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High initial (1, 3) Beta-D-Glucan concentration may be a predictor of satisfactory response of c aspofungin combined with TMP/SMZ for HIV-negative patients with moderate to severe *Pneumocystis jirovecii* pneumonia

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ABSTRACT

Objectives: The aim of this study was to investigate the efficacy of combination therapy of caspofungin and TMP/SMZ (trimethoprim/sulfamethoxazole) in moderate to severe *pneumocystis jirovecii* pneumonia (PJP) in patients without human immunodeficiency virus infection (HIV) and the relationship between therapeutic effect and plasma (1, 3) Beta-D-Glucan (BDG) levels.
Methods: We retrospectively reviewed HIV-negative patients with PJP diagnosed in our department, who were treated with combination therapy of caspofungin and TMP/SMZ or monotherapy of TMP/SMZ during a six and a half year period.
Results: A total of 126 moderate to severe PJP patients were enrolled in the study. In the multivariate analysis, low lymphocyte counts, high serum lactate dehydrogenase levels at the diagnosis of PJP and progression to shock were significant risk factors for death. In all patients, there was no significant difference in risk of death at 3 months. In the group of BDG $\geq$ 800 pg/m, patients receiving combination therapy was associated with a significantly decreased risk of death at 3 months, whereas in the group of BDG<800 pg/ml, there were no statistically significant difference in survival rate between the two treatment regimens.
Conclusion: High initial plasma (1, 3) Beta-D-Glucan concentration may be a predictor of satisfactory caspofungin response to HIV-negative patients with PJP. Based on our findings, we suggest the choice of combination therapy with caspofungin and TMP/SMZ as the initial treatment when BDG $\geq$ 800 pg/ml in moderate to severe HIV-negative patients with PJP.

Background

Pneumocystis jirovecii pneumonia (PJP) is an acute and life-threatening interstitial pneumonia that occurs in immunocompromised patients (White et al., 2017; Wickramasekaran et al., 2017; Roembke et al., 2014). Despite the advent of prophylactic agents to prevent this infection, the morbidity and mortality of patients without human immunodeficiency virus infection have steadily increased (Wickramasekaran et al., 2017). The overall mortality of HIV-negative patients with PJP was 20%–60%, and 84% in severe cases (Wickramasekaran et al., 2017; Roembke et al., 2014). Therefore, early selection of effective therapy is particularly important.

Abbreviations: BALF, bronchoalveolar lavage fluid; BDG, (1, 3) Beta-D-Glucan; CI, confidence intervals; CMV, cytomegalovirus; CT, computed tomography; $D(A\text{-}a)O_2$, alveolar-arterial oxygen difference; FiO_2, fraction of inspiratory oxygen; FRET, fluorescence resonance energy transfer; GGO, ground-glass opacity; GMS, Grocott's methenamine silver; HR, hazard ratio; ICU, intensive care unit; IQR, interquartile range; LDH, lactate dehydrogenase; MSG, major surface glycoprotein; MV, mechanical ventilation; PaO_2, arterial partial pressure of oxygen; PJP, *Pneumocystis jirovecii* peumonia; PCR, polymerase chain reaction; TMP/SMZ, trimethoprim/sulfamethoxazole.

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The first-line therapy of PJP is TMP/SMZ (trimethoprim/sulfamethoxazole), which has remained unchanged for many years (White et al., 2017; Wickramasekaran et al., 2017; Roembke et al., 2014). However, about 25% of patients cannot complete the full course of the TMP/SMZ regimen because of treatment failure or various side effects such as marrow suppression, renal damage, gastrointestinal upset and so on (Benfield et al., 2008). Combination therapy with caspofungin, an antifungal agent that acts on the cyst form of *Pneumocystis jirovecii (P. jirovecii)* by inhibiting (1, 3) Beta-D-Glucan synthesis, was presented (Lobo et al., 2013). Caspofungin has demonstrated efficacy of *P. jirovecii* clearance in experimental mouse models, and Lobo et al suggested that caspofungin combined with a low dose of TMP/SMZ may provide an improved treatment regimen for PJP (Lobo et al., 2013), whereas the clinical evidence of the synergistic effect of caspofungin combined with TMP/SMZ therapy in PJP still remains controversial to date (Kamboj et al., 2006; Li et al., 2016; Armstrong-James et al., 2011).

(1, 3) Beta-D-Glucan (BDG) is an antigenic component of the cell wall of *P. jirovecii,* which partly contributes to the host inflammatory response in the lung (Damiani et al., 2013). High levels of plasma BDG may indicate a higher microbial load (Damiani et al., 2013). Based on the mechanism of caspofungin action and review of literature, some investigators suggested that caspofungin therapy may be more effective in higher BDG levels of PJP patients (Li et al., 2016; Armstrong-James et al., 2011). The present retrospective analysis aimed to comparatively assess caspofungin combined with TMP/SMZ and TMP/SMZ monotherapy to treat moderate to severe HIV-negative patients with PJP, and evaluate the relationship between the level of plasma BDG and caspofungin efficacy.

