J Infect Chemother 25 (2019) 936–942

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Serial change in serum biomarkers during treatment of Non-HIV *Pneumocystis* pneumonia☆

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ARTICLE INFO

Article history:
Received 4 February 2019
Received in revised form
8 May 2019
Accepted 14 May 2019
Available online 8 June 2019

Keywords:
Pneumocystis jirovecii
Non-HIV *Pneumocystis* pneumonia
β-D glucan
Krebs von den Lungen-6 antigen
Surfactant protein-D

ABSTRACT

Background: For patients with non–human immunodeficiency virus (HIV) *Pneumocystis* pneumonia (PCP), data are limited on serial changes in serum biomarkers and the correlations with clinical outcomes.
Objective: This study evaluated serial change in serum biomarkers and clinical outcomes of non-HIV PCP.
Methods: We retrospectively reviewed data from 63 patients treated for non-HIV PCP at Toho University Omori Medical Center. The patients were classified as survivors and nonsurvivors on the basis of 60-day PCP mortality. The groups were compared for clinical course and levels of serum biomarkers (β-D glucan, Krebs von den Lungen-6 antigen [KL-6], and surfactant protein-D [SP-D]), which were measured at baseline, and 7 days and 14 days after starting treatment. In addition, serial changes in serum biomarkers were analyzed in survivors and nonsurvivors.
Results: There were 14 PCP nonsurvivors and 49 survivors. Biomarker values were not different between groups at baseline. At 7 and 14 days after starting treatment, the proportions of patients with elevated β-D glucan and KL-6 did not significantly differ between groups; however, the proportion of patients with elevated SP-D was significantly lower among survivors than among nonsurvivors (57.1% vs. 100%, $p = 0.009$; 30% vs. 100%, $p < 0.001$; respectively). SP-D on day 14 was significantly lower than that at baseline among survivors (99.6 [61.0–190.3] vs. 156 [100.8–283.5]; $p = 0.045$) but significantly higher among nonsurvivors (974 [744.5–1565] vs. 317 [211–448]; $p = 0.03$).
Conclusion: Serum SP-D value continues to increase after failure of treatment for non-HIV PCP and may thus be associated with outcomes for non-HIV PCP patients.

1. Introduction

Pneumocystis pneumonia (PCP), a pulmonary infection caused by *Pneumocystis jirovecii* (*P. jirovecii*), can manifest as an opportunistic infection in persons with human immunodeficiency virus (HIV) or other causes of immunodeficiency. Persons with autoimmune disorders, recipients of organ transplants, and patients with malignant neoplasms often receive massive doses of corticosteroids and immunosuppressants for treatment or palliative care. The prevalence of PCP has thus been increasing in patient groups other than HIV-infected patients [1].

Recent studies of the clinical characteristics, outcomes, and prognostic factors for non-HIV PCP and HIV PCP [2–27] indicate that these conditions differ in clinical manifestations and characteristics and prognosis. Non-HIV PCP tends to have a more rapid clinical course. The mortality rate was estimated to be 0–10% for HIV PCP [5,6,10,16,26,27] and 17.2–66.7% for non-HIV PCP [2–27]. Although factors such as time to start of PCP treatment and use of mechanical ventilation were reported to be prognostic factors for non-HIV PCP [5,13–15,19,20,23], no study has evaluated clinical course, such as oxygen requirement and chest radiography findings, after treatment in non-HIV PCP patients. Previous studies reported that serum concentrations of β-D glucan, Krebs von den Lungen-6 antigen (KL-6), and surfactant protein D (SP-D) are elevated in patients with PCP [28–30], but few reports evaluated serial change in these levels after the start of treatment for non-HIV PCP [15,31].

This study evaluated post-treatment clinical course and serial change in the serum markers including β-D glucan, KL-6, and SP-D

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https://doi.org/10.1016/j.jiac.2019.05.007
1341-321X/

in patients with non-HIV PCP and attempted to identify predictors of non-HIV PCP death at 2 months.

