Clinical Utility of Serum $\beta$-D-Glucan and KL-6 Levels in *Pneumocystis jirovecii* Pneumonia

Hideta Nakamura 1, Masao Tateyama 1, Daisuke Tasato 1, Syusaku Haranaga 1, Satomi Yara 1, Futoshi Higa 1, Yuji Ohtsuki 2 and Jiro Fujita 1

**Abstract**

**Objective** New serum markers (1→3) $\beta$-D-glucan ($\beta$-D-glucan) and KL-6 are reported to be useful for the clinical diagnosis of *Pneumocystis jirovecii* pneumonia (PCP). However, the utility of these markers in PCP with HIV infection (HIV PCP) and without HIV (non-HIV PCP) is unknown. This study was aimed to evaluate the utility of $\beta$-D-glucan and KL-6 for the diagnosis of PCP in patients with HIV infection (HIV PCP) and non-HIV PCP.

**Methods** Retrospective study

**Patients** We reviewed the medical records of consecutive 35 patients. The serum levels of $\beta$-D-glucan and KL-6 in HIV PCP and non-HIV PCP were comparatively evaluated. We evaluated these markers in survivors and non survivors.

**Results** The detection rates of serum $\beta$-D-glucan and KL-6 levels in non-HIV PCP were lower than those in HIV PCP (88% vs. 100%, 66% vs. 88%, respectively). The false positive rates of these markers in both groups were similar (12%, 37%, respectively). Oxygenation index, serum albumin, and mechanical ventilation were the variables which were significantly associated with poor outcome in the univariate analysis.

**Conclusion** In conclusion, $\beta$-D-glucan was a reliable diagnostic marker for PCP. However, the detection rate of $\beta$-D-glucan and KL-6 in non-HIV PCP was lower than in HIV PCP. Neither $\beta$-D-glucan nor KL-6 was associated with the outcome of PCP.

**Key words:** (1→3) $\beta$-D-glucan, human immunodeficiency virus, KL-6, *Pneumocystis jirovecii* pneumonia


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**Introduction**

*Pneumocystis jirovecii* pneumonia (PCP) is a major cause of morbidity and mortality in patients with human immunodeficiency virus (HIV) infection (HIV-related PCP) and other conditions associated with immunosuppressive therapy (1). Invasive diagnostic techniques such as bronchoalveolar lavage (BAL) or transbronchial lung biopsy (TBLB) and specific staining of cyst and/or trophozoites are the gold standard for PCP diagnosis. New techniques such as the identification of *P. jirovecii* DNA by polymerase chain reaction (PCR) from expectorated and induced sputa showed greater sensitivity for PCP diagnosis than conventional histochecmical staining techniques (2).

Yasuoka et al reported that (1→3) $\beta$-D-glucan ($\beta$-D-glucan), one of the major components of the cyst wall of *P. jirovecii*, can be a serological marker for the diagnosis and monitoring of PCP (3); some reports have also supported this evidence (4-6). On the other hand, it has been reported that KL-6, a mucin-like glycoprotein, which is expressed on type II pneumocytes, is another possible diagnostic marker of PCP (7). More recently, Tasaka et al have reported that $\beta$-D-glucan is more reliable as a serum diagnostic marker of PCP than KL-6 or lactate dehydrogenase (LDH), especially when BAL could not be performed because of severe respiratory failure (8).

The clinical characteristics of HIV-related PCP and non-
HIV PCP are different. However, the previous reports failed to show the differences in these serum markers between HIV-related PCP and non-HIV PCP. Therefore, we compared serum β-D-glucan and KL-6 as well as clinical characteristics in patients with different clinical backgrounds; HIV-related PCP and non-HIV PCP. In addition, the influence of these serum markers on the outcome of PCP was investigated.

### Material and Methods

#### Patients

For this study, consecutive 35 patients diagnosed with PCP, 19 with HIV-related PCP and 16 with non-HIV PCP, in our institution between 1989 and 2006 were retrospectively evaluated. This study was approved by the institutional review board for clinical research in the University of the Ryukyus.