Method

Patient selection

The present study was performed at the Peking Union Medical College Hospital (Beijing, China). A total of 684 patients with suspected PJP were enrolled in the study from January 2012 to June 2018. The eligible patients did not have HIV infection, were older than 18 years of age, and had clinical manifestations of PJP such as dyspnea, cough, fever, or abnormal chest radiograph. Enrollment was limited to patients whose room air arterial partial pressure of oxygen/fraction of inspiratory oxygen (PaO_2/FiO_2) $\leq$70 mmHg and/or alveolar-arterial oxygen difference [$D(A\text{-}a)O_2$] $\geq$35 mmHg. These patients were classified as moderate to severe PJP patients. The mycological and morphological diagnoses were confirmed by real-time fluorescence quantitative polymerase chain reaction (PCR) and Grocott's methenamine silver (GMS) staining of respiratory samples respectively. According to previous studies (White et al., 2017; Yasuoka et al., 1996), the diagnosis of PJP was considered as definitive if *P. jirovecii* was found on the morphological analysis of respiratory samples from patients with clinical manifestations (fever, dry cough, or dyspnea), hypoxemia, and radiologic findings compatible with PJP. The diagnosis of PJP was considered as presumptive if patients met all three criteria and had either a positive PCR test for *P. jirovecii* DNA or an increased level of BDG (Yasuoka et al., 1996). In our study, both definitive and presumptive cases were included. Exclusion criteria were: lost to follow-up, initial second-line treatment (clindamycin, primaquine ect.), lacking in testing the level of BDG.

Technical information

PCR was performed using specific primers to amplify the mitochondrial large subunit rRNA (mtLSUrRNA) gene of *P. jirovecii.* The sequences of primer for the *P. jirovecii* target gene, major surface glycoprotein (MSG), were MSG-fw, 5′-CTTAAAATAAATAAT-CAGACTATGTGCGATAAG-3′, and MSG-rv, 5′-GGAGCTTTAAT-TACTTTTTTCTGGC-3′. A dual labeled fluorescence resonance energy transfer (FRET) hydrolysis probe (MSG-probe 5′-FAM-TAGATAGTCGAAAGGGAAA-MGE-3′) was used for detection. The cycle threshold (*Ct*) value was checked for positive samples, and defined as the replicated cycle number at which the fluorescence generated within a reaction crossed the fluorescence threshold. The positive PCR result in our study was defined as *Ct* value $\leq$37. BDG was measured by the alkaline-kinetic chromogenic Limulus method (FUNGITEC G test-MK, Fungitec, cutoff value: 20 pg/ml).

Data collection

Treatment data were collected through chart review, including patient demographic characteristics, underlying disease, laboratory data, presence of co-infection, dates of treatment start. Additionally, development of pneumothorax and pleural effusion, development of shock, need for intensive care unit (ICU) and mechanical ventilation (MV), and outcome were recorded for each patient. Additionally, we observed the drug-related adverse reactions of caspofungin. The PJP treatment regimen administered in the present study was a combination therapy with caspofungin and TMP/SMZ, compared with TMP/SMZ monotherapy.

Treatment regimen

The choice of PJP treatment was consistent during the study. Monotherapy was defined as a 21 day course of TMP/SMZ therapy, using a similar regimen for drug dosage and administration recommended by international guidelines (White et al., 2017; Benfield et al., 2008). Combination therapy was defined as the addition of caspofungin, with a dose of 70 mg on the first day and 50 mg/d from the second day. All patients with moderate to severe PJP received adjuvant glucocorticoids therapy (recommended dose of methylprednisolone or prednisone).

Definitions

The classification of PJP severity was based on PaO_2/FiO_2 while breathing room air or $D(A\text{-}a)O_2$, as follows: Mild, PaO_2/FiO_2 $>$ 70 mmHg or $D(A\text{-}a)O_2$ $<$35 mmHg; moderate, $PaO_2/FiO_2 \leq$ 70 mmHg or $D(A\text{-}a)O_2 \geq$ 35 mmHg; severe, PaO_2/FiO_2 $<$60 mmHg or $D(A\text{-}a)O_2 \geq$ 45mmHg. Treatment failure was defined as clinical deterioration occurring after 4-8 days of monotherapy or combination therapy: (1) progressive clinical deterioration characterized by the inability to maintain a stable PaO_2 despite an increase proportion of FiO_2, and/or persistent fever; (2) progressive deterioration of vital signs (pulse rate, blood pressure, and respiratory rate) and/or radiographic. Survival was defined as being alive 3 months after symptoms onset. Treatment response included one of the following: (1) amelioration or resolution of baseline signs and symptoms, chest radiograph, and hospital discharge; (2) clinical improvement sustained at least 2 to 4 weeks after cessation of antifungal therapy. In terms of co-infection, PCR was used to evaluate the plasma loads of CMV DNA for all patients; a cut-off value of $\geq$500copies/ml was considered positive CMV infection. Pulmonary aspergillosis was diagnosed by recovery in culture from a specimen obtained from respiratory samples or direct microscopic demonstration of appropriate morphologic forms with a distinctive appearance characteristic (De Pauw et al., 2008). Bacteremia was defined as the presence of microorganisms in the blood, which were detected by blood cultures.