2. Patients and methods

2.1. Study design

A total of 65 patients with non-HIV PCP were treated during the period from January 2007 through September 2018 at Toho University Omori Medical Center. This single-center retrospective cohort study analyzed the clinical records of these 63 patients. On the basis of 60-day survival after the start of PCP treatment, the patients were classified as survivors (n = 49) and nonsurvivors (n = 14). Two patients who died of causes other than PCP during PCP treatment were excluded from the analysis. We assessed differences between the groups in clinical characteristics, underlying disease, comorbidities, time from symptom onset to treatment, laboratory data, treatment regimen, and adjuvant corticosteroid therapy. The proportions of patients with increased oxygen requirement and exacerbations on chest radiography findings at 48–72 h, 7 days, and 14 days after the start of treatment compared to baseline were compared between groups. The proportions of patients with higher levels of β-D glucan, KL-6, and SP-D at 7 days and 14 days after the start of treatment than at baseline and with consecutive increases at 7 and 14 days after baseline (The patients following criteria were met: values on baseline < day 7 < day 14) were also compared between groups. Chest radiography findings were assessed by 2 respiratory specialists. Serial changes in β-D glucan, KL-6, and SP-D in survivors and nonsurvivors were analyzed. Values on days 7 and 14 were compared with those at baseline. In addition, univariate and multivariate logistic regression analysis was used to identify factors independently associated with 60-day mortality in non-HIV PCP patients. Data collected from days 6 through 8 were classified as “day 7”, and data collected from days 12 through 16 were classified as “day 14”.

2.2. Diagnosis of non-HIV PCP

A diagnosis of non-HIV PCP was made when all of the following criteria were met: 1) compromised cellular immunity due to corticosteroid or immunosuppressant therapy; 2) presence of symptoms such as dry cough, dyspnea, and fever; 3) diffuse, bilateral interstitial infiltrates on plain chest radiography; and 4) *P. jirovecii* cysts identified by Grocott silver staining, or positive detection of PCP by nested PCR of bronchoalveolar lavage fluid or sputum specimens, as previously described [32].

2.3. Ethics

This study was approved by the Internal Review Board (IRB) of Toho University Omori Medical Center (IRB approval No. M18119).

2.4. Data collection

The following patient data were collected: age, sex, body mass index, smoking history, underlying disease and underlying immunosuppressive conditions, comorbidities, chest CT findings, time from symptom onset to start of PCP treatment, trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis, ratio of partial pressure of arterial O_2 to the fraction of inspired O_2 (PaO_2/FiO_2 ratio), medications and adjuvant corticosteroids used to treat PCP, and 60-day mortality. In addition, we collected laboratory data on serum C-reactive protein (CRP), lactate dehydrogenase, β-D glucan, KL-6, and SP-D at the start of treatment and at 48–72 h, 7 days, and 14 days after starting PCP treatment. Serum β-D glucan was measured by using the Fungitec G test (Seikagaku Corporation, Tokyo, Japan), in 2007–2015, or the WAKO turbidimetric assay (Wako Pure Chemical Industries, Tokyo, Japan), in 2016–2017. Commercially available ELISA kits were used to determine serum concentrations of KL-6 (Eisai Corporation, Tokyo, Japan) and SP-D (Yamasa, Chiba, Japan). The serum cut-off levels were 500 U/ml for KL-6 and 110 ng/ml for SP-D.

2.5. Statistical analysis

The data are presented as numbers and percentages of patients. Age, body mass index, time from symptom onset to treatment, daily prednisolone dose, PaO_2/FiO_2 and laboratory data are expressed as median plus interquartile range. Associations of categorical and continuous variables in survivors and nonsurvivors were tested with the chi-square or Fisher exact test, or the paired t-test, respectively. Odds ratios (ORs) for 60-day PCP mortality were evaluated by univariate and multivariate logistic regression, and multivariate logistic regression was performed by using a stepwise method. A P value of <0.05 was considered to indicate statistical significance. Statistical analyses were performed with SPSS version 22 software (IBM, NY, USA).

3. Results

3.1. Patient characteristics

There were 49 survivors and 14 nonsurvivors. Survivors were significantly younger than nonsurvivors (63 [48–75] vs. 72.5 [70–75] years, respectively; $p < 0.001$), had a lower malignancy rate (22.4% vs. 57.1%; $p = 0.02$) and CRP level (5.4 [2.4–9.1] vs. 12.8 [8.1–19.8] mg/dl; $p = 0.003$), and a higher PaO_2/FiO_2 ratio (282.9 [236.2–356.2] vs. 219.3 [169.8–232.1] mmHg; $p = 0.001$) and albumin concentration (3.0 [2.7–3.4] vs. 2.3 [2.0–2.7] g/dl; $p = 0.001$). The groups did not significantly differ at baseline in β-D glucan (188 [68.4–473] vs. 504.5 [166–665.5] pg/ml, respectively; $p = 0.789$), KL-6 (657 [448–1097] vs. 682 [567.8–1012.3] U/ml; $p = 0.696$) or SP-D (156 [100.8–283.5] vs. 317 [211–448] ng/ml; $p = 0.124$) values (Table 1). No patient received trimethoprim-sulfamethoxazole (TMX-SMX) prophylaxis for PCP.