#### PCP diagnosis and BAL evaluation

**BAL evaluation**

BAL and/or TBLB were/was performed by fiberoptic bronchoscopy after obtaining written informed consent from the patients. BAL was performed by positioning the bronchoscope in the distal airway of the relevant bronchus (middle or lingual lobe in patients with diffuse pulmonary infiltrates) and administering 50 mL sterile normal saline solution per lavage. Conventionally, 3 lavages were performed. The fluid was immediately divided into aliquots and dispatched to different laboratories for examinations and culturing. Routine evaluation for P. jirovecii included 3 stains (Diff Quik<sup>®</sup>, toluidine blue O, and Grocott stain) and polymerase chain reaction (PCR). PCR was performed according to the well-established method of Wakefield et al (9). The diagnosis of PCP was defined by positive results of staining and/or PCR. BAL fluid was also evaluated for other pathogens, including opportunistic bacteria, viruses, fungi, protozoa, and acid-fast bacteria.

#### Data collection

We reviewed the medical records of all patients and collected the following data: (i) epidemiological data (age, gender, predisposing factors for PCP, and duration of symptoms prior to diagnosis), (ii) laboratory data (serum albumin, C-reactive protein (CRP), Lactate dehydrogenase (LDH), oxygenation index, β-D-glucan, and KL-6), and (iii) BAL data. β-D-Glucan was measured with a G test (Seikagaku Corporation, Tokyo, Japan), and KL-6 was measured by an enzyme-linked immunosorbent assay (ELISA) by using a KL-6 antibody kit (ED046; Eisai Co., Tokyo, Japan). The conventional cut-off points of these markers were defined as 20 pg/mL (10) and 520 U/mL (11), respectively. The oxygenation index was determined from the arterial oxygen tension (PaO₂) and fraction of inspired oxygen (FiO₂) values.

We enrolled patients with other infectious lung diseases, asymptomatic HIV-1 infection, and healthy volunteers so that the levels of β-D-glucan and KL-6 could be compared. We examined a total of 24 patients with other infectious lung diseases, including 6 patients with bacterial pneumonia, 6 with lung tuberculosis, and 15 with Legionella pneumonia. Bacterial pneumonia diagnosis was confirmed by the isolation of a bacterial pathogen from sputum. We diagnosed Legionella pneumonia on the basis of specific urine antigen detection (12). Lung tuberculosis was diagnosed on the basis of isolation of Mycobacterium tuberculosis from sputum culture. The CD4 cell count of all asymptomatic HIV carriers was above 300 cells/mm<sup>3</sup>. Healthy volunteers were recruited from among the medical staff in the hospital.

#### Immunohistochemical staining

Pulmonary tissues were obtained by TBLB from the patients who were examined; the tissue slices were fixed in 10% formalin and embedded in paraffin using the standard procedure. The tissue sections (4-μm thick) were dewaxed and stained according to the following method. The tissue sections of each patient were examined after Hematoxylin and Eosin staining, Grocott staining, and periodic acid Schiff (PAS) reaction. Monoclonal antibody to KL-6 (kindly provided by Eisai, Tokyo, Japan) was used at a dilution of 1:5,120 without pretreatment, as previously described (13). The general staining method followed the instructions in the kit manual for LSAB2 kit/horseradish peroxidase (HRP) (DakoCytomation, Kyoto, Japan) with diaminobenzidine as the substrate for HRP.

#### Statistical methods

Statistical analysis was performed using the statistical software (SPSS for Windows, version 15; Chicago, IL). Data were expressed as the median with the interquartile range in parentheses. Comparisons between patients were performed using the chi-square test for categorical variables and the Mann-Whitney U test followed by the Kruskal-Wallis test for continuous variables. The relationships between variables were analyzed by the Spearman rank-over correlation test. Statistical significance was defined as p < 0.05.

