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Comparison of Antigen Detection and PCR Assay Using Bronchoalveolar Lavage Fluid for Diagnosing Invasive Pulmonary Aspergillosis in Patients Receiving Treatment for Hematological Malignancies

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Invasive pulmonary aspergillosis (IPA) is a major threat to leukemic patients with cytotoxic therapy-induced neutropenia and transplant recipients receiving high-dose corticosteroid therapy. Since the diagnosis of IPA in an early stage is seldom possible and the mortality rate is very high (6), successful treatment is directly related to early diagnosis (1). Several methods for obtaining an early and rapid diagnosis of IPA are now under study. Aspergillus DNA can be detected in bronchoalveolar lavage (BAL) fluid by PCR (5, 16, 18, 22, 25), but the numbers of patients and controls tested have been too limited to establish the diagnostic value of this technique. An alternative approach is to detect circulating Aspergillus antigen in body fluids, e.g., serum or urine, by a number of in-house methods (12, 19, 20). The Aspergillus antigen test (Pastorex Aspergillus; Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) is commercially available and uses the rat monoclonal antibody EB-A2 to detect galactomannan (GM), a polysaccharide antigen of Aspergillus species (13). This antigen test has been evaluated in several institutes (4, 8, 9, 26, 29), but it has been found to lack sufficient sensitivity (8, 9, 26, 29). A recently developed sandwich enzyme-linked immunosorbent assay (ELISA) allowed the detection of low levels of circulating Aspergillus GM in sera from patients at high risk of IPA, but the increase in sensitivity was also associated with false-positive results in up to 8% of the serum samples (21, 27). Since IPA is predominantly a pulmonary infection in immunocompromised patients, and Aspergillus fumigatus is known to release GM into the culture medium when it is grown in vitro (13), detectable antigen levels may be present in BAL fluid samples from patients suspected of having IPA and therefore may be of use for the diagnosis of IPA.

We therefore investigated the use of the sandwich ELISA to detect GM with BAL fluid samples which had been collected from patients at high risk and from patients at low risk of IPA. The results were compared with those obtained by culture and Aspergillus genus-specific PCR amplification and were related to the clinical outcome.

MATERIALS AND METHODS

Patients. During a 12-month period 19 consecutive BAL fluid samples had been collected from patients who were neutropenic as a result of cytotoxic therapy or from whom circulating granulocytes were recovered. In each patient a bronchoscopy was performed because fever (temperature, >38.3°C) persisted despite treatment with broad-spectrum antibacterial agents and pulmonary infiltrates developed on the chest roentgenogram. The BAL fluid sample was obtained by wedging a flexible bronchoscope in the area of maximal roentgenographic involvement, after which 150 ml of sterile 0.9% saline was instilled and approximately 100 ml was recovered by suctioning. The medical, histopathological, microbiological, and radiological records of all patients were reviewed for evidence of IPA or other respiratory disease. Since a definitive diagnosis of IPA is very difficult to establish in this patient group, the patients were allocated to three categories by review of the appearance of the pulmonary infiltrates on the chest roentgenogram by two physicians who were blinded to the medical histories of the patients. The patients were allocated to one of the following groups: A, probable IPA (n = 24), with the chest roentgenogram showing focal nonanatomical infiltrates or any coexisting lesion; B, unlikely IPA (n = 10), with the chest roentgenogram showing diffuse infiltrates characterized by nodular, reticular, or reticulonodular lesions which were distributed throughout both lung fields or anatomical focal infiltrates (15); and C, possible IPA (n = 2), which included
Aspergillus was performed. These samples had also been stored at —80°C until testing by ELISA. Patients with infiltrates on the chest roentgenogram who could not be classified V patients at low risk of IPA containing a range of dilutions of GM (range, 0.25 to 10 ng/ml) were added to 0.9% saline and culture- and PCR-negative BAL fluid samples from 35 control patients are given in Table 1. Serum (optical density value, 0.17) (21). The results of Aspergillus ELISA, different concentrations of GM (range, 0.25 to 10 ng/ml) were added to 0.9% saline and culture- and PCR-negative BAL fluid samples spiked with the same concentrations of GM (data not shown). The mean optical density values of the 30 BAL fluid samples from nonneutropenic patients revealed positive amplification reactions for five samples which were all negative by ELISA (25). The ELISA was positive for 2 of 30 PCR-negative BAL fluid samples (Table 1).

