Serodiagnosis and Monitoring of Aspergillus Infections after Lung Transplantation

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Objective: To determine whether quantification of specific antifungal antibody responses in serum can provide supplemental information for the diagnosis of Aspergillus fumigatus infections and the monitoring of antifungal treatment in patients after lung transplantation.

Design: Retrospective study.

Setting: Center for lung transplantation, University Hospital Groningen, the Netherlands.

Patients: 4 patients with proven A. fumigatus infections after lung transplantation and fatal outcome.

Measurements: The IgG antibody response specific for A. fumigatus antigens was measured by enzyme-linked immunosorbent assay and was compared with radiographic features, cytologic findings, microbiological cultures, and clinical diagnosis.

Results: Increasing IgG antibody responses specific for A. fumigatus closely paralleled cytologic or microbiological identification of A. fumigatus from bronchoalveolar lavage fluid and decrease of lung function. Increasing specific IgG antibody responses were found to precede radiographic identification of lung cavitation by 1 to 2 weeks, precede the diagnosis of aspergillosis by 2 to 20 weeks, and detect fungal reinfection. In most cases, successful antifungal treatment decreased specific IgG antibody response. A decrease in specific IgG antibody response correlated with the inability to culture or identify A. fumigatus in bronchoalveolar lavage fluid and with radiographic and clinical improvement.

Conclusions: Specific IgG antibody responses in serum correlate with radiographic, cytologic, and microbiological findings and with the clinical diagnosis of A. fumigatus infections in patients who have had lung transplantation. Increased IgG antibody responses in serum may provide important information that is helpful in the diagnosis and early treatment of pulmonary fungal infections and in monitoring antifungal treatment.

Methods

Patients

Between 1990 and 1994, 56 patients received lung transplants at the University Hospital Groningen, the Netherlands; these patients had a 1-year survival rate of 84%. We reviewed the data of four male patients, 33 to 58 years of age, who had received lung transplants in 1993 and had had at least one episode of proven A. fumigatus infection. The indications for lung transplantation were emphysema in two patients and cystic fibrosis in two patients. The diagnosis of invasive fungal infection required isolation of fungus from a normally sterile site or evidence of fungal tissue invasion in a biopsy specimen. Key clinical and laboratory findings of the patients are summarized in Table 1.

Serum Specimens

Serum samples from all four patients were collected after lung transplantation and stored at −20°C. A positive reference serum sample was prepared by pooling the serum specimens of several patients with diseases related to A. fumigatus. These
Table 1. Clinical and Laboratory Findings of Patients Receiving Lung Transplants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>General information</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication for lung transplantation</td>
<td>Cystic fibrosis</td>
<td>Emphysema</td>
<td>Emphysema</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Age, y</td>
<td>35</td>
<td>44</td>
<td>55</td>
<td>30</td>
</tr>
<tr>
<td>Type of lung transplantation</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Right sided</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Day on which death occurred</td>
<td>696</td>
<td>373</td>
<td>372</td>
<td>469</td>
</tr>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days on which sputum cultures were positive for Aspergillus fumigatus</td>
<td>408, 687</td>
<td>70, 105</td>
<td>275 (LS), 281 (RS), 687</td>
<td>105 (LS, UF), 184 (RS, UF)</td>
</tr>
<tr>
<td>Days on which cavitations were found on radiography (location)</td>
<td>275 (LS), 281 (RS), 687 (LS, RS)</td>
<td>121 (RS, UF), 1631 (RS, MF)</td>
<td>105 (LS, UF), 184 (RS, UF)</td>
<td>231 (LS)</td>
</tr>
<tr>
<td>Days on which cytologic identification of A. fumigatus was made (location)</td>
<td>408, 687†, 688 (LS)‡</td>
<td>59 (RS, LS)§, 166 (RS, UF)</td>
<td>85 (LS)§, 105 (LS, RS)§</td>
<td>19 (LS)‡, 166 (LS, RS)§</td>
</tr>
<tr>
<td>Days on which aspergillosis was diagnosed</td>
<td>408, 687**</td>
<td>166</td>
<td>105</td>
<td>20</td>
</tr>
<tr>
<td>Manifestation of aspergillosis</td>
<td>Aspergilloma, aspergil­</td>
<td></td>
<td>Aspergilloma (LS)</td>
<td>Wound infection</td>
</tr>
<tr>
<td>loma/pneumonia**</td>
<td>8 (408–453), itracon­</td>
<td>azole (454–688)</td>
<td>8 (79–97), liposomal amphotericin 8 (105–145), itraconazole (146–338)</td>
<td>Liposomal amphotericin 8 and local irrigation with amphotericin B (24)</td>
</tr>
<tr>
<td>Days on which A. fumigatus IgG levels first increased</td>
<td>267</td>
<td>170</td>
<td>107</td>
<td>16</td>
</tr>
<tr>
<td>Effect of therapy on A. fumigatus IgG levels</td>
<td>Decrease</td>
<td>Decrease</td>
<td>Decrease†</td>
<td>No change</td>
</tr>
</tbody>
</table>

