BRIEF REPORT

Serum (1→3) β-D-Glucan as a Noninvasive Adjunct Marker for the Diagnosis of *Pneumocystis* Pneumonia in Patients with AIDS

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High serum (1→3) β-D-glucan levels are described in patients with *Pneumocystis* pneumonia (PCP). We evaluated the diagnostic value of β-D-glucan in 111 patients with AIDS who had PCP and confirmed its usefulness. However, it does not correlate with disease severity and is not suitable for monitoring response to treatment.

Pneumocystis pneumonia (PCP) is associated with significant morbidity and mortality in patients with human immnuodeficiency virus type 1 (HIV-1) infection [1, 2]. PCP is usually diagnosed microscopically by identifying *Pneumocystis jirovecii* in bronchoalveolar lavage fluid (BALF) or bronchoscopically obtained lung tissue [3]. Bronchoscopy, however, is invasive, especially in patients with hypoxemia associated with PCP. Therefore, a minimally invasive method is desirable for diagnosis.

Serum (1→3) β-D-glucan (hereafter, β-D-glucan) is a common component of the cell wall of most fungi and is the major component of the cyst of *P. jirovecii*. Therefore, it is measured in patients who are suspected to have PCP, as well as in those with deep-seated mycotic infections [4]. Although β-D-glucan has been used as an adjunct test for the diagnosis of PCP [5], only a few reports have evaluated its level [5–7] and its correlation with other parameters (such as lactate dehydrogenase [LDH] level) in mixed populations that included a small number of HIV-infected patients [6]. For this purpose, we analyzed the correlation between β-D-glucan levels and other parameters among patients with AIDS who have PCP.

Methods. We evaluated data from 111 consecutive HIV-1–infected patients with PCP at the International Medical Center of Japan, an 885-bed tertiary care hospital in Tokyo, from April 1997 through July 2007. This study was approved by the Ethics Review Committee of the hospital (IMCJ-H20-569). Patients who did not undergo diagnostic bronchoscopy were excluded from the study.

Medical records were reviewed, and the following data were collected: age; sex; mode of infection; $CD4^+$ cell count; serum levels of LDH, β-D-glucan, and C-reactive protein (CRP); and alveolar-arterial oxygen tension gradient ($AaDO_2$). Serum β-D-glucan levels were measured using the Fungitec G MK test (Seikagaku). Manipulation was performed described elsewhere [4, 5], in accordance with the manufacturer's instructions. Serum β-D-glucan levels in HIV-1–infected patients without PCP determined during the same period were used as a control. If serum β-D-glucan levels had been determined several times for the same patient, only the first measurement was included. Although oral and esophageal candidiasis are superficial infections, they were included as an independent factor and analyzed. In this report, the term *candidiasis* refers to oral and/or esophageal candidiasis.

The diagnosis of PCP was established by identification of *P. jirovecii* in BALF. Each BALF specimen (100 μL) was centrifuged at 900 *g* for 2 min by means of a Shandon Cytospin III device, and a monolayer of deposited cells were stained using Diff-Quik (Dade Behring) and examined microscopically for the presence of *P. jirovecii*.

Data were expressed as means ± standard deviations (SDs) or as medians. Differences in categorical variables between patients with PCP and control patients were assessed using the Mann-Whitney *U* test. The Mann-Whitney *U* test (for comparison of 2 groups) and the Kruskal-Wallis test (for comparison of 3 groups) were used for analysis of differences in serum β-D-glucan levels. A receiver-operating-characteristic (ROC) curve was constructed to illustrate the cutoff value for β-D-glucan. The relationships were analyzed by linear regression analysis. Differences were considered significant at $P < .05$. Statistical analyses were performed using SPSS, version 17.0 (SPSS).

Results. A total of 111 patients had a definite diagnosis of PCP, and serum β-D-glucan level was measured in each. Of

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these patients, 67 also had candidiasis at admission. Of the control group (425 patients who did not have PCP), 28 had candidiasis, 3 had cryptococcal infection, and 394 had neither.

The patients with PCP were older than the control patients (mean ± SD, 42.3 ± 11.9 vs 38.7 ± 11.7 years; $P < .01$), and $CD4^+$ cell counts were significantly higher in the control patients than in the patients with PCP (mean ± SD, 178.6 ± 155.6 vs 49.1 ± 63.1 cells/μL; $P < .001$). Sex and mode of transmission of HIV were similar in both groups ($P = .81$ and $P = .53$, respectively). All patients with PCP received treatment, and 6 patients died of PCP.

