Saprophytic Fungal Infections and Complications Involving the Bronchial Anastomosis Following Human Lung Transplantation*

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Study objective: To demonstrate an association between saprophytic fungal infections occurring at the bronchial anastomosis (BA) and the development of additional complications arising at this site.

Design: Retrospective review.

Setting: University lung transplant center.

Materials and methods: Review of all single-lung and double-lung transplant (LTX) recipients who underwent transplantation between June 1993 and December 2000. All recipients were subjected to surveillance bronchoscopy with biopsy at predetermined intervals and when clinically indicated. Bronchial wash fluid and biopsy material were examined using appropriate fungal stains and culture techniques. An infection was defined when fungal organisms were identified in tissue specimens.

Results: Fifteen saprophytic fungal infections involving the BA were identified in 61 LTX recipients (24.6%) who survived a minimum of 75 days post-transplantation. Infections were attributed to Aspergillus sp (n = 9), Candida sp (n = 2), Torulopsis sp (n = 1), and mixed flora (ie, Penicillium + Candida, two patients; and Aspergillus + Candida, one patient). Saprophytic fungal infections occurred by a median of postoperative day 35 (range, 13 to 159 days). Airway complications involving the BA ultimately developed in 11 of 61 recipients (18%). These complications included symptomatic bronchial stenosis (nine patients), bronchomalacia (one patient), and fatal hemorrhage (one patient). Bronchial complications arose in 7 of 15 recipients (46.7%) with saprophytic fungal infections of the BA in contrast to 4 of 46 (8.7%) without infections (p = 0.003, Fisher exact test). Also demonstrated was a positive correlation between anastomotic infections and bronchial complications (Φ coefficient = 0.43; p = 0.001), while logistic regression analysis revealed that the absence of anastomotic infections predicted the absence of such complications (p = 0.002). The risk of developing an additional complication following an anastomotic infection in patients with infections was five times that of those recipients without an infection (relative risk, 5.36; 95% confidence interval [CI], 1.82 to 15.79). The odds in favor of a bronchial complication following an infection were eight times greater than in those recipients without infection (odds ratio, 8.31; 95% CI, 1.96 to 35.16).

Conclusions: Following LTX, saprophytic fungal infections of the BA are associated with serious airway complications.

Key words: Aspergillus; bronchial anastomosis; fungal; infection; lung transplantation

Abbreviations: BA = bronchial anastomosis; HE = hematoxylin and eosin; GMS = Gomori methenamine silver; LTX = lung transplantation; RR = relative risk

Over the last decade, lung transplantation (LTX) has emerged as a viable option for treating patients with end-stage lung disease due to a variety of pulmonary disorders. Unfortunately, the inherent use of immunosuppressive medications, which allows for allograft acceptance, subjects the recipient to the risk of opportunistic infections. Such infections currently account for significant mortality in these recipients, especially within the first year following the transplant procedure.1 While these infections do not always involve the transplanted lung, the allograft is potentially susceptible to infections from a variety of sources, including flora from both the recipient and the donor native airways.2,3 Infections may result from bacterial, fungal, or viral infiltration of the graft. The bronchial anastomosis (BA) has been
Materials and Methods

The investigation involved a retrospective review of all LTX procedures performed between June 1993 and December 2000. To allow for an interval of adequate post-transplantation surveillance, a recipient had to survive a minimum of 75 days following transplantation to be included in the study. No recipient having an infection or complication of the BA died before postoperative day 75.