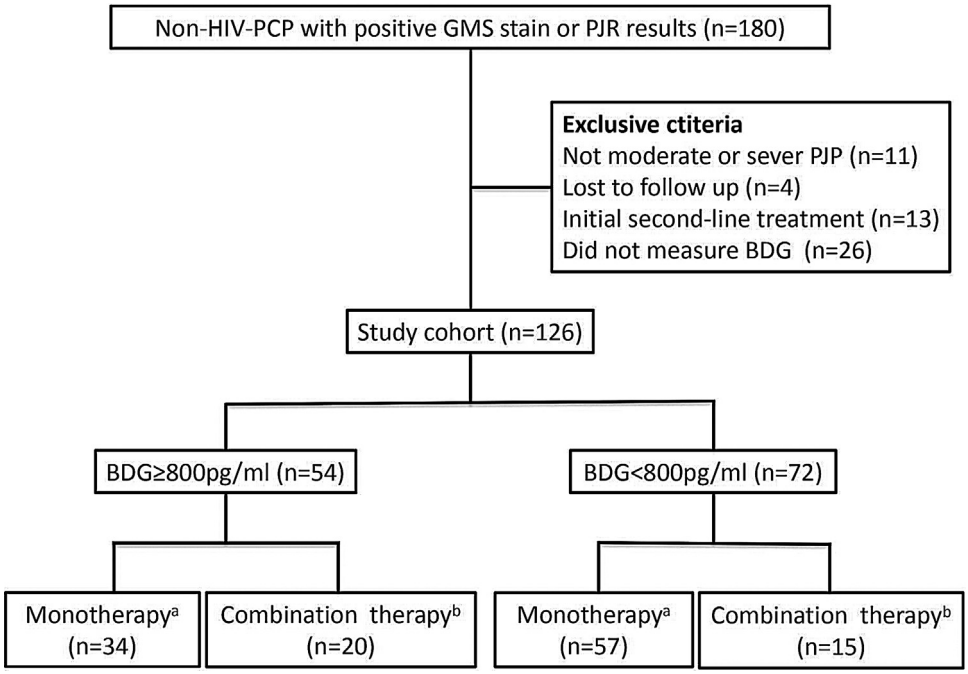

Figure 1. Study flow diagram. GMS, Grocott's methenamine silver; PCR, polymerase chain reaction; BDG, (1, 3) Beta-D-Glucan. [a]monotherapy: therapy of trimethoprim/sulfamethoxazole. [b]combination therapy: therapy of caspofungin combined with trimethoprim/sulfamethoxazole.

Statistical analysis

We compared baseline variables using the chi-square or Fisher exact test. Wilcoxon signed-rank test was used to test continuous variables. We calculated the time of symptom onset to all-cause mortality from Kaplan-Meier methods and multivariable Cox regression models. A stepwise forward method was used, and the likelihood ratio tests were carried out to identify significant independent predictors. Hazard ratios (HRs) were presented with robust 95% confidence intervals (CIs) and determined using the Wald tests. All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). P-values < 0.05 were considered statistically significant.

Results

Baseline characteristics

180 HIV-negative patients had positive PCR and/or GMS staining from respiratory samples (sputum or BALF). A total of 126 patients with moderate to severe PJP were enrolled in the study (including 117 PCR positive and 26 GMS staining positive patients, 17 of them were positive for both PCR and GMS staining). Among them, 91 patients received TMP/SMZ as initial therapy, the remaining 35 patients received caspofungin combined with TMP/SMZ as initial therapy. We divided these patients into two groups according to the level of plasma BDG, one group with BDG levels greater than or equal to 800 pg/ml (n = 54), the other group with BDG levels less than 800 pg/ml (n = 72) (Figure 1). Overall baseline characteristics stratified by the level of BDG were presented in Table 1. Included patients had a median age of 57 years, and 57% of patients (n = 71) were male. The most common underlying diseases were autoimmune diseases (n = 60, 47%), solid tumors (n = 13, 10%), and membranous nephropathy (n = 17, 13%). Most patients were associated with an immune-compromised state in the 3 months preceding PJP onset, 91% of patients (n = 115) received medication with corticosteroids and 75% of patients (n = 94) received immunosuppressant therapy. The median of CD4 T lymphocytes count was 108 cells/mm^3, which was far below the normal level. Most patients showed bilateral ground-glass opacity (GGO) on computed tomography (CT) scan (n = 107, 85%). The complication of pneumothorax occurred in 4 patients (3%) and 29 patients (23%) developed pleural effusion. The impact of concomitant infection on outcome of drug treatment was assessed. Rates of concomitant infection were bacteremia = 12%, pulmonary aspergillosis = 5%, CMV infection = 54%. The median time from symptom onset to PJP treatment was 9 days (interquartile range from 5 to 17 days).

