Aspergillus Airway Colonization and Invasive Disease After Lung Transplantation*

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**Background:** Invasive Aspergillus is an important cause of morbidity and mortality among lung transplant recipients. The diagnosis can be difficult and treatment is often unsuccessful so many centers preemptively treat all Aspergillus airway isolates to prevent invasive disease. This approach is untested as little is known about the relationship between Aspergillus airway colonization and invasive disease. This study was undertaken to evaluate the incidence of Aspergillus airway colonization after lung transplantation and the risk of invasive disease after colonization.

**Design:** All cultures and histologic specimens obtained from a consecutive series of 151 lung transplant cases were reviewed for the presence of Aspergillus and compared with clinical data. 

**Results:** Aspergillus was isolated from the airway in 69 (46%) of 151 transplant recipients. Invasive disease occurred in five cases and was uniformly fatal, accounting for 13% of all posttransplant deaths. Results of cytologic examination of BAL fluid were normal in all cases of invasive disease and cultures were positive in only one of five patients prior to invasion. Invasive disease occurred exclusively in patients who died or were colonized with *Aspergillus fumigatus* within the first 6 months posttransplant. Patients growing *A. fumigatus* from the airway during the first 6 months were 11 times more likely to develop invasive disease relative to those not colonized.

**Conclusion:** Aspergillus airway colonization after lung transplantation is common and in most cases, transient. In contrast, invasive Aspergillus disease is less common, but fatal. Bronchoscopy with cytologic examination and fungal culture are not sensitive or timely predictors of invasive disease. Invasive Aspergillus occurred only in patients initially colonized with *A. fumigatus* within the first 6 months posttransplant. A trial of empirical anti-Aspergillus therapy limited to the first 6 months posttransplant may be warranted.

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**Key words:** antifungal therapy; Aspergillus; bronchoscopy; case series; fungal culture; lung transplantation

Locally invasive or disseminated Aspergillus infection remains an important cause of morbidity and mortality among lung transplant recipients, accounting for 2 to 33% of postlung transplant infections and 4 to 7% of all lung transplant deaths. Since treatment of invasive Aspergillus is often not successful, a variety of approaches have been employed to reduce the impact of this disease, including the use of preemptive antifungal therapy for positive Aspergillus airway cultures. The introduction of the oral azoles and the increased use of aerosolized amphotericin have facilitated the use of preemptive strategies for treatment of Aspergillus airway isolates in lung transplant recipients. However, the efficacy of this approach is not clear as very little is known about the relationship between Aspergillus airway colonization and invasive disease.
The propensity of Aspergillus to invade or disseminate depends on the pulmonary and systemic immune status of the host.\textsuperscript{7-10} Inhalation of fungal spores and airway colonization presumably precede invasive disease, but the incidence of invasive disease after airway colonization is not known.\textsuperscript{11,12} Because the lung allograft is the target of both infection and rejection, lung transplant recipients undergo frequent surveillance bronchoscopy. This provides a unique opportunity to observe the course of Aspergillus in the lung over time. This study was undertaken to evaluate the incidence of Aspergillus airway colonization after lung transplantation and the risk of invasive disease after airway colonization.

**Materials and Methods**

Between May 1986 and October 1994, we evaluated a consecutive series of 147 patients undergoing lung or heart-lung transplantation at the University of Minnesota. A transplantation case was defined as a new transplant or a retransplant that was performed at least 6 months after the original surgery when immunosuppression had been tapered to its lowest level.

After transplantation, all patients were maintained on a regimen of immunosuppression with cyclosporine, azathioprine, and prednisone (0.5 mg/kg/d tapering to 0.1 mg/kg/d over 6 months). Treatment with these medications was continued for the life of the patient. Routine clinic visits with surveillance bronchoscopy, BAL, and transbronchial biopsy of the transplanted lung were performed 4 and 8 weeks posttransplant, every 8 weeks thereafter for the first year, and every 12 weeks during the second year. Surveillance bronchoscopy was discontinued after 12 consecutive rejection-free months. Bronchoscopy was also performed to evaluate clinical, radiographic, or spirometric abnormalities. BAL samples were routinely sent for bacterial, fungal, and viral culture. Sputa and nasal washes were likewise cultured if clinical suspicion of infection was present.

All fungal cultures from sputum, BAL, surgical specimens, and autopsy specimens were reviewed for the presence of Aspergillus. Quantitation of Aspergillus growth was not consistently noted in culture reports and therefore not included in the study. Aspergillus culture rates were examined for evidence of temporal clustering to rule out laboratory contamination and/or epidemic Aspergillus exposure. Multiple positive Aspergillus cultures within a 2-week period were counted as a single positive culture even if more than one species was isolated. A culture growing more than one Aspergillus species was counted as *Aspergillus fumigatus* isolate if one of the species was *A. fumigatus*. The decision to preemptively treat an Aspergillus airway isolate was left to the primary physician. Treatment options included itraconazole and/or inhaled or IV amphotericin. Any case in which an Aspergillus airway isolate was treated for any period of time was considered a treatment case.

