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Original article

Serum (1 → 3) β-D-glucan assay for discrimination between *Pneumocystis jirovecii* pneumonia and colonization

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A B S T R A C T

Polymerase chain reaction (PCR) technique is being increasingly used for the microbiological diagnosis of *Pneumocystis* pneumonia (PCP). As PCR is highly sensitive, it can be positive even in a patient with *Pneumocystis* colonization. In this study, we evaluated whether the β-D-glucan assay could be used to differentiate between PCP and *Pneumocystis jirovecii* colonization in immunocompromised patients with pulmonary infiltrates. We retrospectively evaluated data from 166 consecutive patients who underwent bronchoalveolar lavage for the diagnosis of PCP. Serum levels of β-D-glucan in the negative, colonization, probable PCP, and definite PCP groups were 20.2 ± 6.3, 48.8 ± 15.9, 89.9 ± 20.2, 224.9 ± 25.9 pg/mL, respectively. The β-D-glucan levels in the definite PCP group were significantly higher than those in the other 3 groups ($p < 0.001$). Serum β-D-glucan levels in patients with either definite or probable PCP (173.1 ± 18.8 pg/mL) were significantly greater than those in patients with colonization who had positive PCR results but improved without anti-PCP treatment ($p < 0.002$). The cut-off level for discrimination was estimated to be 33.5 pg/mL, with which the positive predictive value was 0.925. These results indicate that β-D-glucan is a useful marker to differentiate between PCP and *Pneumocystis* colonization. A positive β-D-glucan assay result might be a good indication to begin anti-PCP treatment.

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1. Introduction

Pneumocystis pneumonia (PCP) remains one of the most frequent opportunistic infections in immunocompromised patients, including those infected with human immunodeficiency virus (HIV) [1]. Because *Pneumocystis* cannot readily be cultured in the laboratory, microscopic demonstration of the organism in respiratory specimens has been the gold standard for the diagnosis of PCP [1,2]. The sensitivity of microscopic visualization of *Pneumocystis* is inconsistent and depends on the skill and experience of the observer. In addition, non-HIV patients, who develop PCP, are characterized with a lower fungal burden than HIV-infected patients with PCP [3]. This lower fungal burden may result in false-negative results from microscopic examination [4]. Because of these issues, the highly sensitive polymerase chain reaction (PCR) technique is being increasingly used for the microbiological diagnosis of PCP [5,6]. However, *Pneumocystis* colonization, defined as the detection of its DNA without signs or symptoms of pneumonia, has been reported to be highly prevalent even among the general population [7]. If a patient colonized with *Pneumocystis jirovecii* experiences pneumonia resulting from a different etiology, a positive *Pneumocystis* PCR result may mislead clinicians. In such cases, it is critical to distinguish *Pneumocystis* colonization from PCP, however, an algorithm for the discrimination between these two conditions remains to be established.

(1 → 3)-β-D-glucan (β-D-glucan) is a major structural component of the cell wall of several fungi, including *Pneumocystis* [8]. Previous studies have shown that the serum β-D-glucan assay can be useful for the diagnosis of PCP [9,10]. However, there are few data available on the ability of this assay to detect *Pneumocystis* colonization or differentiate this colonization from PCP [11].

In this study, we evaluated whether β-D-glucan and other serum markers could be used to differentiate between PCP and *P. jirovecii*

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colonization in immunocompromised patients with pulmonary infiltrates.

2. Methods

The ethical committee of Keio University School of Medicine approved the study protocol.

2.1. Patient selection

We retrospectively evaluated data from 166 consecutive patients who underwent bronchoalveolar lavage (BAL) for the diagnosis of PCP at Keio University Hospital (Tokyo, Japan) during the period from April 2006 until November 2011. All of the patients had ground-glass opacities on chest computed tomography and clinical presentations such as fever, cough or dyspea.

2.2. Data collection

We reviewed the medical records of all the patients evaluated for their clinical courses. In sera, five serum markers C-reactive protein (CRP), lactate dehydrogenase (LDH), KL-6, surfactant protein D (SP-D) and β-D-glucan were evaluated. The levels of β-D-glucan were measured with a kinetic turbidimetric assay using Beta−Glucan test WAKO™ (Wako Pure Chemical Industries, Tokyo, Japan).

2.3. BAL procedure

Informed consent to conduct BAL was obtained from either the patient or surrogate. In most cases, BAL was targeted toward affected lung segments as noted on chest CT and performed with 50 mL of 0.9% saline solution per lavage. Usually, three lavages were performed, and the lavage fluid was immediately placed on ice.