Outcomes in all patients

In the study, 54% of patients (n = 68) were admitted to intensive care unit (ICU) and 45% of patients (n = 57) received invasive mechanical ventilation (MV). The overall positive response rate of PJP treatment was 59% (n = 54) and 69% (n = 24) in the monotherapy and combination therapy, respectively (p = 0.339). Patients who received combination therapy had a lower 3 month mortality, but there was no statistically significant difference (23%, n = 8 vs. 36%, n = 33; p = 0.150) (Table 2). Response to treatment of PJP at 21days, both overall and stratified by treatment status, is shown in Table 3 (if the patients died within 21 days after treatment, we selected the final data before death). After patients received TMP/SMZ monotherapy, there was a significant decrease in the level of serum LDH (476 [IQR 323–719] to 382 [IQR 280–523] U/L; p = 0.020). However, there was no significant decline of LDH in patients with combination therapy. Both of the two groups had a significant decline in plasma BDG (monotherapy: $p < 0.001$; combination therapy: p = 0.028). The Kaplan-Meier curve indicates that use of two different treatment regimens were associated with no significant difference (P = 0.842) (Figure 2). In the multivariate analysis using a Cox proportional hazard model, low lymphocyte counts [p = 0.030, HR = 4.937 (95% CI = 1.166–20.902)], high

Table 1
Demographic and clinical features of patients at the time of initiation of treatment.

Variables	All patients (n = 126)	BDG ≥ 800 pg/ml (n = 54)	BDG < 800 pg/ml (n = 72)
Age	57 (40–64)	50 (33–62)	60 (46–65)
Male	71 (57%)	25 (47%)	46 (64%)
Underlying disease			
Autoimmune diseases	60 (47%)	23 (43%)	37 (51%)
Solid tumor	13 (10%)	2 (4%)	11 (15%)
Hematological disease	6 (4%)	3 (6%)	3 (4%)
Membranous nephropathy	17 (13%)	6 (11%)	11 (15%)
Others[a]	30 (24%)		
Immunosuppressions			
Corticosteriod	115 (91%)	49 (91%)	66 (92%)
Immunosuppressants	94 (75%)	37 (69%)	57 (79%)
Laboratory results			
PaO2/FiO_2, mmHg	52 (46–60)	52 (43–61)	53 (47–60)
P(A-a)O_2, mmHg	58 (51–66)	57 (51–66)	59 (51–65)
Neutrophils ($\times 10^9$g/L)	6.0 (4.0–8.7)	6.5 (3.6–8.3)	5.9 (4.2–9.5)
Lymphocytes ($\times 10^9$g/L)	0.5 (0.3–0.9)	0.5 (0.2–0.9)	0.5 (0.3–0.9)
Albumin, g/L	27 (24–31)	26 (24–29)	28 (25–33)
Urea nitrogen, mmol/L	11 (6–36)	12 (6–38)	10 (6–32)
Creatinine, μmol/L	70 (57–107)	74 (58–143)	70 (55–92)
C-reactive protein, mg/dL	9 (6–47)	10 (6–84)	8 (6–35)
Lactate dehydrogenase, U/L	557 (409–751)	575 (346–760)	457 (316–657)
(1, 3) Beta-D-Glucan, pg/mL	618 (240–1308)	1549 (1054–2410)	274 (145–519)
CD4 T lymphocytes, cells/mm^3	108 (46–234)	104 (43–192)	115 (46–287)
Radiographic findings			
Bilateral GGOs	107 (85%)	48 (89%)	59 (82%)
Pleural effusion	29 (23%)	16 (30%)	13 (18%)
Pneumothorax	4 (3%)	2 (4%)	2 (3%)
Co-infections			
Bacteremia	15 (12%)	8 (15%)	7 (10%)
Pulmonary aspergillosis	6 (5%)	3 (6%)	3 (4%)
CMV infection	68 (54%)	27 (50%)	41 (57%)
Symptom onset to PJP treatment, days	9 (5–17)	9 (5–20)	10 (4–16)

PaO_2, arterial partial pressure of oxygen; FiO_2, fraction of inspiratory oxygen; GGO, ground-glass opacity; CMV, cytomegalovirus; PJP, *Pneumocystis jirovecii* pneumonia. Data are presented as median (interquartile range) and n (%).
[a] Others: Thrombotic thrombocytopenic purpura, aplastic anemia, Henoch-Schonlein purpura nephritis, neuromyelitis optica, radiation-induced lung injury and adult Still's disease.