Histologic specimens from all postmortem examinations and/or autopsies were reviewed for fungal elements of Aspergillus. Invasive Aspergillus disease was diagnosed by the presence of characteristic sepiate hyphae in tissue specimens, 3 to 4 μm in diameter, with dichotomous branching at 45°. A positive fungal culture was not required to make the diagnosis of invasive Aspergillus.

**Results**

Between May 1986 and October 1994, 147 patients (62 men and 85 women) with advanced pulmonary or pulmonary vascular disease underwent 153 lung or heart-lung transplantation procedures at the University of Minnesota. The median patient age at the time of transplantation was 44 years (range, 3 to 64 years). Six of the 153 transplants were retransplant surgeries for acute or chronic graft failure. Four of the retransplants were performed more than 6 months after the original surgery and were thus considered new transplant cases. The total number of transplant cases was 151. Clinical characteristics of the subjects are listed in Table 1. The mean follow-up for cases was 22 months (range, 0 to 89 months).

Eighty-two (54%) of the 151 cases had no evidence of Aspergillus airway colonization after transplantation despite routine surveillance bronchoscopy with fungal culture of BAL fluid. Twenty-three of these patients died in the course of the study. Complete or limited postmortem examinations and/or antemortem surgical specimens were obtained in 19 (83%) of the 23 decedents with no evidence of colonization. Invasive Aspergillus disease was found at autopsy in 2 of the 19 patients. One patient had clinically unsuspected Aspergillus invasion of the transplanted lung and the kidney, and one patient had multiple Aspergillus brain abscesses without evidence of lung involvement. In the latter case, Aspergillus was the suspected pathogen despite normal results of a cytologic examination and negative fungal culture of aspirated abscess material. Invasive disease was not diagnosed definitively until autopsy.

Sixty-nine (46%) of the 151 lung transplant cases cultured Aspergillus from the airway at some point after transplantation. Forty-four (64%) of the 69 cases first grew Aspergillus in the initial 6 months posttransplantation when steroid therapy was most intensive, airway complications (anastomotic stenosis, tracheobronchomalacia, and accumulation of anastomotic granulation tissue) were common, and surveillance bronchoscopy was frequently performed (Fig 1). A *fumigatus* was most commonly isolated, followed by *Aspergillus niger*, *Aspergillus versicolor*, and *Aspergillus flavus* (Table 2). In 43 (62%) of the 69 cases, Aspergillus was isolated only once in the posttransplant course, while in 26 (38%) cases, the fungus grew repeatedly from the airway posttransplantation. There was no temporal clustering of Aspergillus isolates over the course of the study. The underlying disease, patient age at the time of transplantation, and gender were not predictive of Aspergillus airway colonization or invasive disease after
transplantation. Receiving a single lung transplant was a significant risk factor for Aspergillus airway colonization when compared to a bilateral single lung transplant, but was not a risk factor for invasive disease (Table 1).

There were a total of 16 deaths among the 69 cases with Aspergillus airway isolates during the course of the study. In 3 of the 16, invasive Aspergillus was documented antemortem. Nine (54%) of the 16 decedents, including 2 of the 3 with invasive disease, underwent postmortem examination. No additional cases of invasive Aspergillus were found at autopsy. All three patients with Aspergillus airway isolates who developed invasive disease grew \textit{A. fumigatus}. Cytologic examination of BAL fluid did not demonstrate hyphae in any of the cases of invasive disease. In two of the cases, invasive disease was documented before positive Aspergillus airway cultures were reported. Both patients were treated with Amphotericin once invasive disease was documented, but both died of invasive Aspergillus. In one patient, \textit{A. fumigatus} was first isolated from the airway 3 months posttransplantation but was not treated. The patient died 26 months after transplantation, and in the 4 months prior to death, \textit{A. fumigatus} was repeatedly cultured from the airway despite treatment with itraconazole followed by amphotericin. At autopsy, typical branching septate hyphae were identified in the lung parenchyma and brain.

The initial 6 months posttransplantation appears to be a high-risk period for the development of invasive Aspergillus disease. All three patients who died with invasive disease after airway colonization was noted first grew \textit{A. fumigatus} within 6 months of transplantation. In total, 18 (12%) of the 151 cases first grew \textit{A. fumigatus} in the 6 months posttransplant and 3 (17%) of the 18 developed invasive disease (Fig 1). Among the 133 patients who did not grow \textit{A. fumigatus} in the first 6 months posttransplantation, 2 (1.5%) cases of invasive disease occurred (relative risk=11, confidence interval=2 to 62). In both of these cases, death occurred within 6 months of their transplant. None of the 25 patients first colonized with any Aspergillus species more than 6 months posttransplant and none of the 26 patients colonized with non-\textit{A. fumigatus} species in the first 6 months posttransplant developed invasive disease.