2.4. BAL fluid processing for analysis

The BAL fluid was pooled, filtered through sterile gauze to remove mucous strands, and centrifuged at 200 × g for 5 min at 4 °C. The cell pellets were used for the differential counts on Wright-Giemsa-stained preparations. For the detection of *P. jirovecii*, a 10 mL aliquot of BAL fluid was centrifuged at 1875 × g for 10 min, and a smear was microscopically examined for the presence of *P. jirovecii* with Grocott-Gomori methenamine stain (GMS) and Calcofluor white stain (Fungifluor; Polysciences, Inc., Warington, PA), following the manufacturer's recommendations.

2.5. Detection of Pneumocystis DNA

Template DNAs were extracted from BAL fluid samples by means of proteinase K digestion and phenol/chloroform extraction and subsequently subjected to PCR as templates. The PCR analysis for *Pneumocystis* DNA was performed in 50 μl of amplification reaction mixtures, with denaturation at 94 °C for 90 s, annealing at 50 °C for 90 s, and extension at 72 °C for 2 min (40 cycles). The following oligonucleotide primers were used at 100 pmol: 5′-GAT GGC TGT TTC CAA GCC CA-3′ and 5′-GTG TAC GTT GCA AAG TAC TC-3′. DNA products with lengths of 376 bp were amplified from template DNAs. This analysis was done at SRL Inc., (Tachikawa, Japan). The details of this method were described by Wakefield and colleagues [12].

2.6. Diagnosis of PCP

The diagnosis of definite PCP was established by the microscopic identification of *P. jirovecii* in BAL fluid. A diagnosis of probable PCP was made when the patient did not have microscopically visible *P. jirovecii* but had a positive PCR results and a clinical presentation compatible with PCP, including complete resolution of pulmonary infiltrates after a full course of anti-PCP treatment [5]. The patients who had positive PCR results but improved without anti-PCP treatment were diagnosed as ‘pulmonary infiltration with *P. jirovecii* colonization’ (colonization). Those having neither observable *P. jirovecii* nor positive PCR results were classified as negative patients.

2.7. Statistical methods

All data are expressed as the mean ± SE. The data were analyzed by one-way analysis of variance with Scheffe's *post hoc* test using SPSS Windows 15.0 statistical analysis software (SPSS, Inc., Chicago, IL). In patients with PCP (either definite or prpbable), serum β-D-glucan levels were compared between HIV and non-HIV subjects using unpaired *t*-test. To evaluate the sensitivity and specificity of β-D-glucan assay, a receiver operating characteristic (ROC) curve was constructed. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Patient characteristics

During the study period, 64 patients, of whom 16 (25.0%) were HIV-positive, were diagnosed with definite PCP. Another 38 patients had probable PCP, of whom 6 (15.8%) were HIV-positive. Twenty-four patients were colonized with *P. jirovecii*; none of the colonized patients were HIV-positive. Forty patients were negative for *P. jirovecii*, of which 1 (2.5%) was HIV-positive.

The patient characteristics and laboratory data are summarized in Table 1. The patients with definite PCP were significantly younger than those of other 3 groups ($p < 0.001$). As an underlying disease, 22 of the patients with definite and probable PCP had HIV infection, whereas none of those with *Pneumocystis* colonization did. A chi-square test showed heterogeneity of the underlying diseases across the groups ($p < 0.02$).

Table 1
Patient's characteristics and laboratory data.

	Definite PCP ($n = 64$)	Probable PCP ($n = 38$)	Colonization ($n = 24$)	Negative ($n = 40$)
Age (year)	47 ± 2*	59 ± 3	66 ± 3	60 ± 3
Male/female	37/27	22/16	11/13	20/20
Underlying disease				
HIV infection	16	6	0	1
Hematological malignancy	15	6	7	8
Collagen vascular disease	15	14	6	12
Solid tumor	9	4	4	9
Organ transplantation	6	1	1	1
Others	3	7	6	9
Serum marker				
CRP (mg/dL)	6.87 ± 0.74	6.73 ± 0.91	5.14 ± 0.89	5.63 ± 0.83
LDH (IU/L)	436 ± 24	352 ± 28	397 ± 38	339 ± 24
KL-6 (U/mL)	1.216 ± 203	1037 ± 161	791 ± 180	588 ± 66
SP-D (ng/mL)	291 ± 117	268 ± 71	212 ± 36	181 ± 49
β-D-glucan (pg/mL)	224.9 ± 25.9*	89.9 ± 20.2	48.8 ± 15.9	20.2 ± 6.3
PaO_2/FIO_2 (mmHg)	270 ± 19	308 ± 18	269 ± 21	318 ± 18

Mean ± SE. *$p < 0.001$ vs other 3 groups.

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