**DISCUSSION**

The diagnosis of IPA in an early stage of the disease is of great importance since early antifungal treatment is associated with an improved outcome (1). The early diagnosis of IPA is difficult, and a definitive diagnosis is often made at autopsy. The presence of severe thrombocytopenia in neutropenic patients limits the use of invasive diagnostic procedures to obtain a specimen for culture and histopathological examination. BAL has been advocated as a safe procedure for obtaining a specimen for culture and histopathological examination. The presence of severe thrombocytopenia in neutropenic patients limits the use of invasive diagnostic procedures to obtain a specimen for culture and histopathological examination. BAL has been advocated as a safe procedure for obtaining a specimen for culture and histopathological examination.

<table>
<thead>
<tr>
<th>Microbiological analysis result</th>
<th>Hematologic patients</th>
<th>Nonneutropenic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive, ELISA positive</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>PCR positive, ELISA negative</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>PCR negative, ELISA positive</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>PCR negative, ELISA negative</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>35</td>
</tr>
</tbody>
</table>

**TABLE 1. Results of Aspergillus genus-specific PCR amplification and sandwich ELISA of BAL fluid samples obtained from 19 patients with hematological malignancies and 35 nonneutropenic patients**
viously described the use of a radioimmunoassay to detect an uncharacterized purified *A. fumigatus* carbohydrate in BAL fluid (2, 3, 30). This radioimmunoassay detected *Aspergillus* antigen in 91% of the BAL fluid samples from rabbits with IPA, and in 27% of these animals antigen was detected in BAL fluid only and not in serum (2). The radioimmunoassay was also used to detect *Aspergillus* antigen in BAL fluid samples from nine immunocompromised patients with pulmonary infiltrates, and two patients were found to be positive (3). GM was detected by a latex agglutination test (Pastor *et al.*, 1982) in BAL fluid samples from three patients with AIDS and IPA (14). However, GM could not be detected by the same test under laboratory conditions in BAL fluid samples from 14 untreated rabbits with IPA, while serum samples from 10 (71%) animals were positive (7). Although the present study was limited by the difficulty in reliably classifying patients with IPA, GM was detected by sandwich ELISA in BAL fluid samples from five of seven patients with radiological evidence of IPA, and therefore, this test may be promising for use in the diagnosis of this infection.

The increase in the level of sensitivity required to diagnose early IPA, however, is hampered by an increase in the number of false-positive results. For this reason, it was interesting to compare DNA and GM detection, since these two assays have a different target. In the case of diagnosis by PCR, a positive result obtained from the use of a BAL fluid sample can be due to the presence of contaminating conidia without any mycelial development (see, for example, data for patients C1 and C2). Indeed, previous studies found a rate of 15% positive PCR amplification in BAL fluid samples from nonneutropenic patients (22, 25). The occurrence of a negative PCR result for patient A6, whose BAL fluid and serum samples were GM positive and who had radiological and clinical evidence of IPA, could be due to the presence of inhibitors of the PCR in the BAL fluid (23).

False-positive results were also obtained by the sandwich ELISA. The reasons for the false-positive reactions are unknown, but it has been shown that molecules which are responsible for the false positivity appear to mimic the epitope recognized by anti-GM monoclonal antibodies (21). In 30 culture- and PCR-negative BAL fluid samples from nonneutropenic patients, a 7% false-positive rate was found, which is similar to the 8% false-positive rate found in serum (21). A 10-fold concentration of the samples by acetone precipitation resulted in a false-positive rate of 27% (data not shown). Analysis by PCR and ELISA can be done in less than 24 h (PCR, 24 h; ELISA, 4 h), and the results can be forwarded to the clinician on the day following the BAL.

An excellent correlation was found between serum and BAL fluid ELISA results, and GM was detected in the serum even
before the BAL was performed. Since a bronchoscopy was performed only after pulmonary infiltrates were present on the chest roentgenogram, GM may be detectable in the serum before infiltrates become visible on the plain chest film. It has been shown that in neutropenic patients abnormalities are difficult to detect on plain chest film (17) and that other radiologic methods, such as high-resolution computed tomography, are more sensitive for the detection of pulmonary infiltrates in these patients (10, 11, 28) and may even be used for therapeutic monitoring (28).

This preliminary investigation suggests that both Aspergillus genus-specific PCR and sandwich ELISA might be beneficial before infiltrates become visible on the plain chest film. It has been shown that in neutropenic patients abnormalities are difficult to detect on plain chest film (17) and that other radiologic methods, such as high-resolution computed tomography, are more sensitive for the detection of pulmonary infiltrates in these patients (10, 11, 28) and may even be used for therapeutic monitoring (28).

REFERENCES