3.2. Clinical course of non-HIV PCP

Table 2 shows the clinical course of non-HIV PCP. At 48–72 h after starting PCP treatment, the proportion of patients with exacerbation in relation to oxygen requirement was significantly lower for survivors than for nonsurvivors (38.8% vs. 78.6%, respectively; $p = 0.014$), but the proportion of patients with exacerbation on chest radiography did not significantly differ between groups (54.5% vs. 78.6%; $p = 0.13$).

At 7 days after starting treatment, the proportions of patients with exacerbation in relation to oxygen requirement and chest radiography were significantly lower among survivors than among nonsurvivors (12.2% vs. 92.9%, respectively, for oxygen requirement, $p < 0.001$; 11.1% vs. 78.6%, respectively, for chest radiography, $p < 0.001$). Although the proportions of patients with elevated β-D glucan and KL-6 did not significantly differ between groups, the proportion of patients with elevated SP-D was significantly lower among survivors (57.1%, vs. 100% for nonsurvivors; $p = 0.009$).

At 14 days after starting treatment, the proportions of patients with exacerbation in relation to oxygen requirement and chest radiography were significantly lower among survivors than among nonsurvivors (2.1% vs. 100%, respectively, for oxygen requirement, $p < 0.001$; 0% vs. 81.8%, respectively, for chest radiography, $p < 0.001$). Although the proportions of patients with elevated β-D

Table 1
Characteristics and laboratory findings for survivors and nonsurvivors.

Characteristic	Survivors	Nonsurvivors	*P* Value
No. of patients	49	14	
Median age, years (range)[a]	63 (48–75)	72.5 (70–75)	**<0.001**
Gender: female, n (%)	31 (63.3)	6 (42.9)	0.223
Median BMI, kg/m[b](range)[a]	20.2 (17.9–23.4)	19.7 (18.9–21.0)	0.416
Smoking: never, n (%)	29 (55.1)	7 (50)	0.557
Underlying disease, n (%)			
Kidney transplantation	14 (28.6)	1 (7.1)	0.156
Autoimmune disorder	24 (49.0)	5 (35.7)	0.229
Malignancy	11 (22.4)	8 (57.1)	**0.02**
Comorbidities, n (%)			
Chronic kidney disease	21 (42.9)	5 (35.7)	0.762
Diabetes mellitus	6 (12.2)	0	0.323
Chronic liver disease	4 (8.2)	2 (14.3)	0.177
Underlying pulmonary disease, n (%)			
Emphysema	4 (8.2)	4 (28.6)	0.065
Interstitial pneumonia	4 (8.2)	3 (21.4)	0.177
Median time from symptom onset to treatment, days (range)[a]	7 (5–10)	8.5 (4.3–13.8)	0.403
Corticosteroid use at diagnosis, n (%)	45 (91.8)	13 (92.9)	1
Median daily prednisolone dose, mg (range)[a]	10 (5–20)	20 (10–25)	0.093
PCP prophylaxis before diagnosis, n (%)	0 (0)	0 (0)	
PaO_2/FiO_2 ratio, mmHg (range)[a]	282.9 (236.2–356.2)	219.3 (169.8–232.1)	**0.001**
Laboratory Data: median (range)[a]			
WBC, (/μl)	7900 (6100–9800)	9300 (3950–11325)	0.855
Lymphocytes, (/μL)	697 (578.8–1102.5)	512.5 (244.3–918.8)	0.125
Albumin, (g/dL)	3.0 (2.7–3.4)	2.3 (2.0–2.7)	**0.001**
LDH, (IU/L)	398 (306–560)	476.5 (413.5–576.3)	0.407
CRP, (mg/dL)	5.4 (2.4–9.1)	12.8 (8.1–19.8)	**0.003**
β-D glucan, (pg/mL)	188 (68.4–473)	504.5 (166–665.5)	0.789
KL-6, (U/mL)	657 (448–1097)	682 (567.8–1012.3)	0.696
SP-D, (ng/mL)	156 (100.8–283.5)	317 (211–448)	0.124
Treatment regimen, n (%)			
1st line used Trimethoprim-sulfamethoxazole	42 (85.7)	14 (100)	0.325
1st line used Pentamidine	5 (10.2)	0 (0)	**<0.001**
1st line used Atovaquone	2 (4.1)	0 (0)	0.734
Adjuvant corticosteroid therapy[b],n (%)	42 (85.7)	14 (100)	0.333
Methylprednisolone 1000 mg/day	14 (28.6)	12 (85.7)	**<0.001**
Prednisolone 80 mg/day	11 (22.4)	1 (7.1)	0.716
Prednisolone 1 mg/kg	5 (10.2)	0	0.578
Prednisolone 0.5 mg/kg	12 (24.5)	1 (7.1)	1

PCP, *Pneumocystis jirovecii* pneumonia; WBC, white blood cells; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6, SP-D, Surfactant protein-D; CRP, C-reactive protein.
[a] Inter quartile range.
[b] Maximum dose.