As previously described, in patients undergoing single-LTX, implantation of the donor organ was achieved by creating an end-to-end or telescoping BA, while double-LTX procedures were performed using the bilateral sequential technique.\(^6,7\) Immediately following LTX, routine immunosuppression was employed utilizing standard regimens consisting of cyclosporine with azathioprine and corticosteroids (initially, IV methylprednisolone and later oral prednisone). LTX recipients received empiric systemic antibiotic therapy with differing antimicrobial agents (mostly clindamycin and ceftazidime) for 10 to 14 days following transplantation. Recipients receiving allografts for septic pulmonary disorders received antibiotics that were specific for respiratory tract flora that had been identified on pretransplant cultures of the sputum. All recipients received fungal prophylaxis with oral itraconazole in a dose of 200 mg per day for a minimum of 3 months. Serum levels of this medication were not routinely followed. In addition to continuing the oral administration of itraconazole, for those recipients ultimately identified with a saprophytic fungal infection of the BA, therapy was undertaken with both IV and inhaled amphotericin B. For systemic therapy, a liposomal preparation of amphotericin B was initiated in a dose of 5 mg/kg. The inhaled administration of amphotericin B was accomplished by placing 10 mg of drug in 5 to 10 mL of sterile water delivered twice a day via a nebulizer.

Surveillance bronchoscopy was performed routinely by one of two experienced transplant pulmonologists in all recipients approximately at postoperative days 7, 14, 28, 56, 84, 180, and 365. As determined by the physician, additional bronchoscopic procedures were performed for any deterioration in the recipient’s clinical status, as manifested by cough, dyspnea, fever, and/or decline in spirometric values. If the recipient was receiving mechanical ventilatory support, the bronchoscope was passed via the endotracheal or tracheostomy tube. If the patient was spontaneously breathing, the bronchoscope was passed either through the nose and nasopharynx or the mouth and oropharynx. A 2% solution of lidocaine was used as a regional anesthesia of the glottic structures. BAL with a minimum of 120 mL saline solution was performed as a routine part of each bronchoscopic procedure from either the middle lobe or the lingula. An examination of the BA was performed during each procedure. If, in the judgment of the bronchoscopist, the BA appeared to be abnormal, endobronchial biopsy specimens of this site were obtained. Characteristics that constituted an abnormal-appearing BA site included the following: mucopurulent secretions, grayish or devitalized mucosa; or sloughed mucosa partially or completely occluding the airway lumen. Additionally, a bronchial wash specimen from this area was collected. Tissue samples obtained from bronchoscopy were fixed in 10% neutral buffered formalin. Three sections from each tissue sample were stained with hematoxylin-eosin (HE). An additional section was stained by the Gomori methenamine silver (GMS) method to identify fungal organisms. All biopsy and cytopathologic specimens were interpreted by a skilled pulmonary pathologist. Both bronchial wash and BAL specimens were forwarded to a cytology laboratory where they were centrifuged and the cell pellet was recovered. These cells were examined utilizing GMS and periodic acid-Schiff stains. The recovered bronchial fluid also was sent to a microbiology laboratory where the fluid was examined using Gram staining and then was plated onto standard bacterial and fungal growth media.

Definitions

A saprophytic fungal infection of the BA was suspected when the bronchial wash fluid obtained from this site was plated onto appropriate culture media and resulted in the growth of fungal organisms. Before being acknowledged as an infection, all positive cultures were confirmed by the presence of fungal organisms in tissue biopsies of the BA. A complication of the BA was defined as any compromise of the anastomotic integrity and/or patency, as well as any symptomatic hemorrhage that was noted at the site. When visually confirmed by fiberoptic bronchoscopy, and often displaying the inability to pass through the bronchoscope beyond the stenotic segment, a stenosis of the BA was considered significant if the recipient experienced symptomatic wheezing and airflow limitation that was not explained by active infection or acute/chronic rejection.

Statistical Analysis

The Fisher exact test was employed for comparisons between nominal variables utilizing 2 × 2 contingency tables, while the phi coefficient (Φ) was used to assess for the presence of a relationship between such variables. Logistic regression analysis was utilized to identify a relationship between independent and dependent variables. The relative risk (RR) and odds ratio were calculated to compare probabilities between different groups. A p value of < 0.05 was considered to be significant.