### Results

#### Patient characteristics

During the study period, 35 episodes of PCP were confirmed in 34 individuals. Nineteen patients presented as HIV-related PCP patients and 16 had other conditions associated with immunosuppression. Of the 19 HIV-infected patients, 13 presented with PCP as the first manifestation of acquired immunodeficiency syndrome (AIDS). One patient had been previously diagnosed with PCP. The underlying diseases in patients with non-HIV PCP were described in Table 1. All patients with non-HIV PCP had been adminis-
Table 1. Comparison of Patient Characteristics between HIV-related PCP and Non-HIV PCP

<table>
<thead>
<tr>
<th></th>
<th>HIV related PCP (n = 19)</th>
<th>Non-HIV PCP (n = 16)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>39.0 (29–58)</td>
<td>57.5 (43–75)</td>
<td>0.001</td>
</tr>
<tr>
<td>Male/Female</td>
<td>17/2</td>
<td>9/7</td>
<td>0.05</td>
</tr>
<tr>
<td>Diagnostic methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAL/TBLB</td>
<td>17(89.4)</td>
<td>11(68.7)</td>
<td></td>
</tr>
<tr>
<td>PCR assay</td>
<td>19(100)</td>
<td>16(100)</td>
<td></td>
</tr>
<tr>
<td>Predisposing factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV infection</td>
<td>19 (100)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hematological malignancy</td>
<td>0</td>
<td>9 (56)</td>
<td></td>
</tr>
<tr>
<td>Solid tumor</td>
<td>0</td>
<td>3 (18)</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
<td>4 (25)</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive agents</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Previous PCP*</td>
<td>1 (5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Duration**, days</td>
<td>42 (1–112)</td>
<td>5.5 (2–28)</td>
<td>0.001</td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2/FiO2</td>
<td>281 (117–461)</td>
<td>153.5 (150–260)</td>
<td>0.07</td>
</tr>
<tr>
<td>LDH, U/mL</td>
<td>398 (245–1043)</td>
<td>594.5 (368–912)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin, mg/dL</td>
<td>2.75 (2.4–3.8)</td>
<td>3.15 (2.4–3.8)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>5.78 (1.15–12.2)</td>
<td>6.08 (1.83–26.0)</td>
<td>NS</td>
</tr>
<tr>
<td>β-D-glucan, pg/mL</td>
<td>300(32-4822)</td>
<td>85.4(7.2-429)</td>
<td>0.027</td>
</tr>
<tr>
<td>KL-6, U/mL</td>
<td>1120(331-4330)</td>
<td>644(49.6-1860)</td>
<td>0.045</td>
</tr>
<tr>
<td>CD4, cells/μL</td>
<td>39.0 (1.14–308)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>HIV-RNA, ×10^5 copy/mL</td>
<td>1.1 (0.004–5.6)</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Unless otherwise indicated, data are presented as number (%) or median (range)
NS: No significant, ND: No data
*Previous PCP: Patient who had a PCP in the past
**Duration of symptoms prior to diagnosis

tered immunosuppressive agents, except those with adult T-cell leukemia. PCP was diagnosed by microscopic methods in 17 of the 19 patients with HIV-related PCP and 11 of 16 patients with non-HIV PCP. All of them also had positive results in the PCR assay. Seven patients (2 with HIV-related PCP and 5 with non-HIV PCP) with negative results in the microscopic analyses were diagnosed based on the PCR assay of BAL fluid.

Serum β-D-glucan and KL-6 values on admission

The serum levels of β-D-glucan were significantly higher in patients with HIV and non-HIV PCP than in patients with other infectious lung diseases and the controls (p<0.05) (Fig. 1a). Serum β-D-glucan levels were significantly higher in patients with HIV PCP than in those with non-HIV PCP. Serum KL-6 levels were significantly higher in patients with HIV-related PCP than in those with non-HIV PCP and other pulmonary infections (Fig. 1b). Although the levels of serum KL-6 were significantly higher in non-HIV PCP patients than in the controls, these levels were not significantly different from those of patients with other infectious lung diseases. Considering the conventional cut-off point (10,11), the detection rate and false positive rate of β-D-glucan were 100% and 12%, respectively, in the HIV-related PCP patients and 88% and 12% in the non-HIV PCP patients, respectively. The detection rate and false positive rate of KL-6 were 88% and 37%, respectively, in the HIV-related PCP patients, and 66% and 37% in the non-HIV PCP patients, respectively. In cases in which both β-D-glucan and KL-6 were positive, the detection rate in HIV-related PCP and
Correlations between these markers in the sera and laboratory findings were evaluated in all cases (Fig. 2). There were significant correlations between LDH levels and PaO₂/FiO₂ ($p=0.016$, $R^2 0.18$); the proportion of BAL fluid neutrophils and PaO₂/FiO₂ ($p=0.001$, $R^2 0.36$). There was a significant correlation between the KL-6 levels and the duration of symptoms prior to PCP diagnosis ($p=0.003$, $R^2 0.32$). There was no significant correlation between the levels of β-D-glucan and KL-6.