Table 2
Disease exacerbation and changes in biomarkers after the start of treatment, by survival status of non-HIV PCP patients.

Characteristic	Survivors	Nonsurvivors	*P* Value
48–72 h after the start of treatment: % (n)			
Increased oxygen requirement[a]	38.8% (19/49)	78.6% (11/14)	**0.014**
Exacerbations on chest X-ray[a]	54.5% (24/44)	78.6% (11/14)	0.13
7 days after the start of treatment: % (n)			
Increased oxygen requirement[a]	12.2% (6/49)	92.9% (13/14)	**<0.001**
Exacerbations on chest X-ray[a]	11.1% (5/45)	78.6% (11/14)	**<0.001**
Increased in β-Dglucan[a]	22.7% (10/44)	30.8% (4/13)	0.715
Increased in KL-6[a]	80.6% (29/36)	100% (11/11)	0.175
Increased in SP-D[a]	57.1% (16/28)	100% (11/11)	**0.009**
14 days after the start of treatment: % (n)			
Increased oxygen requirement[a]	2.1% (1/48)	100% (11/11)	**<0.001**
Exacerbations on chest X-ray[a]	0% (0/46)	81.8% (9/11)	**<0.001**
Increased in β-Dglucan[a]	7.9% (3/38)	25% (2/8)	0.203
Increased in KL-6[a]	80.6% (25/31)	100% (9/9)	0.192
Increased in SP-D[a]	30.8% (8/26)	100% (8/8)	**0.001**
Consecutive increase 7 and 14 days from baseline: % (n)			
Increased in β-Dglucan[a]	0% (0/33)	25% (2/8)	0.033
Increased in KL-6[a]	46.2% (12/26)	85.7% (6/7)	0.095
Increased in SP-D[a]	4.8% (1/21)	66.7% (4/6)	0.004

PCP, *Pneumocystis jirovecii* pneumonia; LDH, lactate dehydrogenase; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6, SP-D, Surfactant protein-D.
[a] The rate of patients.

glucan and KL-6 did not significantly differ between groups, the proportion of those with elevated SP-D was significantly lower among survivors (30.8% vs. 100% among nonsurvivors; $p = 0.001$).

Although the proportion of patients with consecutive increases in KL-6 at days 7 and days 14 from baseline did not significantly differ between groups, the proportions of those with consecutive increases in β-D glucan and SP-D were significantly lower among survivors than among nonsurvivors (0% vs. 25%, respectively, for β-D glucan, $p = 0.033$; 4.8% vs. 66.7%, respectively, for SP-D, $p = 0.004$).

3.3. Serial changes in serum markers

Fig. 1 shows serial changes in serum β-D glucan level. Among survivors, the mean value on day 7 did not significantly differ from that at baseline (102.6 [37.7–462.5] vs. 188 [68.4–473] pg/ml, respectively; $p = 0.184$); however, the value on day 14 was significantly lower than that at baseline (36.9 [19–83.1] vs. 188 [68.4–473] pg/ml, respectively; $p = 0.004$). Among nonsurvivors, β-D glucan levels on days 7 and 14 did not significantly differ from that at baseline (150 [98.4–570] vs. 504.5 [166–665.5] pg/ml, respectively, for day 7, $p = 0.883$; 88.2 [29.3–365.3] vs. 504.5 [166–665.5] pg/ml, respectively, for day 14, $p = 0.697$).