Table 2
PJP treatment and patients outcomes.

	All patients				BDG≥800 pg/ml				BDG < 800 pg/ml			
Variables (n, %)	Total (n = 126)	Monotherap (n = 91)	Combination therapy (n = 35)	P-value	Total (n = 54)	Monotherapy (n = 34)	Combination therapy (n = 20)	P-value	Total (n = 72)	Monotherapy (n = 57)	Combination therapy (n = 15)	P-value
Admission to ICU	68 (54%)	47 (52%)	20 (57%)	0.663	35 (65%)	24 (71%)	11 (55%)	0.247	33 (46%)	23 (40%)	10 (67%)	0.069
Invasive mechanical ventilation	57 (45%)	38 (42%)	19 (54%)	0.206	32 (59%)	21 (62%)	11 (55%)	0.625	25 (35%)	17 (30%)	8 (53%)	0.089
Shock	28 (22%)	20 (22%)	8 (23%)	0.915	17 (32%)	12 (35%)	5 (25%)	0.432	11 (15%)	8 (14%)	3 (5%)	0.687
Positive response rate	78 (62%)	54 (59%)	24 (69%)	0.339	29 (54%)	13 (38%)	16 (80%)	<0.001	49 (68%)	41 (72%)	8 (53%)	0.216
3 month all-cause mortality[a]	43 (34%)	33 (36%)	8 (23%)	0.150	23 (43%)	19 (56%)	4 (20%)	0.010	20 (28%)	14 (25%)	4 (27%)	0.867

ICU, intensive care unit.
[a] Five patients were excluded, two patients died from other causes (one was gastrointestinal bleeding, the other was cerebral hernia), and three died from primary disease progression.

LDH levels [p = 0.025, HR = 3.542 (95% CI = 1.171–10.716)] and progression to shock [p < 0.001, HR = 22.277 (95% CI = 5.077–97.745)] were significant risk factors for death (Table 4). Similarly, there was no significant difference in risk of death at 3 months in Cox regression analysis, after adjustment for possible confounders (the level of LDH, lymphocyte counts, albumin, PaO2/FiO2 at the diagnosis of PJP and had bacteremia and shock) [p = 0.809, HR = 1.100 (95% CI = 0.508–2.380)] (Table 5).

Outcomes in the group of BDG ≥ 800 pg/ml

In patients with BDG levels greater than or equal to 800 pg/ml, the combination therapy showed a significantly higher positive response rate compared with monotherapy (80% vs. 38%; p < 0.001). Meanwhile, patients who received combination therapy had a significantly lower 3 month mortality (20% vs. 56%; p = 0.010) (Table 2). Comparing the LDH and BDG levels 21 days after

Table 3
Response to treatment at 21 days.[a]

Variables (all patients)	Total (n = 126)			Monotherapy (n = 91)			Combination therapy (n = 35)		
	Baseline	21 days	P-value	Baseline	21 days	P-value	Baseline	21 days	P-value
LDH, U/l	557 (409–751)	374 (270–679)	0.073	476 (323–719)	382 (280–523)	0.020	476 (349–757)	345 (260–1036)	0.478
BDG, pg/ml	618 (240–1308)	177 (61–490)	<0.001	527 (221–1185)	157 (50–345)	<0.001	834 (271–2003)	351 (151–675)	0.028
Variables (BDG≥800 pg/ml)	Total (n = 54)			Monotherapy (n = 34)			Combination therapy (n = 20)		
	Baseline	21 days	P-value	Baseline	21 days	P-value	Baseline	21 days	P-value
LDH, U/l	575 (346–760)	366 (281–511)	0.009	600 (302–757)	405 (286–479)	0.004	476 (374–848)	321 (263–518)	0.037
BDG, pg/ml	1549 (1054–2410)	423 (176–1097)	0.011	1540 (1068–2392)	639 (115–2605)	0.215	1943 (1013–2465)	368 (311–649)	0.007
Variables (BDG < 800 pg/ml)	Total (n = 72)			Monotherapy (n = 57)			Combination therapy (n = 15)		
	Baseline	21 days	P-value	Baseline	21 days	P-value	Baseline	21 days	P-value
LDH, U/l	457 (316–657)	381 (262–691)	0.941	450 (335–657)	381 (270–665)	0.414	475 (72–657)	606 (222–1557)	0.069
BDG, pg/ml	274 (145–519)	91 (45–187)	0.003	294 (149–544)	91 (41–181)	<0.001	265 (129–387)	182 (52–680)	0.779

LDH, Lactate dehydrogenase; BDG, (1, 3) Beta-D-Glucan.
Data are presented as median (interquartile range).
[a] If the patients died within 21 days after treatment, we selected the final data before death.