**Histopathological and immunohistochemical analysis of the TBLB tissues of an HIV case**

Histopathological findings revealed eosinophilic foamy exudates in the intra-alveolar spaces, which were characteristic of *P. jirovecii* infection (Fig. 3A, arrow); these exudates also tested positive with the PAS reaction (Fig. 3B, arrow). Grocott staining clearly revealed black round or crescent *P. jirovecii* cysts in the identical part with Fig. 3B (Fig. 3C, arrow). Strong positive immune reaction with anti-KL-6 antibody was detected not only on the surface of proliferating type II alveolar epithelial cells (Fig. 3D, double arrow) but also within the exudates in a granular or fine vesicular pattern (Fig. 3D, arrow).

**Clinical findings on admission and outcome**

To clarify the prognostic factors of PCP at diagnosis, the clinical findings on admission of the survivors and non-survivors were compared (Table 2). The mortality was higher in the non-HIV PCP group than in the HIV-related PCP group. On admission, the following parameters were...
Figure 2. Correlations between several parameters that were evaluated. a: Serum levels of LDH and PaO2/FiO2 are inversely correlated ($p=0.016$, $R^2 0.18$). b: The proportion of neutrophils in BAL fluid is inversely correlated with that of PaO2/FiO2 ($p=0.001$ $R^2 0.36$). c: Duration prior to the diagnosis of PCP and serum KL-6 levels are positively correlated ($p=0.003$, $R^2 0.32$).

significantly different: age, serum albumin, PaO2/FiO2, the proportion of neutrophils in the BAL fluid, and the requirement of mechanical ventilation. The values of both serum KL-6 and β-D-glucan were not significantly different between the survivors and non-survivors. In addition, the serum LDH levels did not significantly influence the progno-
Figure 3. Histopathological and immunohistochemical staining of PCP tissues. Eosinophilic foamy exudates were observed by Hematoxylin and Eosin staining (A, arrow), and these showed strong positive reaction with PAS (B, arrow). Grocott stain revealed cysts of *P. jirovecii* (C, arrow), and these exudates were KL-6 immunopositive (D, arrow). KL-6 was also expressed on the surface of type II alveolar epithelial cells (D, double arrows). A−D: ×400

Discussion

In the present study, we evaluated the laboratory and BAL findings to compare the clinical features of HIV-related PCP and non-HIV PCP. The main finding of our study is that β-D-glucan is the most reliable adjunctive diagnostic marker for detecting PCP, particularly in HIV-related PCP patients, but the detection rate of β-D-glucan in the diagnosis of non-HIV PCP is inferior to that of HIV PCP. The levels of β-D-glucan on admission have no influence on the prognosis of this disease. Serum KL-6 was a less useful marker because of its low detection rate and high false positive rate in both groups. The LDH level and the proportion of neutrophils in BAL fluid were correlated with oxygenation impairment.

Our data confirmed the observation of previous studies which reported that mortality is influenced by 3 factors present on admission; these factors are severe hypoxia, low serum albumin level, and BAL neutrophilia as well as the need for mechanical ventilation during therapy (14-16). Although LDH and pneumothorax are described as prognostic factors in previous reports (17), in the present study, these factors were not significantly different between survivors and non survivors. The discrepancy might have resulted from the fact that this study included PCP with different clinical backgrounds.

β-D-Glucan is one of the major components of the yeast cell wall and has been used for the presumptive diagnosis of invasive fungal infections (18). The utility of serum β-D-glucan in the adjunct diagnosis of PCP has been proved in several studies (3-6). However, these studies have been conducted on a small number of patients, Tasaka et al have reported its usefulness in a large number of patients (8). The present result is consistent with those of previous studies, and we observed that the detection rate of β-D-glucan was higher in patients with HIV-related PCP than in those with non-HIV PCP. This result can be associated with the fact that the number of *P. jirovecii* in the lungs of HIV-related PCP patients is significantly increased as compared with that in non-HIV PCP patients (19). This marker was not a predictor of outcome. Serum S-adenosylmethionine is reported to be another marker for PCP (20), but the role in the outcome of the marker is unknown, which needs further study.

