Plasma (1→3)- β -D-glucan Measurement and Polymerase Chain Reaction on Sputum as Practical Parameters in *Pneumocystis carinii* Pneumonia

Keiji OKAMOTO, Tetsuro YAMAMOTO, Daisuke NONAKA*, Hirokazu SHIN*,
Kaoru OKADA*, Jun HAYASHI and Seizaburo KASHIWAGI

We report a case of *Pneumocystis carinii* pneumonia in a patient with acquired immunodeficiency syndrome diagnosed and monitored with polymerase chain reaction (PCR) for *Pneumocystis carinii* on sputum and measurement of plasma (1→3)- β -D-glucan (G-test). Results of both studies paralleled the clinical and radiographic improvement. However, the plasma (1→3)- β -D-glucan level remained higher than normal when PCR for *Pneumocystis carinii* became negative in sputum. Both PCR for *Pneumocystis carinii* on sputum and measurement of plasma (1→3)- β -D-glucan are useful for noninvasive diagnosis and monitoring of *Pneumocystis carinii*, although further investigation is necessary to quantify their relationship.
(Internal Medicine 37: 618–621, 1998)

Key words: G-test, human immunodeficiency virus (HIV)

Introduction

Pneumocystis carinii pneumonia (PCP) is the most common opportunistic infection in acquired immunodeficiency syndrome. Diagnosis is established with the demonstration of typical oocysts in respiratory secretions, particularly in bronchoalveolar lavage fluid (sensitivity >95%) (1). However, in patients with severe respiratory failure, bronchoscopy with bronchoalveolar lavage is dangerous. Recently, polymerase chain reaction (PCR) on induced sputum has become an alternative diagnostic and monitoring method (2). In addition, measurement of plasma (1→3)- β -D-glucan (G-test) has been shown to be useful in the diagnosis and monitoring of PCP (3). We present a case of PCP diagnosed and monitored with both PCR on sputum and G-test on plasma.

Case Report

A 35-year-old South African man was admitted to Kyushu University Hospital on October 8, 1996, because of severe pneumonia. In June 1996, the patient began to lose appetite and weight, and on September 25, high fever and cough developed. The patient was admitted to a local hospital. He was administered several antibiotics, including piperacillin and minocycline,

however these antibiotics were not effective, and the patient was transferred to Kyushu University Hospital on October 8.

The patient had contracted malaria in 1988, and denied any risk factors for human immunodeficiency virus (HIV) infection. He had smoked about 20 cigarettes per day for the past 5 years. Family history was unremarkable.

Body temperature was 39°C, pulse was 96 beats per minute, and respiratory rate was 40 per minute. Blood pressure was 110/80 mmHg. Oxygen was administered at a rate of 4 l/min by face mask. Analysis of arterial blood gas showed that the partial pressure of oxygen was 53.9 mmHg, the partial pressure of carbon dioxide was 27.2 mmHg, and pH was 7.412. Physical examination showed slightly anemic conjunctiva, white-coated tongue, and bilateral diffuse fine crackles over the lung bases.

Blood laboratory tests were performed (Table 1). Neutrophilia and anemia were present with elevated C-reactive protein. Hepatic dysfunction was also shown probably due to right heart failure secondary to acute respiratory failure. The CD4 count was 4 cells/ml. The patient refused HIV antibody test at that time; however, 6 months later (April 18, 1997) he agreed to an HIV antibody test, which was positive. An electrocardiogram revealed sinus tachycardia at a rate of 110/minute. A chest radiograph showed diffuse interstitial infiltrations (Fig. 1). Chest computed tomography (CT) also showed diffuse

From the Department of General Medicine and *the First Department of Internal Medicine, Kyushu University Hospital, Fukuoka

Received for publication October 28, 1997; Accepted for publication March 24, 1998

Reprint requests should be addressed to Dr. Tetsuro Yamamoto, the Department of General Medicine, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582

