Invasive Pulmonary Aspergillosis Diagnosed Early by Polymerase Chain Reaction Assay

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We compared the usefulness of a polymerase chain reaction (PCR) assay for the early diagnosis of invasive pulmonary aspergillosis with the serodiagnosis of sufficient concentrations of galactomannan using the same serum samples. A patient was treated with prednisolone for the management of hepatitis. Computed tomography (CT) scan of the chest showed the nodular shadow with a cavity containing a clear fungus ball. DNA of Aspergillus spp. from a serum sample was detected and using the same serum sample, both latex agglutination and sandwich enzyme-linked immunosorbent assay (ELISA) of galactomannan were negative. PCR assay provides an early diagnosis of invasive pulmonary aspergillosis compared with ELISA of galactomannan.

(Key words: serodiagnosis, Aspergillus fumigatus, enzyme-linked immunosorbent assay (ELISA), galactomannan, prednizolone)

Introduction

Invasive aspergillosis has a poor prognosis in immunocompromized patients. A reliable method, including a sensitive serodiagnostic test, for the early diagnosis of aspergillosis is not yet available. The latex agglutination test for the detection of circulating galactomannan of Aspergillus spp. is widely used in clinical laboratories for the diagnosis of invasive aspergillosis; however, the sensitivity of this assay is low (1). In this regard, a polymerase chain reaction (PCR) assay provides a highly sensitive diagnosis of a variety of infectious diseases. The detection of Aspergillus DNA in bronchoalveolar lavage fluid samples of patients with invasive pulmonary aspergillosis is useful, but the bronchofiberscopic technique is impractical for routine use and could be hazardous in the immunocompromized patient (2). Detection of the Aspergillus DNA by PCR assay using serum samples for the diagnosis of invasive pulmonary aspergillosis has been reported (3). In the present case, we evaluated the usefulness of PCR assay for the early diagnosis of pulmonary aspergillosis and compared these results with serodagnosis by detection of galactomannan.

Case Report

A 45-year-old man was admitted on June 7, 1997 to Nagasaki University Hospital for management of type B hepatitis. There were no abnormal shadows on radiological examination of the chest on admission. Hepatitis was treated with oral prednisolone (40 mg/day). A nodular shadow appeared in the lower lobe of the right lung in the chest X-ray on July 15, 1997 (Fig. 1). On laboratory data, leukocyte count was 8,600/μl and C-reactive protein (CRP) was 0.84 mg/dl. The shadow was suspected to be a lung abscess on the finding of chest X-ray (Fig. 2) and the patient was treated with antibiotics consisting of a course of 2 g/day piperacillin for 7 days followed by 1 g/day meropenem for 7 days. This was followed by a change in the radiological appearance of the nodular shadow with a cavity containing a clear fungus ball (Fig. 3). DNA of Aspergillus spp. from a serum sample was detected by PCR on July 28, 1997. Using the same serum sample, both latex agglutination test (Pastorex Aspergillus, Sanofi Diagnostics Pasteur, France) and sandwich enzyme-linked immunosorbent assay (ELISA) test (Platelia Aspergillus, Sanofi Diagnostics Pasteur, France) for the detection of circulating galactomannan were negative. To confirm the PCR results, the patient was examined bronchoscopically on July 30, 1997. Aspergillus fumigatus was isolated from bronchoalveolar lavage fluid (BALF) samples obtained from the right B6a bronchus. The serum sample obtained on the same day was positive on both PCR assay and sandwich ELISA test. Thus, the diagnosis of invasive pulmonary aspergillosis was established and intravenous amphotericin B commenced on August 7, 1997. However, renal dysfunc-
PCR Assay for Pulmonary Aspergillosis

Figure 1. The chest X-ray showed a circumscribed mass in the right lower lobe on July 15, 1997.

Figure 2. The shadow was suspected to be a lung abscess on the finding of chest X-ray on July 28, 1997.

Figure 3. The chest CT scan showed a nodular shadow with a cavity containing a fungus ball on August 11, 1997.

Section developed after intravenous amphotericin B therapy (total dose, 501 mg). Accordingly, amphotericin B was replaced with 200 mg/day itraconazole. PCR for *Aspergillus* DNA became negative in serum samples obtained after treatment with amphotericin B. Another bronchoscopic examination was performed after one month of treatment with itraconazole. BALF samples were negative for *A. fumigatus* at that stage (Table 1).

Discussion

In this report, we compared the usefulness of PCR assay for the early diagnosis of pulmonary aspergillosis with that by galactomannan detection tests. Pulmonary aspergillosis is a common opportunistic fungal infection in neutropenic patients, particularly after intensive chemotherapy or immunosuppressive therapy. Triazole antifungal agents are commonly used as a prophylaxis against fungal infections in neutropenic patients. However, amphotericin B is considered the drug of choice for aspergillosis.

The most important factor related to the prognosis of pulmonary aspergillosis is the early use of amphotericin B at an appropriate dosage (4). Accordingly, an early confirmative diagnosis is essential for the commencement of treatment at an early stage. Mycological and histopathological examination under bronchofiberscopy was considered as the confirmative method for the diagnosis of pulmonary aspergillosis, but the use of bronchoscopy is potentially associated with some risk in the immunocompromized patient. Detection of the circulating galactomannan of *Aspergillus* spp. is currently used for the serodiagnosis of pulmonary aspergillosis. Detection of galactomannan by latex agglutination test is widely used as a serodiagnostic test in clinical laboratories. However, this test may require at least 15 ng/ml of galactomannan in the serum sample for a positive result, suggesting a low sensitivity but high specificity. The new test for the detection of galactomannan based on the sandwich ELISA is reported to be very useful for the early diagnosis of invasive aspergillosis (5). Sandwich ELISA is usually positive when the concentration of galactomannan in the serum is >1 ng/ml (5).
Another strategy for the diagnosis of infectious diseases is the use of PCR amplification of the target DNA. In aspergillosis, the detection of Aspergillus DNA by PCR assay using serum samples was successful in the present case. We compared the usefulness of the PCR assay for the early diagnosis of aspergillosis with the serodiagnosis of sufficient concentrations of galactomannan using the same serum samples. While both tests were useful for the diagnosis of pulmonary aspergillosis, the PCR detection of DNA of Aspergillus was positive two days earlier than that of sandwich ELISA test of galactomannan. Sandwich ELISA was positive in only one sample obtained just before treatment with amphotericin B. Furthermore, the PCR became negative after treatment with amphotericin B when BALF samples became negative for A. fumigatus.

In conclusion, these results showed that based on the detection of Aspergillus DNA, PCR assay may be the most useful test for the early diagnosis of pulmonary aspergillosis.

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References