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Serum (1→3)-β-D-Glucan Levels in Primary Infection and Pulmonary Colonization with *Pneumocystis jirovecii*[▽]

Céline Damiani,[1,2] Solène Le Gal,[3,4] Delphine Lejeune,[1] Naima Brahimi,[1] Michèle Virmaux,[3] Gilles Nevez,[3,4] and Anne Totet[1,2]*

Parasitology and Mycology Department, University Hospital, Amiens, France[1]; University of Picardy Jules Verne, EA 4285 UMI INERIS 01, Amiens, France[2]; UEB, University of Brest, LUBEM EA 3882, IFR 148, Brest, France[3]; and Parasitology and Mycology Department, University Hospital, Brest, France[4]

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This article describes positive (1→3)-β-D-glucan levels in serum from infants with primary *Pneumocystis* infection and from immunosuppressed patients with *Pneumocystis* pneumonia (PCP) and negative levels in serum from patients colonized by *Pneumocystis jirovecii*. Glucan detection is a complementary tool for the diagnosis of the diverse clinical presentations of *P. jirovecii* infection.

Pneumocystis jirovecii (the human-specific *Pneumocystis* species) is an atypical fungus that has been recognized for a long time as a cause of severe pneumonia (*Pneumocystis* pneumonia [PCP]) in immunocompromised individuals. More recently, the use of PCR assays for *P. jirovecii* detection in pulmonary samples has revealed that immunocompromised patients and patients with lung diseases can be infected by only a small number of microorganisms, which are usually not detected by microscopy (13). The term colonization is frequently used in this context. It has also been established that *P. jirovecii* can be detected by PCR in nasopharyngeal aspirates (NPA) from immunocompetent infants who develop primary *Pneumocystis* infection contemporaneously with acute respiratory syndromes (18, 19).

(1→3)-β-D-Glucan (BG) represents a major structural component of the cell wall of most fungi and is abundant in *Pneumocystis* cysts (4). Previous studies have reported high levels of BG in serum samples from patients with PCP (2, 3, 5–9, 11, 12, 14, 15, 17, 20). In contrast, this marker has been investigated in only one study concerning pulmonary colonization with *P. jirovecii* (16) and has not yet been studied in primary *Pneumocystis* infection. In this study, we retrospectively investigated BG levels in serum samples from infants developing primary *Pneumocystis* infection, adults colonized by *P. jirovecii*, and adults developing PCP, who were followed up at Amiens University Hospital (France).

Fourteen immunocompetent term infants (mean age, 6 months [range, 1.7 to 15.7]; 9 boys and 5 girls) with primary *Pneumocystis* infection were enrolled. They were hospitalized between November 1999 and April 2001. NPA and serum samples were initially collected from all infants to investigate an acute respiratory syndrome. Serum samples were collected over an interval ranging from 2 days before to 3 days after NPA retrieval. No infants presented immunodeficiency and risk factors or clinical signs of invasive fungal infection. No infants had received antibiotics before sampling. *P. jirovecii* was detected by a real-time PCR assay targeting the mitochondrial large subunit rRNA (mtLSU rRNA) gene, as previously reported (18). The diagnosis of primary *Pneumocystis* infection was based on positive results of *P. jirovecii* detection and the low mean age of the infants (6 months), compatible with first contact with the fungus. Clinical improvement was obtained in all infants in response to respiratory physiotherapy, despite the absence of any specific treatment for the fungus. Eight patients colonized by *P. jirovecii* (mean age, 50.8 years [range, 23 to 77]; 5 men and 3 women) hospitalized between February 2008 and June 2009 were enrolled. Bronchoalveolar lavage (BAL) and serum samples were initially collected from patients to investigate pulmonary symptoms (abnormal chest X-ray, cough) or fever. Serum samples were collected over an interval ranging from 2 days before to 10 days after BAL fluid retrieval. None of the patients presented clinical or laboratory signs of invasive fungal infection. Detection of *Aspergillus* galactomannan and *Candida* mannan antigens in serum samples using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Platelia Candida Ag plus and Platelia Aspergillus Ag; Bio-Rad, Marnes-la-Coquette, France) and blood cultures (Bactec Mycosis; Becton, Dickinson and Company, Sparks, MD) were negative. *P. jirovecii* was not detected in BAL specimens by microscopic examination but was detected by a real-time PCR assay directed at the mtLSU rRNA gene, as previously described (10). Alternative diagnoses of PCP were bacterial pneumonia (six patients), pulmonary sarcoidosis (one patient), and bronchial carcinoma (one patient). Clinical improvement was observed in all patients except for the patient with bronchial carcinoma, despite the absence of any specific treatment for the fungus. Patients with bacterial pneumonia and pulmonary sarcoidosis were successfully treated by extended-spectrum beta-lactam antibiotics and corticosteroids, respectively. The follow-up with no PCP occurrence ranged from 2 to 21 months. The patient with lung cancer died 6 weeks after *P. jirovecii* detection in a context of terminal cancer with no evidence of a contribution of *P. jirovecii* to death. A diagnosis of PCP was therefore excluded by the physicians, and all eight patients were considered to be colonized by *P. jirovecii.*

* Corresponding author. Mailing address: Laboratoire de Parasitologie-Mycologie, CHU, Centre Hospitalier Sud, 1 Avenue René Laennec, 80054 Amiens, France. Phone: 33 3 22 45 59 75. Fax: 33 3 22 45 56 53. E-mail: totet.anne@chu-amiens.fr.