Table 1. Blood Laboratory Test Results

Variable	Oct 8	Oct 25	Nov 15
White cell count (/mm ³)	11,000	3,130	980
Differentiation (%)			
Stab	14	3.5	2.0
Segmental neutrocyte	71	76	40
Lymphocyte	4	8	17
Monocyte	9	4.5	10
Eosinocyte	2	5.0	29
Hematocrit (%)	33.9	28.7	26.4
Platelet count ($\times 10^3$ /mm ³)	176	116	78
Total protein (g/dl)	5.4	6.0	5.8
Albumin (g/dl)	2.4	3.3	3.7
Blood urea nitrogen (mg/dl)	42	15	10
Serum creatinine (mg/dl)	1.2	1.0	1.0
Aspartate aminotransferase (U/l)	251	21	18
Alanine aminotransferase (U/l)	200	38	27
Lactate dehydrogenase (U/l)	2,178	820	500
C-reactive protein (mg/dl)	25.2	1.8	0.1

Figure 1. Chest X-ray film on admission.

ground-glass-like infiltrations (Fig. 2).

Imaging studies suggested that the most likely diagnosis was an interstitial pneumonia, such as that due to *Pneumocystis carinii* or cytomegalovirus. Bronchoalveolar lavage was attempted, but was not tolerated by the patient. Trimethoprim and sulfamethoxazole (TMP/SMX) at 160 mg/800 mg every 8 hours with glucocorticoid therapy was empirically started on October 9. (One gram of methylprednisolone daily for the first 3 days, followed by 80 mg of prednisolone daily for 2 days. The prednisolone was gradually tapered.) Sputum culture yielded no bacteria. Sputum cytology revealed no protozoa. PCRs on sputum for *Mycobacterium tuberculosis* and *Mycobacterium avium complex* were negative. However, *Pneumocystis carinii* was detected in sputum with PCR, and the plasma (1 $\rightarrow$ 3)- β -D-glucan level was increased to 272.6 pg/ml on G-test (Seikagaku Co., Tokyo). Blood culture yielded no bacteria or fungus. Plasma cytomegaloviral antigenemia and serum immunoglobulin G (IgG) and immunoglobulin M (IgM) to Cytomegalovirus were negative. Based on the above findings, PCP was diagnosed on October 21. On October 15, 1 week after TMP/SMX and glucocorticoid were started, arterial blood gas analysis and plain chest X-ray films showed marked improvement. On October 25, 2 weeks after the start of treatment, respiratory symptoms, such as cough, sputum, and dyspnea had mostly resolved, and the partial pressure of oxygen was 82.9 mmHg on room air despite the remaining infiltration on plain chest X-ray films, and a positive PCR for *Pneumocystis carinii* in sputum. At the same time, the plasma (1 $\rightarrow$ 3)- β -D-glucan level decreased to 67.9 pg/ml and further to 49.1 pg/ml on November 1. On November 5, chest CT still showed infiltration (Fig. 3), and PCR for *Pneumocystis carinii* in sputum was positive. On November 14, chest CT showed no active infiltrations (Fig. 4). On November 15, PCR on sputum became negative for

Figure 2. Chest CT on admission.

Pneumocystis carinii and the plasma (1 $\rightarrow$ 3)- β -D-glucan level was 70.5 pg/ml. Therefore, TMP/SMX was reduced to a prophylactic dose (one double strength) and glucocorticoids were discontinued on November 21, 1996. The patient was discharged on November 25, 1996.

Figure 3. Chest CT on November 5.

Figure 4. Chest CT on November 14.

Discussion

In the treatment of patients with acquired immunodeficiency syndrome, an early and correct diagnosis of PCP is essential. Definitive diagnosis is made by morphologic demonstration of

the organism in sputum or bronchoalveolar lavage fluid or both. However, the sputum sensitivity is low (60%) and bronchoalveolar lavage is sometimes difficult to perform in

Figure 5. The relationship between plasma (1→3)-β-D-glucan levels and PCR on sputum for *Pneumocystis carinii*.

patients with severe respiratory failure despite its high sensitivity (>95%) (1). Follow-up transbronchial lung biopsy has shown that some patients have PCP for as long as 25 days, although the recommended treatment for PCP lasts only 3 weeks (4). Therefore, noninvasive methods are necessary for both monitoring and diagnosis of PCP. Recently, PCR has been shown to be more sensitive and specific for detecting *Pneumocystis carinii* in sputum (2); however, it also detects dead *Pneumocystis carinii*, producing false-positive results (5). On the other hand, according to the recent evidence that *Pneumocystis carinii* might be a fungus (6), (1 $\rightarrow$ 3)- β -D-glucan, which composes a portion of the cell wall of most fungi, has become a new marker for *Pneumocystis carinii* infection (3).