**DISCUSSION**

Invasive fungal infection caused by Aspergillus is an often fatal complication of lung transplantation.\textsuperscript{2,3,6,13} The lung is the presumed portal of entry for fungal spores, and direct invasion of the lung or airway is present in the vast majority of transplant recipients dying with invasive Aspergillosis.\textsuperscript{9,14-16} This was confirmed in our study where invasive disease had a 100% mortality rate and four of the five patients with invasive disease had fungal invasion of the transplanted lung.
The presentation of Aspergillus disease in the lung transplant patient can be occult, with the extent of disease at the time of diagnosis far out of proportion to the severity of symptoms. Often, the diagnosis is not confirmed before invasion or dissemination has occurred. Early diagnosis of invasive disease is hampered both by inability to grow the fungus from clinical specimens when histologic proof of disease is present and the common isolation of the fungus from the airway when disease is absent.

In this study, Aspergillus airway colonization was documented in 46% of lung transplant cases at some point in the posttransplant course. This striking incidence of airway colonization has been confirmed in other lung transplant centers (personal communication, J. Dauber, MD; University of Pittsburgh; October, 1996) and may be related to impaired mucociliary clearance, disruption of lymphatic drainage, a diminished cough reflex, anastomotic problems, or an incompatible human leukocyte antigen microenvironment where macrophages and lymphocytes of the recipient live in the milieu of the donor. The fact that the transplanted lung is the only organ transplant in direct communication with the environment may also facilitate Aspergillus airway colonization in these patients.

Although almost half of our patients had Aspergillus airway colonization at some point posttransplantation, invasive Aspergillus disease was found in only five (3%) patients (accounting for 13% of all posttransplant deaths). The true incidence of invasive disease is probably higher, as not all decedents had autopsies and not all autopsies were complete. Only 9 of 16 (56%) decedents with Aspergillus airway colonization underwent postmortem examination. Despite the disparity between the incidence of airway colonization and invasive disease in this study, patients with A fumigatus airway colonization in the first 6 months posttransplant were 11 times more likely to develop invasive disease relative to those not colonized with A fumigatus during this period.

In this study, all patients with invasive disease and positive fungal cultures grew A fumigatus exclusively and did so during the first 6 months after transplantation. Both patients with invasive disease first diagnosed at autopsy also died within 6 months of transplantation. These data imply that invasive Aspergillus disease results from A fumigatus colonization during the first 6 months posttransplant. This finding is consistent with published data from other lung transplant centers. Yelandi et al found that 6 (16%) of 37 lung transplant recipients grew A fumigatus posttransplant. Four of the six died, two with documented invasive disease. Both invasive disease deaths occurred within 4 months of transplantation. In the study of Paradis and Williams, the majority of invasive Aspergillus disease occurred within 6 months of lung transplantation. Steroid therapy is known to predispose to Aspergillus invasive disease, and intensive steroid therapy likely accounts for the excess risk of Aspergillus invasive disease in lung transplant recipients during this initial posttransplant period.

Unfortunately, BAL with cytologic examination and fungal culture was not a timely indicator of Aspergillus airway colonization prior to invasion for two patients in this study and surveillance lavage, cytologic examination, and fungal culture were negative in an additional two patients with invasive disease. In only one case was airway culture positive prior to the diagnosis of invasive disease, but preemptive therapy was unsuccessful in eradicating the fungus prior to invasion. Since intensive surveillance followed by preemptive therapy could not have prevented four of the five invasive disease cases in our series (even with the dubious assumption that

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**Table 2—Frequency of Aspergillus Species Isolated**

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fumigatus</em></td>
<td>68</td>
<td>44</td>
</tr>
<tr>
<td>*A. niger</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td><em>A. versicolor</em></td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>*A. flavus</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>*A. nidulans</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>*A. glaucus</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>*A. terreus</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>100</td>
</tr>
</tbody>
</table>

*A. fumigatus* was the most common species isolated. Other refers to two *A. ochraceus* isolates, and one isolate each of *A. clavatus*, *A. ustus*, and *A. wentii*. 
preemptive therapy is 100% effective), an alternate strategy is needed. We suggest a randomized trial of empiric antı-Aspergillus therapy for the first 6 months posttransplant, with a control arm comprising intensive surveillance followed by preemptive therapy for patients with *A fumigatus* isolates.

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