwent endotracheal intubation and mechanical ventilation following tracheostomy due to severe respiratory distress, and another patient received non-invasive mechanical ventilation due to unbearable hypoxic symptoms.

In the TMP-SMZ monotherapy group, the mean duration of treatment was 20.1 days. After 1 week of treatment, chest x-ray showed varying degrees of improvement and reduced inflam-

mation. In two patients, inflammation was reduced by > 50%; it was > 25% in five cases. However, increased inflammation was observed in two patients. Kidney function also improved after withdrawal of the oral immunosuppressants.

*Comparison of treatment outcomes between the combination therapy and TMP-SMZ groups*

Neutropenia was observed in three patients (23.1%) in the combination group and six patients (66.7%) in the TMP-SMZ group 1 week after treatment. Folic acid or folinic acid was prescribed, and patients received a subcutaneous injection of colony-stimulating factor. Thrombocytopenia was not observed in either group.

As shown in **Table 3**, the combination group had a significantly shorter duration of time required for their temperature to return to normal (2 vs. 7 days; $P < 0.001$), experienced a shorter respiratory intensive care unit (RICU) stay (5 vs. 12 days; $P < 0.001$), had higher $PaO_2$ (67.2 vs. 56.0 mmHg; $P = 0.004$), and had increased $PaO_2/FiO_2$ (320.0 vs. 200.0 mmHg; $P = 0.007$) than the TMP-SMZ group (**Table 3**). In addition, no patient progressed to acute respiratory distress syndrome (ARDS) in the combination group while four (44.4%) patients in the TMP-SMZ group developed ARDS, which necessitated mechanical ventilation ($P = 0.017$). In addition, mechanical ventilation, was required for 55.6% of patients that received TMP-SMZ monotherapy as compared to 0% of those that were treated with combination therapy ($P = 0.005$), No significant difference was found in LDH level between two groups (173.0 vs. 239.0 U/L; $P = 0.144$). However, significant reductions in LDH level after treatment were found in both groups ($P = 0.001$ in combination group and $P = 0.008$ in TMP-SMZ group; data not shown). Finally, while two patients in the TMP-SMZ monotherapy group died from complications resulting from PCP, no deaths were observed in those patients receiving combination therapy ($P = 0.156$; **Table 3**).

*Safety evaluation*

No adverse events associated with caspofungin, including chills, fever, skin rash, nausea, vomiting, and diarrhea, were observed in the nine patients, who received caspofungin treatment. In addition, liver or kidney dysfunction was not observed in these patients.

## Discussion

PCP is a common opportunistic infection of immunocompromised patients; mortality due to PCP in AIDS patients can reach as high as 59% [23]. Thus, identifying a safe and effective treatment for PCP is extremely important. In the present study, we found that the outcomes of patients receiving the combination therapy were significantly better than those observed for patients receiving the standard TMP-SMZ monotherapy. In the combination therapy group, patients recovered more quickly, and progression to ARDS was effectively blocked. In addition, the duration of TMP-SMZ administration was decreased as compared to the monotherapy group (12.6 versus 20.1 days, respectively, $P < 0.01$). Furthermore, leukopenia was observed in a smaller proportion of patients receiving the combination therapy. Finally, whereas two patients in the TMP-SMZ monotherapy group died, no deaths were observed in the combination therapy group. Thus, combination therapy may be better at eliminating the infection in a shorter amount of time and may be associated with fewer side effects than TMP-SMZ monotherapy.

Few clinical studies have analyzed the effects of caspofungin in the treatment of PCP and have reported varying efficacy. While Beltz et al. [14], Annaloro et al. [16] and Mu et al. [24] successfully treated three cases of severe PCP using caspofungin, Kamboj et al. [15] reported poor outcomes associated with caspofungin treatment in two tumor patients with PCP. In four patients with PCP after organ transplantation, two of which experienced treatment failure of TMP-SMZ monotherapy, TMP-SMZ combined with caspofungin resulted in a complete cure without any side effects [17], which is in agreement with the present study.

In the present study, the duration of caspofungin administration ranged from 6 to 15 days in the combined treatment group without inducing any significant adverse events. The improvement in kidney function during the treatment, indicated by decreased serum creatinine level, might be related to the withdrawal or reduced administration of calcineurin inhibitor immunosuppressant which have been well-known for

their nephrotoxicity. Given the adverse effects on kidney and bone marrow function associated with large doses of sulfa drugs, it is critical to shorten the duration of their application during PCP treatment. In the current study, the duration of TMP-SMZ was relatively shorter in patients receiving the combination therapy, which may have reduced the incidence of leucopenia in these patients.

Increased LDH is common in patients with PCP. However, it is not considered to be a specific indicator of PCP but a reaction of the lung to inflammation and injury [25]. In the current study, we also found that the LDH level decreased after treatment in the both groups, indicating an improvement in lung condition.

The present study is limited in that there were significantly more patients in the monotherapy group that required mechanical ventilation at admission. Therefore, further studies with a larger number of patients are required to rule out selection bias.

## Conclusions

This is the largest study to examine the efficacy of caspofungin and TMP-SMZ co-treatment and compare it with TMP-SMZ monotherapy in renal transplant patients with PCP to date. Patients that received the combination therapy experienced reduced fever duration, length of RICU stay, duration of large-dose sulfa drug administration and the incidence of side effects as well as improved $PaO_2/FiO_2$. Although this is by far the largest study to assess the efficacy of TMP-SMZ and caspofungin co-treatment in PCP patients, the sample size is still small, and the time between the individual cases is long. Furthermore, there were significant differences in the baseline intubation requirement between the treatment groups. Therefore, large-scale, prospective studies are needed to confirm the results of the present study.

## Disclosure of conflict of interest

None.

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