In our patient, both PCR on sputum and the G-test were used to monitor PCP during treatment (Fig. 5). Results of both studies paralleled clinical and radiographic improvement of PCP. Before treatment, plasma (1 $\rightarrow$ 3)- β -D-glucan was high (272.6 pg/ml) and PCR on sputum was positive for *Pneumocystis carinii*. Then, the plasma (1 $\rightarrow$ 3)- β -D-glucan level rapidly fell from 272.6 pg/ml to 70 pg/ml during the first 2 weeks of treatment, parallel to clinical and radiographic improvement. However, for the next 3 weeks, the plasma (1 $\rightarrow$ 3)- β -D-glucan level was almost unchanged despite further clinical and radiographic improvement. When PCR on sputum for *Pneumocystis carinii* became negative, the plasma level of (1 $\rightarrow$ 3)- β -D-glucan (70.5 pg/ml) was still higher than normal (<10 pg/ml) (7).

This sustained elevation of (1 $\rightarrow$ 3)- β -D-glucan despite negative PCR results might be due to another subclinical deep fungal infection. However, the patient had only oral candidiasis which resolved with fluconazole, and blood culture showed no fungemia. Yasuoka et al also reported that six patients with PCP showed decreases in plasma (1 $\rightarrow$ 3)- β -D-glucan levels to 50% or less of peak levels with clinical improvement (3). These findings suggest that plasma (1 $\rightarrow$ 3)- β -D-glucan does not nec-

essarily return to normal in PCP. In the case of other deep fungal infections, plasma (1 $\rightarrow$ 3)- β -D-glucan levels have been reported to return to normal with clinical improvement (7). Therefore, there seems to be a characteristic pattern change in the plasma (1 $\rightarrow$ 3)- β -D-glucan level in improving PCP. Further investigation is necessary for plasma (1 $\rightarrow$ 3)- β -D-glucan to be clinically useful as a quantitative marker of PCP.

In summary, the combination of PCR for *Pneumocystis carinii* on sputum and the G-test is useful in the diagnosis and monitoring of PCP, especially when bronchoalveolar lavage cannot be done. In our patient, the plasma (1 $\rightarrow$ 3)- β -D-glucan level decreased to about one-fourth that before treatment, although it remained higher than normal when the PCR on sputum became negative for *Pneumocystis carinii*.

References

- 1) Bartlett GJ. Medical care of patients with HIV infection. Williams & Wilkins, Baltimore, 1996, p. 57.
- 2) Oka S, Kitada K, Kohjin T, Nakamura Y, Kimura S, Shimada K. Direct monitoring as well as sensitive diagnosis of *Pneumocystis carinii* pneumonia by the polymerase chain reaction on sputum samples. *Mol Cell Probes* 7: 419, 1993.
- 3) Yasuoka A, Tachikawa N, Shimada K, Kimura S, Oka S. (1 $\rightarrow$ 3)- β -D-glucan as a quantitative serological marker for *Pneumocystis carinii* pneumonia. *Clin Diagn Lab Immunol* 3: 197, 1996.
- 4) Delorenzo LJ, Maguire GP, Wormser GP, Davidian MM, Stone DJ. Persistence of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Evaluation of therapy by follow-up transbronchial lung biopsy. *Chest* 88: 79, 1985.
- 5) Lipschik GY, Gill VJ, Lundgren JD, et al. Improved diagnosis of *Pneumocystis carinii* infection by polymerase chain reaction on induced sputum and blood. *Lancet* 340: 203, 1992.
- 6) Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of fungal. *Nature* 334: 519, 1988.
- 7) Obayashi T, Yoshida M, Mori T, et al. Plasma (1 $\rightarrow$ 3)- β -D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 345: 17, 1995.