* All patients were men. † Days: are days after transplantation. LS = left side; MF = middle field; RS = right side; UF = upper field.
|† Coinciding with malaise, dyspnea, and chest pain.
|§ Identified in material obtained by transthoracic puncture.
|** Probably as a contamination from the throat.
|§ Identified in bronchoalveolar lavage fluid.
|†† Identified in infected wound.
|** For fungal reinfection.
|††† After initial increase.

Patients (who had pulmonary aspergilloma or allergic bronchopulmonary aspergillosis) showed high IgG antibody titers specific for A. fumigatus on enzyme-linked immunosorbent assay (ELISA) and had high numbers of precipitins on double immunodiffusion tests.

Immunologic Procedures

We measured the IgG antibody response specific for A. fumigatus antigens (A. fumigatus IgG) retrospectively and in batches by a direct ELISA procedure (8) with late-phase Sabouraud dextrose culture filtrates as previously described (9). The results are expressed as ELISA units as previously described (8).

Results

The A. fumigatus IgG levels for patient 1 during disease progression and treatment are shown in Figure 1. Before lung transplantation, this patient’s A. fumigatus IgG levels were low (data not shown). After lung transplantation, these levels remained low for 246 days. Within 21 days after this period (246 to 267 days after transplantation), the levels increased sharply, approximately 1 to 2 weeks before cavitations were seen in the left lung (day 275 after transplantation) and in the right lung (day 281 after transplantation) (Figure 1). In the following period, A. fumigatus IgG levels remained high and even increased slightly. When aspergilloma could be diagnosed by transthoracic puncture on day 408 after transplantation, the levels were still elevated (Figure 1). During antifungal treatment, the levels steadily decreased until day 587 after transplantation, although sputum samples repeatedly grew A. fumigatus. When fungal reinfection was diagnosed by transthoracic puncture on day 688 after transplantation, the A. fumigatus IgG level had sharply increased.

Data from the four patients are summarized in Table 1. Increasing A. fumigatus IgG levels closely paralleled cytologic or microbiological identification of A. fumigatus from bronchoalveolar lavage fluid (patient 3), deterioration of lung function, the development of an infiltrate on the radiograph, clinical symptoms, and the diagnosis of organizing pneumonia with A. fumigatus (patient 2).

Increasing A. fumigatus IgG levels were found to precede radiographic identification of lung cavitations (by 1.5 weeks in patient 3 and by 1 to 2 weeks in patient 1), the diagnosis of aspergillosis by radiologic and cytologic examination of bronchoalveolar lavage fluid (by 1.5 weeks in patient 3) or by cytologic examination of material obtained by trans-
thoracic puncture (by 20 weeks in patient 1), and
the diagnosis of thoracotomy wound infection (by 2
weeks in patient 4) (Table 1). In patient 1, highly
elevated \textit{A. fumigatus} IgG levels were found at the
time of fungal reinfection and may have increased
before reinfection was diagnosed (Figure 1). How­
ever, because no serum specimens were available
between 587 and 688 days after transplantation, we
could not test this assumption. The rate of increase
of \textit{A. fumigatus} IgG levels was remarkable; titers
changed within 2 weeks from negative to strongly
positive values in all patients studied (data not
shown).

Successful antifungal treatment was reflected by
clinical improvement in all patients and by decreas­
ing \textit{A. fumigatus} IgG levels in three patients (pa­
tients 1, 2, and 3). Decreasing \textit{A. fumigatus} IgG
levels correlated with the inability to culture or
identify \textit{A. fumigatus} from bronchoalveolar lavage
fluid (patients 1 and 3) and with radiographic im­
provement (patients 1 and 2). In all patients, \textit{A.
fumigatus} IgG levels peaked 2 to 3 weeks after
initiation of liposomal amphotericin B treatment
and again after itraconazole treatment (Figure 1), as
previously described; this outcome may have re­
lected the liberation of antigen after the killing of
the microorganism (10).

Discussion

We present data from four retrospectively stud­
ied patients, who had proven \textit{A. fumigatus} infections
after lung transplantation and fatal outcome. These
data show a close correlation between \textit{A. fumigatus}
IgG levels measured in serum and radiographic fea­
tures, cytologic and microbiological findings, and
the clinical diagnosis of fungal disease.