Results

During the study period, 66 transplant procedures were performed in 65 recipients. One recipient received a second single-LTX after the initial allograft was compromised by the development of obliterative bronchiolitis. Of these 65 recipients, 17 were recipients of double-LTXs and 48 were recipients of single-LTXs. Sixty-one of these recipients survived a minimum of 75 days following the transplant procedure, and these recipients comprised the study group. Of these 61 recipients, 38 were men and 23 were women. Their mean age at the time of transplantation was 45.7 years. The pulmonary diseases for which this group required transplantation are outlined in Table 1.

Saprophytic Fungal Infection

Microbiological cultures from one or more bronchial washings were positive for fungal growth in 15
of the 61 recipients (24.6%). In all 15 recipients, the staining of tissue sections from endobronchial biopsy material with HE and GMS demonstrated the presence of a necrotic bronchial wall with infiltrating organisms. In 12 of these recipients, only one organism was identified by culture and was presumed to be the infecting agent. These organisms were Aspergillus sp (nine patients) [Fig 1], Candida sp (two patients) [Fig 2], and Torulopsis sp (one patient). The remaining three recipients had bronchial wash cultures that were positive for two fungal organisms as well as stained endobronchial material displaying cellular morphologies that were consistent with both saprophytes. Of these three recipients, two had positive cultures for both Penicillium sp and Candida sp, while the remaining recipient had positive cultures for both Aspergillus sp and Candida sp. Saprophytic fungal infections were identified by median postoperative day 35 (range, 13 to 159 days). In addition to therapy with oralitraconazole, all recipients with identified infection were hospitalized and received therapy that consisted of IV and inhaled amphotericin B. Recipients were treated for a minimum of 8 weeks, with the total duration of therapy decided following sequential surveillance and biopsy of the anastomotic site. Following the institution of this therapy, all recipients were monitored for potential toxicities and side effects. While urine creatinine clearance was not measured, all recipients experienced a rise in serum creatinine levels during the course of the amphotericin B treatment, which required adjustments in cyclosporine and tacrolimus dosing. With the conclusion of therapy, a return to baseline serum creatinine values was observed in all but one recipient. This recipient progressed to complete renal failure necessitating hemodialysis.

In addition to the 15 recipients identified with fungal infections of the BA, 16 other recipients were noted to have bronchial anastomoses that appeared to be abnormal and were subjected to endobronchial biopsies. These biopsies demonstrated only granulation tissue with no infectious elements identified. Utilizing this subset of 31 recipients who all were subjected to

### Table 1—Pulmonary Diseases Requiring LTX

<table>
<thead>
<tr>
<th>Disease</th>
<th>No.</th>
<th>Single</th>
<th>Double</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD*</td>
<td>29</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis</td>
<td>16</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Primary pulmonary hypertension</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Eisenmenger syndrome</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Sarcoiosis</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Obliterative bronchiolitis</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>46</td>
<td>15</td>
</tr>
</tbody>
</table>

*Includes α1-antitrypsin deficiency.
endobronchial biopsy, the probability that a visually suspicious BA was predictive of a fungal anastomotic infection was calculated. Thus, the positive predictive value was only 0.48 for a visually suspect BA actually predicting a fungal infection.

Airway Complication

Airway complications involving the BA ultimately developed in 11 of the 61 recipients (18%). These complications included the following: symptomatic bronchial stenosis (nine patients), bronchomalacia (one patient), and hemorrhage from the anastomotic site (one patient). Bronchial complications arose in 7 of 15 recipients (46.7%) who experienced saprophytic infections of the BA. Table 2 outlines those recipients with saprophytic anastomotic infections, their corresponding BA complications, treatments, and survival status. In all seven of these recipients, the bronchial complication was identified subsequent to the diagnosis of the anastomotic infection. Included in this group of seven recipients were three who died for reasons directly related to their bronchial complication. The first of these recipients experienced bronchial stenosis, which progressed despite multiple interventions and resulted in death from respiratory failure. The second recipient experienced a sudden and fatal hemorrhage arising from the BA. The infection had been identified previously and was aggressively being treated with amphotericin B, but was presumed to be still active at the time of death. The third recipient experienced significant stenosis, which resulted in bronchial disruption during an attempted stent placement. This recipient subsequently died secondary to the development of mediastinitis.