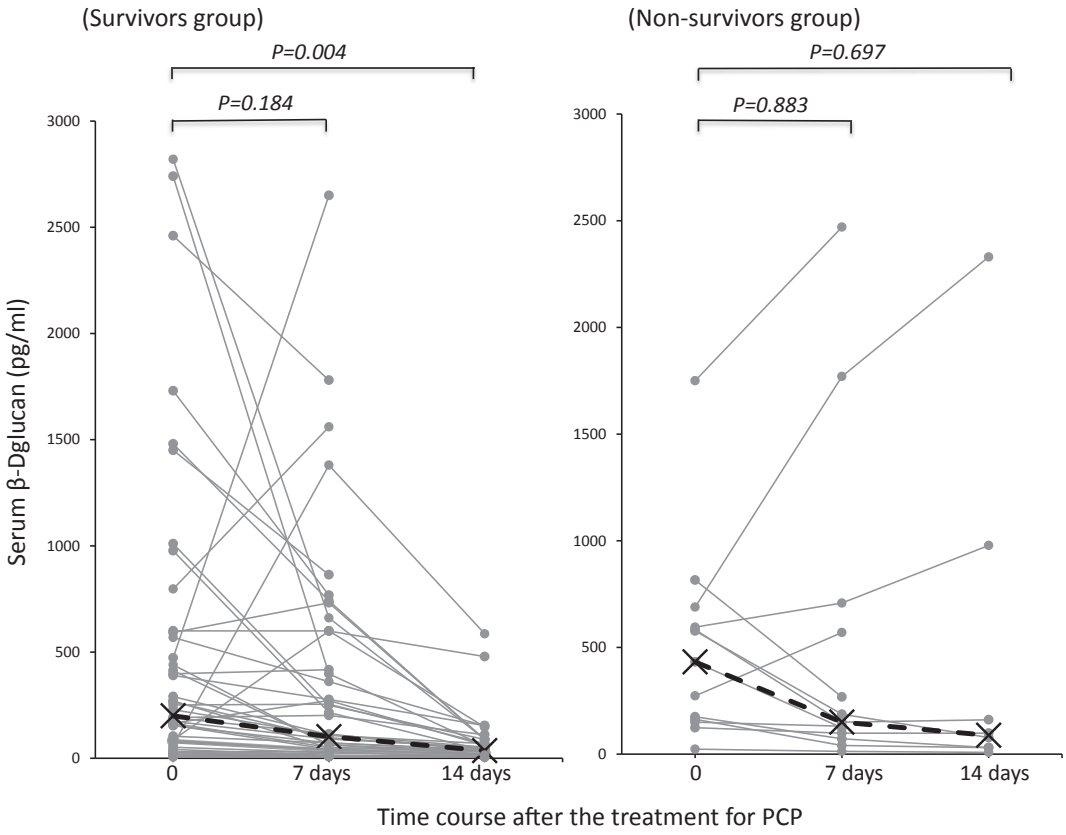

Fig. 1. The gray lines show change in serum β-D glucan levels in each patient after starting PCP treatment. The black dotted lines show the median for all patients. The paired t-test was used to compare values at 1 week and 2 weeks with baseline values.

Fig. 2 shows serial changes in serum KL-6 level. Among survivors, the mean values on days 7 and 14 were significantly higher than at baseline (997 [642.5–1391.3] vs. 657 [448–1097] U/ml, respectively, for day 7, $p < 0.001$; 1001 [749–1409.5] vs. 657 [448–1097] U/ml, respectively, for day 14, $p < 0.001$). Among nonsurvivors, the mean values on days 7 and 14 were also significantly higher than that at baseline (1590 [1144.5–2192] vs. 682 [567.8–1012.3] U/ml, respectively, for day 7, $p = 0.014$; 2777 [2334–5080] vs. 682 [567.8–1012.3] U/ml, respectively, for day 14, $p = 0.003$).

Fig. 3 shows serial changes in serum SP-D level. Among survivors, the mean value on day 7 was not significantly different from that at baseline (180.5 [101.8–376.3] vs. 156 [100.8–283.5] ng/ml, respectively; $p = 0.234$); however, the mean on day 14 was significantly lower than that at baseline (99.6 [61.0–190.3] vs. 156 [100.8–283.5] ng/ml, respectively; $p = 0.045$). Among nonsurvivors, the mean values on days 7 and 14 were significantly higher than that at baseline (1140 [717.5–1330] vs. 317 [211–448] ng/ml, respectively, for day 7, $p = 0.004$; 974 [744.5–1565] vs. 317 [211–448] ng/ml, respectively, for day 14, $p = 0.03$).

3.4. Risk factors for non-HIV PCP 60-day mortality

Table 3 shows the results of univariate and multivariate logistic regression analyses of independent associations with PCP 60-day mortality. Univariate analysis showed that higher malignancy rate (ORs, 4.606; 95% confidence interval [CI], 1.315–16.13; $p = 0.017$) older age (ORs, 1.066; 95% CI, 1.008–1.128; $p = 0.025$), lower PaO_2/FiO_2 ratio (ORs, 0.99; 95% CI, 0.983–0.998; $p = 0.01$), lower serum albumin (ORs, 0.169; 95% CI, 0.05–0.569; $p = 0.004$), higher serum CRP (ORs, 1.252; 95% CI, 1.094–1.432; $p = 0.001$) and consecutive increases in SP-D at 7 and 14 days from baseline (ORs, 40; 95% CI, 2.884–554.711; $p = 0.006$) were independently associated with PCP 60-day mortality. Multivariate analysis showed that a consecutive increase in SP-D at 7 and 14 days from baseline (ORs, 36.425; 95% CI, 1.911–694.172; $p = 0.017$) was independently associated with 60-day mortality.