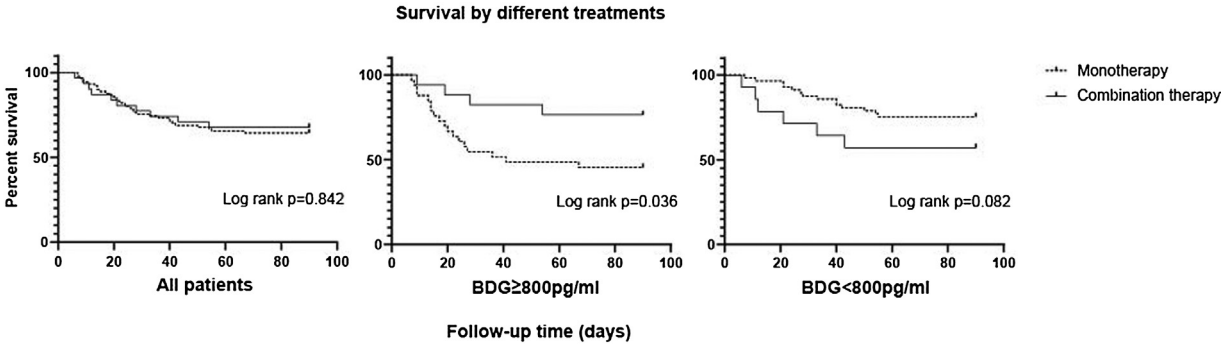

Figure 2. Kaplan-Meier plot of 3 month mortality according to treatment by combination therapy with caspofungin and TMP/SMZ and TMP/SMZ monotherapy. TMP/SMZ, trimethoprim/sulfamethoxazole; BDG, (1, 3) Beta-D-Glucan.

treatment and before treatment, we found that both LDH and BDG showed a significant decline in patients with combination therapy (LDH: 476 to 321U/L, p = 0.037; BDG: 1943 to 368 pg/ml, p = 0.007). However, there was no significant decline of BDG levels in patients with monotherapy (p = 0.215) (Table 3). The Kaplan-Meier curve indicated that patients who received combination therapy had a statistically significantly higher survival rate compared with monotherapy (P = 0.036) (Figure 2). Additionally, patients who received combination therapy were associated with a significantly decreased risk of death at 3 months in Cox regression analysis [p = 0.039, HR = 0.279 (95% CI = 0.083–0.940)] (Table 5).

Outcomes in the group of BDG < 800 pg/ml

In patients with BDG levels less than 800 pg/ml, the positive response rate of combination therapy was lower than that in the patients receiving TMP/SMZ as initial therapy (53% vs. 72%, p = 0.216). 3 month mortality in patients who received combination therapy was similar to the monotherapy group (27% vs. 25%; p = 0.867) (Table 2). Both positive response rate and mortality had no significant difference. In response to treatment of PJP at 21 days, patients received TMP/SMZ monotherapy produced the decline in the level of LDH and BDG, and they had a statistically significant decrease in LDH (294 [IQR 149–544] to 91 [IQR 41–181]; p < 0.001). However, patients who received combination therapy had increased LDH and decreased BDG (Table 3). Similar to the statistical result of all patients, there was no statistically significant difference in survival rate between two treatment regimens according to Kaplan-Meier analysis (P = 0.082) and Cox regression analysis [p = 0.171, HR = 2.216 (95% CI = 0.709–6.930)] (Figure 2 and Table 5).

Serious adverse events

All reported adverse events of patients were recorded. In our study, there were no serious events caused by caspofungin therapy, and no patients discontinued caspofungin therapy due to clinical or laboratory adverse events.

Discussion

In the year of 2006, Annaloro et al. reported the first case of a patient successfully treated with caspofungin as a replacement therapy because of TMP/SMZ intolerance (Annaloro et al., 2006). Several case series have been reported since then, and the efficacy of caspofungin was found to be favourable when it was used as a first-line or salvage therapy for PJP (Kamboj et al., 2006; Li et al., 2016; Armstrong-James et al., 2011). Additionally, caspofungin has

Table 4
Univariate and multivariable analysis of 3 month survival from PJP according to different treatments.