Of the patients with PCP, 67 had candidiasis and 44 did not; of the control patients, 28 had candidiasis, 3 had cryptococcal infection, and 394 did not have any fungal infection. The median (range) serum β-D-glucan level in each group was 171.2 (14.9–2966), 209.6 (2.4–2469), 7.40 (1.0–73.0), 22.7 (9.3–69.7), and 8.25 (1.0–310) pg/mL, respectively (Figure 1). The median serum level of β-D-glucan among all patients with PCP (174.8 [2.4–2966] pg/mL) was significantly higher than that among the control patients (8.2 [1.0–310.1] pg/mL) ($P < .001$). The presence of candidiasis in both the PCP group and the control group and of cryptococcal infection in the control group did not significantly influence serum levels of β-D-glucan ($P = .53$, $P = .83$, and $P = .08$, respectively).

With respect to the diagnostic value of β-D-glucan, the area under the ROC curve for β-D-glucan level was 0.964 (95% confidence interval, 0.945–0.984) (Figure 2). A β-D-glucan cutoff value of 23.2 pg/mL (which represented the technique's threshold of detection) had a sensitivity of 96.4% and a specificity of 87.8%.

There was no correlation between serum levels of β-D-glucan and $AaDO_2$ at room air ($r = 0.125$; $P = .30$), LDH ($r = .030$; $P = .76$), or CRP ($r = .002$; $P = .62$). In 42 instances, serum β-D-glucan levels were measured before and after treatment. On the basis of a cutoff value of 23.2 pg/mL, normalization of serum β-D-glucan levels was noted in 7 patients. In contrast, serum β-D-glucan levels slightly increased in 9 patients despite clinical improvement being noted at week 3. This finding indicates that β-D-glucan levels reflected the clinical course in only 16.7% of patients (7 of 42) within 3 weeks of treatment.

Discussion. The present study has reported 3 major findings. The first major finding is the usefulness of quantitative measurement of serum β-D-glucan levels for the diagnosis of PCP. With a cutoff value of 23.2 pg/mL, β-D-glucan level had

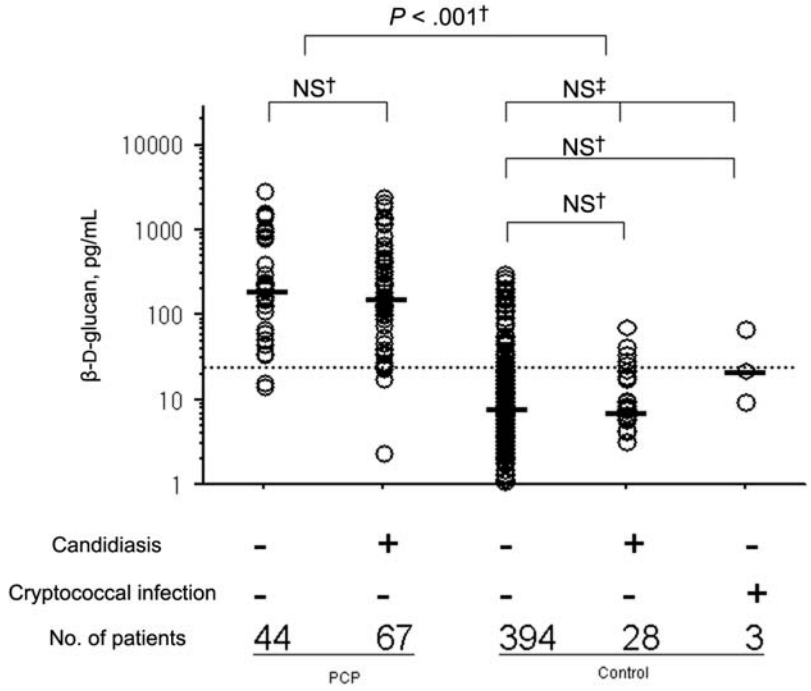

Figure 1. Serum levels of (1→3) β-D-glucan. Levels of β-D-glucan in serum were examined before treatment of *Pneumocystis* pneumonia (PCP), candidiasis, and cryptococcal infection. The Mann-Whitney *U* test (†) and the Kruskal-Wallis test (‡) were used for comparison of serum β-D-glucan levels. Individual values are plotted, and horizontal bars represents medians. The presence of candidiasis in both the PCP group and the control group and of cryptococcal infection in the control group did not significantly influence serum β-D-glucan levels ($P = .53$, $P = .83$, and $P = .08$, respectively). Serum β-D-glucan levels were significantly higher in patients with PCP than in those without PCP, despite the presence of candidiasis and cryptococcal infection ($P < .001$). NS, not significant.