4. Discussion

Previous studies of non-HIV PCP patients reported a mortality rate of 17.2–60%; however, when studies of patients with acute respiratory failure and those requiring mechanical ventilation are excluded [2,4–17,19,20,22,23,25–27], the mortality rate ranged from 20% to 40% [2,4–9,12,14,15,19,20,22,23,25,26], which was similar to the rate of 22.2% observed in the present study.

The present nonsurvivors had a higher malignancy rate. A previous study reported that patients with malignancies had a higher mortality rate than did those with other immunosuppressive conditions [8]. The general condition of patients with malignancy as the underlying disease may be worse than that of patients with other underlying diseases. This hypothesis is supported by the fact that the present nonsurvivors were older and had lower albumin levels. Poor performance status was reported to be an independent risk factor for poor prognosis in non-HIV PCP [2]. Another possibility is that inflammatory cytokine (IC) storm, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8, is associated with poor outcomes. Patients with malignancies release numerous ICs, and ICs such as IL-6 and TNF-α are associated with cancer cachexia syndrome [33]. A previous study reported that non-HIV PCP is a systemic inflammatory reaction caused by

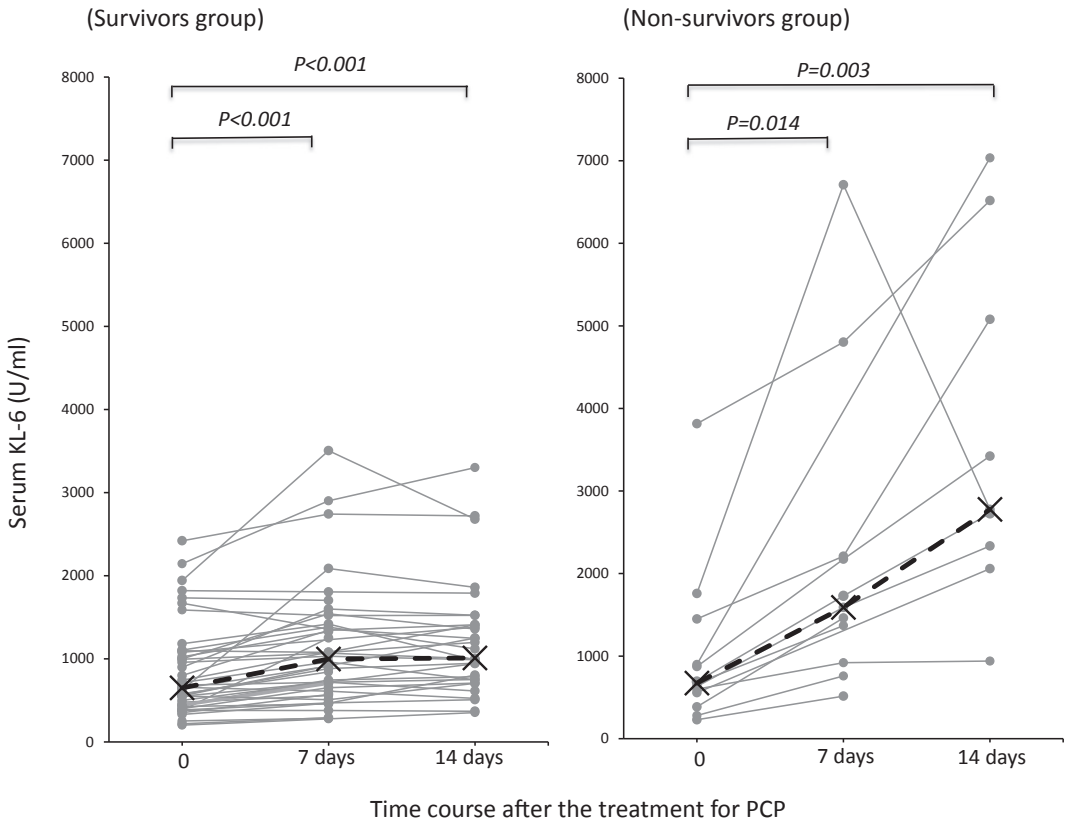

Fig. 2. The gray lines show change in serum KL-6 levels in each patient after starting PCP treatment. The black dotted lines show the median for all patients. The paired t-test was used to compare values at 1 week and 2 weeks with baseline values.