Characteristics	Univariate analysis		Multivariate analysis	
	χ^2 test	P-value	HR (95% CI)	P-value
Age	2.856	0.091		
Solid tumor	0.090	0.764		
Hematological disease	0.413	0.520		
$PaO2/FiO_2$, mmHg				
≥60	4.746	0.029	1	
<60			1.184 (0.342–4.096)	0.789
$D(A\text{-}a)O_2$, mmHg	1.926	0.165		
Neutrophils (×109 g/L)	1.310	0.252		
Lymphocytes (×109 g/L)				
≥0.8	9.625	0.002	1	
<0.8			4.937 (1.166–20.902)	0.030
Albumin, g/L				
≥25	6.019	0.014	1	
<25			1.181 (0.366–3.806)	0.780
Creatinine, μmol/L	1.830	0.176		
LDH, U/L				
<700	17.194	<0.001	1	
≥700			3.542 (1.171–10.716)	0.025
BDG, pg/mL				
<800	3.512	0.061	1	
≥800			1.182 (0.407–3.431)	0.758
Pleural effusion	0.847	0.357		
CMV infection	2.574	0.109		
Bacteremia				
No	4.471	0.034	1	
Yes			1.241 (0.262–5.881)	0.785
Pulmonary aspergillosis	0.011	0.916		
Shock				
No	35.234	<0.001	1	
Yes			22.277 (5.077–97.745)	<0.001
Symptom onset to PJP treatment, days	0.606	0.436		

PaO_2, arterial partial pressure of oxygen; FiO_2, fraction of inspiratory oxygen; $P(A\text{-}a)O_2$, alveolar-arterial oxygen difference; LDH, Lactate dehydrogenase; BDG, (1, 3) Beta-D-Glucan; CMV, cytomegalovirus; PJP, *Pneumocystis jirovecii* pneumonia.

Table 5
Risk of mortality at 3 months according to treatment by time-updated Cox regression analysis.

Variables	Unadjusted		Adjusted[c]	
	OR (95% CI)	P-value	OR (95% CI)	P-value
All patients				
Monotherapy[a]	1		1	
Combination therapy[b]	0.943 (0.464–1.919)	0.872	1.100 (0.508–2.380)	0.809
BDG ≥ 800 pg/ml				
Monotherapy[a]	1		1	
Combination therapy[b]	0.335 (0.113–0.991)	0.048	0.279 (0.083–0.940)	0.039
BDG < 800 pg/ml				
Monotherapy[a]	1		1	
Combination therapy[b]	2.137 (0.821–5.567)	0.120	2.216 (0.709–6.930)	0.171

BDG, (1, 3) Beta-D-Glucan.
[a] Monotherapy: therapy of trimethoprim/sulfamethoxazole.
[b] Combination therapy: therapy of caspofungin combined with trimethoprim/sulfamethoxazole.
[c] Adjusted for the level of LDH, lymphocyte counts, albumin, PaO_2/FiO_2, bacteremia and shock.

demonstrated prophylactic and therapeutic efficacy in animal models of PJP (Lobo et al., 2013). Nevertheless, the clinical use of caspofungin against PJP in the treatment of PJP is still controversial and questionable. A trial involving the caspofungin salvage therapy of PJP showed a high success rate (80%) among HIV patients (Armstrong-James et al., 2011). In contrast, Kim et al. described four HIV-negative patients with PJP who had no response to caspofungin as a salvage therapy (Kamboj et al., 2006). Up to now, there have been no multi-center randomized controlled clinical trials to further verify the efficacy and safety of caspofungin in the treatment of PJP.

In our study, 126 patients were included, and the results showed that there was no significant difference in positive response rate and 3 month all-cause mortality between the treatment regimen of combination therapy with caspofungin and TMP/SMZ and TMP/SMZ monotherapy. However, when we divided these patients into two groups according to the level of plasma BDG, we found that caspofungin and TMP/SMZ as initial therapy were more effective than TMP/SMZ monotherapy in patients when the level of BDG≥800 pg/ml. However, combination therapy had no advantage when the level of BDG<800 pg/ml. The results of the present study suggested that caspofungin and TMP/SMZ exhibit synergistic effects in higher initial BDG concentration. When the level of BDG ≥ 800 pg/ml, the positive response rate of the combination therapy increased from 38% to 80%. Additionally, there was a 36% reduction in all-course mortality rates among

patients receiving combination therapy. Additionally, patients with combination therapy required less ICU hospitalization and invasive mechanical ventilation, and they were less likely to experience shock.