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Six patients diagnosed with PCP (mean age, 53 years [range, 31 to 69]; 5 men and 1 woman) hospitalized between August 2008 and April 2009 were enrolled and were used as positive controls for BG detection. The diagnosis of PCP was based on the criteria described by the Centers for Disease Control and Prevention for HIV-infected patients (1). BAL and serum samples were initially collected from all patients to investigate pulmonary symptoms. Serum samples were collected on the same day as BAL fluid retrieval. None of the patients presented any clinical or laboratory signs of invasive fungal infection other than PCP. Serum samples were negative for *Aspergillus* galactomannan and *Candida* mannan antigens. Blood cultures were also negative. None of the patients had received antibiotics before sampling. The BAL specimens tested positive for *P. jirovecii* by microscopic examination with Giemsa stain and an immunofluorescence assay (MonofluoKit *Pneumocystis*; Bio-Rad, Marnes-la-Coquette, France) and by a real-time PCR assay directed at the mtLSU rRNA gene. Underlying conditions were HIV infection, bronchial carcinoma, colonic adenocarcinoma, acute alcoholic hepatitis treated with high-dose corticosteroids (one patient each), and kidney transplantation (two patients). All patients received anti-*Pneumocystis* treatment immediately after the diagnosis of PCP was established. Clinical improvement was obtained after 3 weeks of treatment in five patients. One patient died from stroke 4 days after PCP diagnosis.

Serum samples from the three populations were stored at −80°C. BG levels in stored serum samples were determined using the Fungitell test kit (Associates of Cape Cod, Inc., Cape Cod, MA) according to the manufacturer's instructions. A BG level of ≥80 pg/ml was considered to be positive. The results of BG detection in the three populations were compared using Student's test and Wilcoxon's test. Statistical significance was defined as a P value of <0.05.

The results of BG detection in infants ranged from 56 to 394 pg/ml, with a median value of 217.6 pg/ml. Thirteen of the 14 infants had a positive result, i.e., ≥80 pg/ml. The results in colonized patients ranged from 45 to 84 pg/ml, with a median value of 69.5 pg/ml. Six of the 8 colonized patients had a negative result. BG levels in the remaining two patients were 82.7 and 84 pg/ml. Patients with PCP had positive BG results, with high values ranging from 184 to 2,710 pg/ml and a median value of 1,768.5 pg/ml.

BG levels in infants and colonized patients were significantly lower than those in the PCP control group ($P < 0.05$). BG levels in infants were significantly higher than those in colonized adults ($P < 0.05$). Results are shown in Fig. 1.

This study reports high BG levels for patients with PCP. Similar results have previously been reported (2, 3, 5–9, 11, 12, 14–17, 20). None of these patients presented any factors that interfere with BG levels, especially antibiotics prior to sampling or invasive fungal infection. These BG levels can then be correlated with the presence of *P. jirovecii* cysts in the lungs, which were effectively numerous and easily observed on microscopic examination of BAL samples.

Although the number of infants is limited, this is the first study to report serum BG levels during primary *Pneumocystis* infection. Positive serum BG levels were observed in 93% (13/14) of infants, with a median value of 217.6 pg/ml. None of these infants presented any factors that interfere with serum BG levels. Furthermore, BG was not detected in a control group that consisted of 14 infants hospitalized for an acute respiratory syndrome and negative for *P. jirovecii* detection (results are shown in Fig. 1). These results are therefore consistent with the presence of *P. jirovecii* cysts in the lungs of infants with primary *Pneumocystis* infection, as was established in adults with PCP. However, none of the infants presented BG levels higher than 400 pg/ml, in contrast with adult patients with PCP. The burden of *P. jirovecii* cysts in the lungs may be limited during the course of primary infection. Indeed, although infants are immune naive for *P. jirovecii*, they are not immunocompromised and their immune response is sufficiently effective to finally clear the fungus without the need for specific treatment.

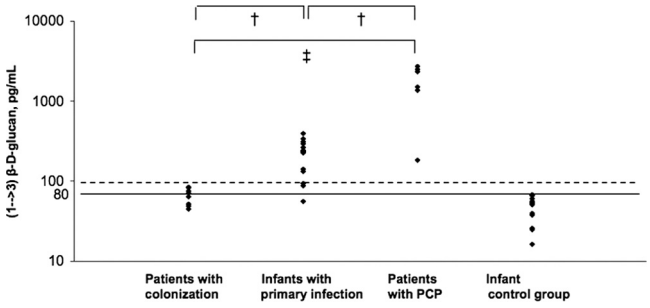

FIG. 1. Serum (1→3)-β-D-glucan levels in infants with primary *Pneumocystis* infection, patients with *Pneumocystis* colonization, patients with *Pneumocystis* pneumonia (PCP), and an infant control group. Serum BG levels were significantly higher in the PCP control group than in infants with primary infection (†, Student's test, $P < 0.05$) and colonized patients (‡, Wilcoxon's test, $P < 0.05$). Serum BG levels were significantly higher in infants with primary infection than in colonized patients (†, Student's test, $P < 0.05$).

In contrast, 6/8 colonized patients had negative serum BG levels, i.e., less than 80 pg/ml. The two remaining patients presented positive BG results, but with values clearly lower than 100 pg/ml, i.e., 82.7 and 84 pg/ml. These results may be explained by the absence or rarity of *P. jirovecii* cysts in pulmonary alveoli.

Taking into account the results of BG detection, *Pneumocystis* infection in immunocompetent infants appears to be a clinical entity which may be closer to PCP than to pulmonary colonization. Moreover, serum BG levels combined with PCR assay may discriminate between PCP and pulmonary colonization with *P. jirovecii.* A serum BG assay is a noninvasive test which appears to be a complementary tool for the diagnosis of the diverse presentations of *P. jirovecii* infection.

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