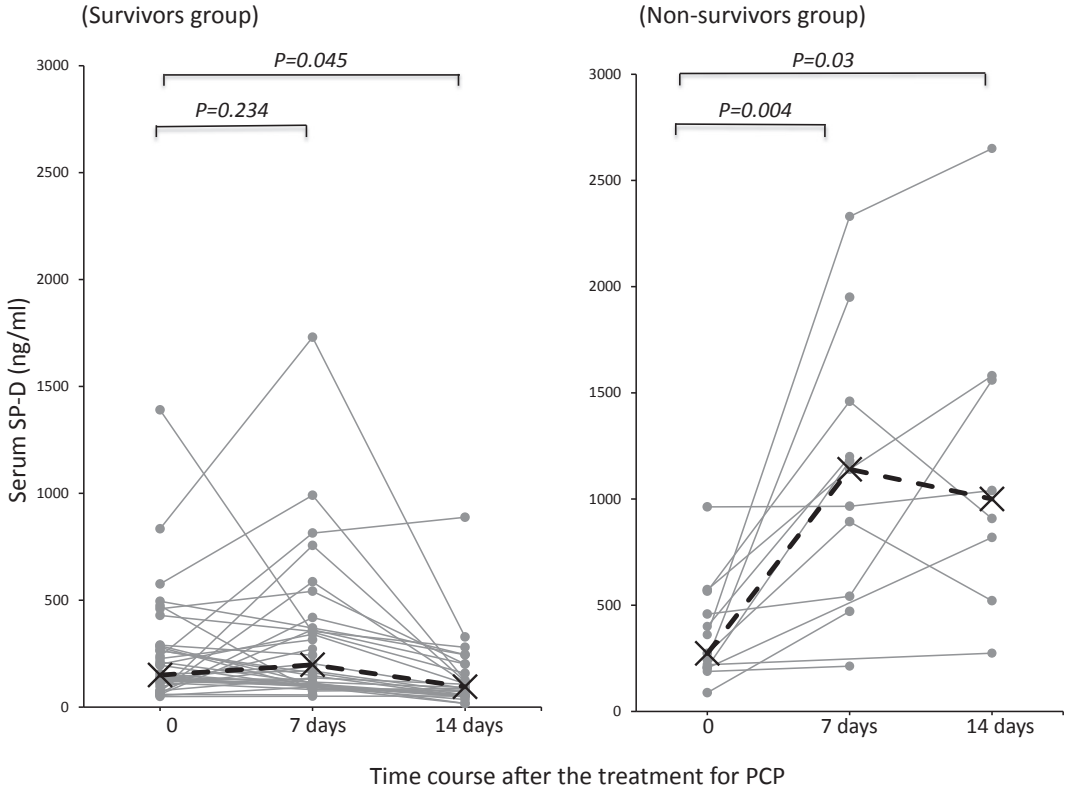

Fig. 3. The gray lines show change in serum SP-D levels in each patient after starting PCP treatment. The black dotted lines show the median for all patients. The paired t-test was used to compare values at 1 week and 2 weeks with baseline values.

Table 3
Univariate and multivariate logistic regression analysis of independent associations with 60-day mortality in non-HIV PCP patients.

Variable	Univariate logistic regression			Multivariate logistic regression		
	ORs	95%CI	p value	ORs	95% CI	p value
Malignancy	4.606	1.315–16.13	**0.017**			
Age	1.066	1.008–1.128	**0.025**			
PaO2/FiO2	0.99	0.983–0.998	**0.01**			
Albumin	0.169	0.05–0.569	**0.004**			
CRP	1.252	1.094–1.432	**0.001**	1.212	0.977–1.503	0.080
β-Dglucan at baseline	1	0.999–1.001	0.829			
KL-6 at baseline	1	0.999–1.001	0.615			
SP-D at baseline	1.002	0.999–1.004	0.163			
Increased in KL-6[a]	7	0.736–66.617	0.09			
Increased in SP-D[a]	40	2.884–554.711	**0.006**	36.425	1.911–694.172	**0.017**

PCP, *Pneumocystis jirovecii* pneumonia; KL-6, Krebs von den Lungen-6; SP-D, Surfactant protein-D.
ORs, odds ratios; CI, confidence interval.
[a] Consecutive increase 7 and 14 days from baseline.

neutrophilic lung inflammation [34] and that neutrophilic overactivation results in alveolar damage due to the release of ICs.

No study has comprehensively evaluated the clinical course of non-HIV PCP, including oxygen requirement, chest radiography findings, and the serum markers β-D glucan, KL-6, and SP-D. At 48–72 h after starting PCP treatment, the proportion of patients with exacerbation on chest radiography compared to baseline was not significantly different between survivors and nonsurvivors. Indeed, 54.5% of survivors had exacerbation on chest radiographs on day 2 or 3, perhaps because the effect of TMP-SMX is slow (a curative effect is usually noted at 5–8 days [35]) or because of paradoxical reactions (PR) to increased exposure to *P. jirovecii* antigens after chemotherapy. Although PR are not rare after the start of highly active antiretroviral therapy for HIV PCP [36], little is known about PR in non-HIV PCP. In both cases, chest radiographic findings are inadequate for early prediction of PCP outcomes.