(1, 3) Beta-D-Glucan is a reliable adjunctive diagnostic marker for PJP. Consistently decreasing BDG during treatment has been shown to produce favorable therapeutic responses in multiple groups of PJP patients (Theel and Doern, 2013; Held and Wagner, 2011). Echinocandins are antifungal drugs that non-competitively inhibit (1, 3) Beta-D-Glucan synthase. Therefore, echinocandins are toxic to fungi in which the glucans play an important role in maintaining the integrity of the fungal cell wall and partly contribute to the host inflammatory response in the lung (Damiani et al., 2013; Song and Stevens, 2015). This may be the basis for the efficiency of combination therapy with caspofungin in PJP patients with high BDG concentration. The other synergistic mechanisms may be as follows: (1)TMP/SMZ only affects trophic forms by interfering with folate metabolism, but caspofungin can affect cyst formation by inhibiting (1, 3) Beta-D-Glucan synthesis. Since we have no way to determine how the trophic and cystic forms change during the treatment course, elevated BDG levels hinted that cysts had been producing glucans on which caspofungin might have an effect (Ekholm, 2009). (2) TMP/SMZ acts slowly and requires 5 to 8 days to induce a curative effect, while caspofungin has a rapid onset (Roux et al., 2014). Therefore, it may be inferred that the combination therapy with caspofungin can compensate for the defects of TMP/SMZ in patients with delayed PJP treatment. (3) Increasing evidence of mutations in the genes that encode dihydropteroate synthase may lead to drug resistance of sulfa agents, and several studies have demonstrated that it was associated with treatment failure (Wickramasekaran et al., 2017; Nahimana et al., 2003). With the attention paid to the prevention of PJP, long-term oral administration of TMP/SMZ may increase the risk of drug resistance. Therefore, a prompt and effective treatment is urgently needed in critical situations. (4) PJP is an opportunistic infection. A part of PJP cases are associated with other fungi such as aspergillosis, which is considered a risk factor increasing the mortality of PJP (Markantonatou et al., 2017). Additionally, the level of BDG can also rise with some fungi such as aspergillosis and candida. Therefore, caspofungin may achieve a dual therapeutic result in some cases of co-infection.

In our study, the combination therapy had no advantage when the level of BDG < 800 pg/ml. Additionally, there was a trend indicating that the combined therapy was associated with increased risk of death. We have the following considerations. Firstly, we preferred to choose combination therapy rather than TMP/SMZ monotherapy for patients with more severe clinical symptoms and imaging manifestations, which may have a worse prognosis. Secondly, it reflected that the effect of caspofungin may not be so satisfactory when the BDG levels were less than 800 pg/ml. Thirdly, the number of patients who received combination therapy when the level of BDG < 800 pg/ml was small, which cannot represent the therapeutic effects of all patients.

Regarding drug side effects, caspofungin does not inhibit the CYP system and does not induce CYP3A4 drug metabolism; the incidence of adverse events from caspofungin is very low (Song and Stevens, 2015). A review of clinical trials showed that less than 3% of patients experienced serious adverse events or discontinued therapy due to caspofungin-related adverse events (Ngai et al., 2011). In our study, none of the serious adverse events were attributed to caspofungin therapy, and none of the patients discontinued caspofungin due to clinical or laboratory adverse events. Therefore, the combination therapy of caspofungin and TMP/SMZ dose not increase this risk compared with TMP/SMZ monotherapy. Caspofungin has few serious adverse events, but it dose have some limitations. Since caspofungin chiefly targets the cysts and is less effective against the trophic forms, treatment of PJP with caspofungin alone will not likely result in eradication of infection (Lobo et al., 2013; Cushion et al., 2010). Additionally, owing to the high molecular weight of echinococcins, oral administration of echinococcins has little absorption, so only intravenous dosage forms are currently available.

In this study, we investigated prognostic factors of HIV-negative patients with PJP; multivariate analysis identified lower lymphocyte count, higher LDH level at the diagnosis of PJP and progression to shock as risk factors for death. These risk factors for death were similar to the known risk factors for occurrence of PJP (Liu et al., 2017; Brakemeier et al., 2016). Most of our patients were immunosuppressive individuals. We assume that lymphocyte count represents the overall effect of immunosuppressive therapy and can be a detectable biomarker. Because low lymphocyte count is associated with poor prognosis, continuous monitoring of lymphocyte count is important to understand the increased risk of PJP in individuals receiving various immunosuppressive treatments. In this study, the level of serum LDH was significantly higher in the non-survivors than in the survivors. Additionally, we found a significant decline in LDH in patients with effective treatment. We considered that the level of serum LDH is related to the severity of the disease; it can contribute to assessing the condition of patients during treatment.

This present study had several limitations. Firstly, certain confounding factors in BDG testing carry a risk of confusion, such as previous antifungal use, treatment with albumin or immunoglobulin, renal insufficiency, catheter removal, or hemodialysis (Kanamori et al., 2009) This would create systematic errors in grouping according the level of BDG. Moreover, due to the small sample size, the study may not be able to accurately identify the differences between different treatment groups. To overcome these limitations and obtain more evidence, larger and multi-center randomized controlled trials are necessary.

Conclusion

The implications of our study indicate that high initial plasma (1, 3) Beta-D-Glucan concentration may be a predictor of satisfactory caspofungin combined with TMP/SMZ response in HIV-negative patients with PJP. Based on our findings, we suggest the choice of combination therapy with caspofungin and TMP/SMZ as the initial treatment when plasma BDG≥800 pg/ml in HIV-negative patients with moderate to severe *Pneumocystis jirovecii* pneumonia. We found equivalent efficacy from combination therapy and TMP/SMZ monotherapy in all patients without BDG grouping, therefore, we agree with the consistent efficacy of first-line treatment with TMP/SMZ.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical standards

Compliance with ethical standards: All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

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