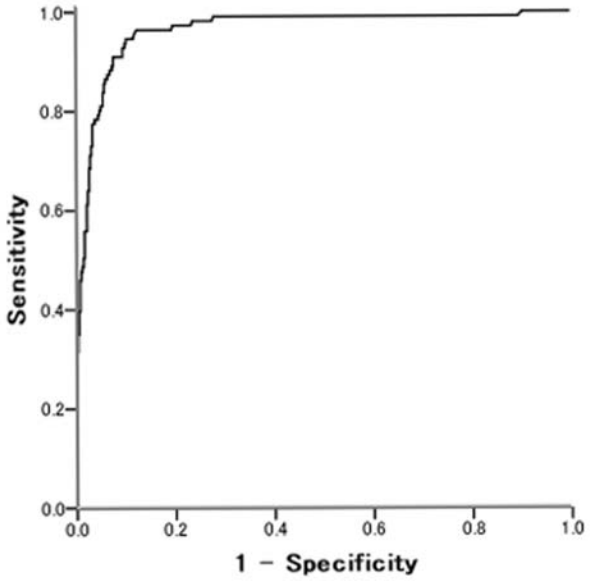

Figure 2. Receiver-operating-characteristic (ROC) curve for the (1→3) β-D-glucan cutoff. The area under the ROC curve for β-D-glucan was 0.964 (95% confidence interval, 0.945–0.984). A β-D-glucan cutoff value of 23.2 pg/mL (which represented the technique's threshold of detection) had a sensitivity of 96.4% and a specificity of 87.8%.

a high sensitivity (96.4%) and specificity (87.8%) for the diagnosis of PCP. Interestingly, serum β-D-glucan levels among those with PCP were not affected by the presence of superficial fungal infection (ie, oral and/or esophageal candidiasis). Deep-seated mycosis other than PCP and cryptococcal infection are quite rare in Japan, and no patients were suspected to have aspergillosis in this study. Hence, we could not analyze the effect of aspergillosis. According to our data and those of others [4], β-D-glucan level increases during cryptococcal infection, but the level is significantly lower than that observed during PCP. The number of *P. jirovecii* organisms in the lungs of patients with AIDS may be significantly higher than that in patients without AIDS [8]. In a meta-analysis of 7 reports in which PCP was diagnosed by staining, the average sensitivity of induced sputum was 56%, whereas that of BALF was >95% [9]. To eliminate false-positive and false-negative results, we analyzed data obtained only from patients who underwent BALF analysis and had a definite diagnosis of PCP.

The second major finding was that the serum level of β-D-glucan does not reflect the severity of PCP in patients with AIDS. Although Shimizu et al [10] reported that β-D-glucan is a negative prognostic marker for PCP in patients with connective tissue diseases, there was no significant difference in β-D-glucan level between survivors and nonsurvivors in our study. Furthermore, Tasaka et al [6] reported that serum levels of LDH correlated with those of β-D-glucan in patients with PCP, whereas our data showed no such relationship. These differences are probably the result of differences in the patient populations studied, especially regarding whether the patients have HIV-1 infection. Considered collectively, these results emphasize the need for further studies to define the exact relationship between β-D-glucan and prognosis as well as LDH.

The third major finding of the present study was that β-D-glucan level did not reflect the effectiveness of therapy. In nearly 85% patients, serum β-D-glucan levels did not decrease to normal despite clinical improvement. Furthermore, 20% of patients had increased levels of β-D-glucan during the early phase of treatment. However, β-D-glucan levels normalized several months or years after treatment in all patients. These results mean that β-D-glucan levels increase transiently early during treatment and decrease thereafter but do not always return to normal during treatment. The transient increase in β-D-glucan level is probably due to lysis of *P. jirovecii* shortly after treatment.

PCP is usually suspected on the basis of chest radiographic findings, clinical symptoms, and low $CD4^+$ cell counts in HIV-infected patients. In the present study, a high serum level of β-D-glucan (especially >23.2 pg/mL by the MK test) was found to be highly indicative of PCP in practically all patients with AIDS. Therefore, the β-D-glucan test is useful for the diagnosis of PCP, especially in HIV-infected patients who are unable to undergo bronchoscopy owing to severe hypoxemia. In conclusion, the present study has demonstrated that β-D-glucan is a useful, noninvasive adjunct marker for the diagnosis of PCP in patients with AIDS. However, its serum levels do not reflect the severity of the disease, and it is not suitable for monitoring response to treatment.

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