Two of the four patients had a clear diagnosis of
aspergilloma formation either in one of the trans­
planted lungs (patient 1) or in the native lung (pa­
tient 3). One patient (patient 2) presented with
organizing pneumonia with \textit{A. fumigatus}, and an­
other patient (patient 4) developed \textit{A. fumigatus}
infection of the thoracotomy wound. All patients
were treated systemically with itraconazole, liposo­
amal amphotericin B, or both; they improved consid­
erably with treatment. Antifungal treatment of the
fungal reinfection in patient 1 was not successful.
The increase in \textit{A. fumigatus} IgG levels in patient 2

![Graph showing Aspergillus fumigatus IgG readings from enzyme-linked immunoassay (ELISA) of patient 1 plotted against time. Arrow 1 indicates day of cavitation of left upper lobe, arrow 2 indicates day of cavitation of right upper lobe, arrow 3 indicates day of diagnosis of aspergilloma of the left upper lobe, and arrow 4 indicates day of diagnosis of fungal reinfection of the left upper lobe. The dashed line represents an interpolation of the \textit{A. fumigatus} IgG response; no serum specimens were available during this period.]

1 August 1996 • \textit{Annals of Internal Medicine} • Volume 125 • Number 3 199
that occurred 222 days after transplantation may have indicated reinfection with *A. fumigatus* at a subclinical level. However, no evidence of reinfection was found at autopsy. In patient 1, however, reinfection with *A. fumigatus* was reflected by an increased *A. fumigatus* IgG response and was confirmed by transthoracic puncture and autopsy. In patient 4, *A. fumigatus* IgG levels did not decrease after liposomal amphotericin B treatment was stopped until 243 days after transplantation (data not shown).

After lung transplantation, patients are at high risk for infections of the lung and airways (5). This is generally attributed to a decrease in both the humoral and the cellular immune defenses, which is caused by immunosuppressive therapy. This assumption was supported by recent in vitro studies (11) that showed decreased IgG formation by B cells in the presence of alveolar macrophages in patients after lung transplantation.

In contrast to these in vitro studies, we found *A. fumigatus* IgG levels to be as high as or higher than the levels found in serum specimens from patients with aspergillosis (without lung transplantation) or exacerbation of allergic bronchopulmonary aspergillosis. Additionaly, dramatic changes in *A. fumigatus* IgG levels from negative to strongly positive values within 2 to 3 weeks indicate that the immune suppression therapy used to prevent rejection does not impair the humoral immune response to invading fungi. Thus, the high susceptibility to fungal infection of this patient group is not explained by a diminished humoral immune response. Our observations are similar to those seen in patients with cytomegaloviral infections after lung transplantation, in whom high antibody responses in serum can be detected during infection episodes (12).

Fungal infections are a major concern; those caused by *A. fumigatus* are especially serious given the high mortality rate caused by this microorganism (13). In addition to being a direct cause of death, frequent infection may lead to long-term sequelae associated with increased episodes of rejection and possibly to the development of oblitative bronchiolitis. An association of pulmonary bacterial infections (14) and particularly viral infections (15) with oblitative bronchiolitis has been described.

The diagnosis of fungal infection is difficult and often is not established before death (16). A slight increase in body temperature and new infiltrations on chest radiography are not specific for fungal infection. Chest radiographs (17) and spirometric findings (18) are abnormal in both rejection and infection of the lungs. Besides, patients may have pulmonary infections with *A. fumigatus* even though chest radiography shows a normal appearance (19). Recent studies (5) have shown that invasive *A. fumigatus* can be found during the autopsies of patients not suspected of having invasive disease.

Patients are susceptible to lung rejection and infection after lung transplantation, two recurrent problems that require early and accurate diagnosis and treatment. Transbronchial biopsy improves the accuracy of diagnosis of acute lung rejection and often allows the distinction to be made between rejection and infection (20). Our study, although limited in size, suggests that elevated *A. fumigatus* IgG serum levels in lung allograft recipients may provide supplemental information helpful in diagnosing and treating pulmonary *Aspergillus* infections and in monitoring the success of antifungal treatment. The sensitivity and specificity of *A. fumigatus* IgG serologic testing in a larger group of patients who have had lung transplantation are currently being studied.

Acknowledgments: The authors thank Professor Dr. Lou de Ley (Department of Clinical Immunology, University Hospital, Groningen, the Netherlands) for providing serum samples from the patients in this study and Mr. Hein Lange (Department of Allergology, University Hospital, Groningen, the Netherlands) for his skillful technical assistance on ELISA procedures.

Grant Support: In part by grant 94.15 from the Dutch Asthma Foundation.

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