The serum KL-6 level is known to be a sensitive indicator of various interstitial lung diseases and acute lung injuries (11, 21). Furthermore, the serum KL-6 level is elevated in some infectious lung diseases such as *Legionella pneumonia* (22) and severe lung tuberculosis (23). There has been a report describing elevated serum KL-6 levels in a small number of PCP patients (7). A new finding in the present study is that the serum KL-6 levels seem to correlate with the duration of symptoms prior to the diagnosis; therefore, the KL-6 level was significantly higher in patients with HIV-related PCP than in those with non-HIV PCP because the clinical progression is more rapid in patients with non-HIV PCP than in those with HIV-related PCP.

The positive results of β-D-glucan and KL-6 contributed
**Table 2. Comparison of Clinical Findings on Admission between Survivors and Non Survivors**

<table>
<thead>
<tr>
<th></th>
<th>Survivors (n = 26)</th>
<th>Non survivors (n = 9)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>48 (29–70)</td>
<td>66 (34–70)</td>
<td>0.042</td>
</tr>
<tr>
<td>Male/Female</td>
<td>21/5</td>
<td>5/4</td>
<td>NS</td>
</tr>
<tr>
<td>HIV related PCP</td>
<td>17</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Non-HIV PCP</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>PaO2/FiO2</td>
<td>251.5 (150–461)</td>
<td>117 (87.5–154)</td>
<td>0.001</td>
</tr>
<tr>
<td>LDH, U/mL</td>
<td>398 (226–1043)</td>
<td>499 (381–912)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin, g/dL</td>
<td>3.0 (2.4–3.8)</td>
<td>2.5 (1.9–3.3)</td>
<td>0.028</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>5.78 (1.15–26)</td>
<td>7.06 (6.67–10.3)</td>
<td>NS</td>
</tr>
<tr>
<td>β-D-glucan, pg/mL</td>
<td>217 (7.2–4822)</td>
<td>278 (85.4–429)</td>
<td>NS</td>
</tr>
<tr>
<td>KL-6, U/mL</td>
<td>767 (275–3840)</td>
<td>780 (49.6–4330)</td>
<td>NS</td>
</tr>
<tr>
<td>BAL fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellularity, ×10⁵</td>
<td>1.8 (0.67–8.1)</td>
<td>3.04 (0.3–22.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>37.5 (4.9–82)</td>
<td>19.0 (7–67.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>2.9 (0.7–43.4)</td>
<td>16.6 (7.8–23.7)</td>
<td>0.028</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.5 (0–5.3)</td>
<td>0.95 (0–2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>51.9 (3.3–90.1)</td>
<td>63.9 (15.1–75.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>3 (11.5)</td>
<td>2 (22.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>1 (3.8)</td>
<td>6 (66.6)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Unless otherwise indicated, data are presented as number (%) or median (range)
NS: No significance

only a slight improvement in the false positive rate, but a decline of the detection rate in the diagnosis of PCP.

A limitation of this study was the small number of patients. Another limitation might be that we included cases of PCP that were diagnosed on the basis of not only microscopic findings but also on the results of PCR assays. A recent study showed that PCR of BAL fluid can detect asymptomatic colonization of *P. jirovecii*, particularly in patients receiving corticosteroid therapy or in immunocompromised patients with lung disease (24). However, all PCR positive cases in this study were symptomatic and successfully treated with Trimethoprim-sulfamethoxazole, and we believe that PCR tests in those cases were not false positive.

In summary, β-D-glucan is a reliable marker for adjunctive diagnosis of PCP, but physicians should be aware of its lower detection rate in non-HIV PCP. Factors influencing outcome are the underlying disease, oxygenation index, serum albumin, and the association of mechanical ventilation. It is important to note that neither β-D-glucan nor KL-6 was associated with the prognosis of PCP.

**Acknowledgement**

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