Serum β-D glucan and KL-6 were reported to be reliable markers of PCP outcomes [28]. The sensitivity and specificity of the combination of β-D glucan and KL-6 for diagnosis of PCP were 94.3% and 89.6%, respectively [29]. In addition, a case report noted that SP-D was helpful for diagnosis of PCP [30].

β-D glucan was not elevated in most survivors and nonsurvivors at days 7 and 14 after starting PCP treatment compared to baseline. Furthermore, analysis of serial changes in β-D glucan showed no significant difference between values at baseline and day 7. These results are consistent with those of previous studies, which reported that serum β-D glucan does not reflect the severity or prognosis of PCP infection and thus may not be suitable for monitoring response to treatment [15,31,37]. Matsumura et al. reported that β-D glucan values after treatment were significantly lower in survivors and nonsurvivors [15]. These past findings suggest that lung injury due to PCP may not be directly caused by *P. jirovecii*, since β-D glucan is a cell wall component of *P. jirovecii*, and that β-D glucan value may directly reflect organism burden. Limper et al. reported that respiratory impairment and mortality were not associated with organism burden but rather with neutrophil counts in the lower respiratory tract [37]. The direct pathogenicity of *P. jirovecii* is weak, and lung injury and respiratory failure in patients with PCP closely correlate with host immune response [38].

KL-6 is a mucin-like glycoprotein with a molecular weight greater than 200 kDa, and SP-D is a member of the C-type lectin superfamily and has a molecular weight of 43 kDa [39,40]. Both are extensively produced by regenerating alveolar type II pneumocytes [41], and both are useful biomarkers in the diagnosis and management of various interstitial lung diseases (ILD) [42–45].

Analysis of serial changes in KL-6 and SP-D showed that KL-6 values at days 7 and 14 were significantly higher than baseline values for survivors and nonsurvivors. In contrast, SP-D was significantly higher at days 7 and 14 of treatment among nonsurvivors but was significantly lower at day 14 among survivors. In addition, multivariate analysis showed that a consecutive increase in SP-D at 7 and 14 days from baseline was independently associated with 60-day PCP mortality. Although serum KL-6 and SP-D both reflect lung injury, SP-D would likely enter the bloodstream more readily than would KL-6, as it has a lower molecular weight than KL-6 [45]. SP-D might improve earlier than KL-6 when lung injury improves; however, prolonged lung injury would likely result in continued elevation of both KL-6 and SP-D. Nakamura et al. suggested that ΔSP-D is more suitable than ΔKL-6 in evaluating ILD induced by anticancer agents [42]. Our findings suggest that the immune response against *P. jirovecii* injures alveolar type II pneumocytes. Furthermore, SP-D might reflect the severity of lung injury due to *P. jirovecii* and remain elevated in PCP patients with prolonged lung injury that may lead to respiratory failure.

Although some reports indicate that prophylaxis reduces PCP incidence in non-HIV immunocompromised patients [46], none of the present patients received TMP-SMX as a prophylactic agent for PCP. Current guidelines recommend PCP prophylaxis for immunesuppressed patients without HIV when the prednisone dose exceeds 20 mg/day for longer than 1 month [47]. In this study, the median daily prednisolone dose for survivors was lower than the cut-off recommended for PCP prophylaxis. Perhaps PCP prophylaxis should be considered even when the prednisolone dose is 10 mg/day if the patient has other immune-suppressive conditions, such as malignancies, or is being treated with other immune-suppressive regimens.

This study has limitations: it was a single-center retrospective study of a small sample and relevant serum markers were not measured in all patients. PCP was diagnosed by positive *P. jirovecii*-PCR along with clinical symptoms and radiological findings, but *P. jirovecii* colonization was detected in 30% of patients with chronic pulmonary disease [48]. Thus, we cannot completely exclude the possibility of other diseases. Many of the present nonsurvivors had malignancy as the underlying disease; therefore, if the proportions of underlying diseases in both groups are similar, the results would be unclear. A future prospective study should enroll a large number of patients with the same underlying disease.

In conclusion, serum SP-D remains elevated in non-HIV PCP patients who do not respond to treatment. Elevated serum SP-D may therefore be a more useful marker than KL-6 for management in acute phase of non-HIV PCP patients.

Acknowledgments

The authors received no funding from the public, commercial, or not-for-profit sectors.

Conflicts of interest

None.

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