In contrast to the 7 of 15 recipients with infections who developed a complication, of the remaining 46 recipients who did not experience an anastomotic infection, only 4 (8.7%) subsequently developed a bronchial anastomotic complication (p = 0.003, Fisher exact test) [Fig 3]. Three of these four recipients also experienced symptomatic stenosis involving the BA. The fourth recipient likewise experienced symptomatic stenosis of a right-sided BA, which, over time, extended distally to involve the bronchus intermedius of the allograft.

While only 3 of 15 recipients (20%) who developed a saprophytic fungal infection subsequently died secondary to a complication of that infection, there was substantial morbidity related to bronchial complications in those recipients who survived. In addition to prolonged treatment with IV and inhaled amphotericin, a variety of interventional modalities were required in the seven recipients who developed complications arising from infections. These interventions included the following: bronchial stenting; balloon dilatation; electrocauterization; laser debridement; and radiation brachytherapy (Table 2).
While a difference in the number of recipients with BA infections who developed an anastomotic complication compared to those without infection was demonstrated, logistic regression analysis further revealed that the absence of a BA infection was associated with the lack of a subsequent anastomotic complication (p = 0.002; β = 2.22; SE = 0.74; Wald value = 9.08). The data then were analyzed to assess the risk of developing a complication of the BA as a direct consequence of a saprophytic anastomotic infection. Those experiencing an infection were five times more likely to develop an additional anasto-

### Table 2—LTX Recipients With Saprophytic Fungal Infections of the BA*

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Infection</th>
<th>POD</th>
<th>BA Complication</th>
<th>Treatment</th>
<th>Survived Complication?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspergillus sp</td>
<td>64</td>
<td>Bronchomalacia</td>
<td>Bronchial stent</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Aspergillus sp</td>
<td>33</td>
<td>Bronchial stenosis</td>
<td>Balloon dilatation</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Penicillium sp, Candida sp</td>
<td>33</td>
<td>Bronchial stenosis</td>
<td>Bronchial stent</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Aspergillus sp, Candida sp</td>
<td>35</td>
<td>None</td>
<td>Balloon dilatation</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Penicillium sp, Candida sp</td>
<td>36</td>
<td>None</td>
<td>Electrocauterization</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>Aspergillus sp</td>
<td>21</td>
<td>None</td>
<td>Laser debridement</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>Aspergillus sp</td>
<td>13</td>
<td>None</td>
<td>Laser debridement</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>Aspergillus sp</td>
<td>139</td>
<td>None</td>
<td>Brachytherapy</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>Aspergillus sp</td>
<td>92</td>
<td>None</td>
<td>Laser debridement</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>Aspergillus sp</td>
<td>28</td>
<td>Hemorrhage</td>
<td>Laser debridement</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Torulopsis sp</td>
<td>58</td>
<td>Bronchial stenosis</td>
<td>Balloon dilatation</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>Candida sp</td>
<td>28</td>
<td>None</td>
<td>Electrocauterization</td>
<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>Candida sp</td>
<td>23</td>
<td>Bronchial stenosis</td>
<td>Balloon dilatation</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>Aspergillus sp</td>
<td>43</td>
<td>None</td>
<td>Laser debridement</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>Aspergillus sp</td>
<td>60</td>
<td>Bronchial stenosis</td>
<td>Laser debridement</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*POD = postoperative day; NA = not applicable.

![Bronchial Anastomotic Complications](image)

**Figure 3.** Bronchial anastomotic complications are shown.
motic complication (RR, 5.36; 95% confidence interval, 1.82 to 15.79). The odds in favor of a BA complication following such a saprophytic infection were 8 times greater than the odds of developing a similar complication in those recipients without infection (odds ratio, 8.31; 95% confidence interval, 1.96 to 35.16).

**Discussion**

The bronchial circulation, being the primary source of blood flow to the major bronchi, is disrupted during the single-LTX and double-LTX procedures. Consequently, in the early post-transplantation period the BA site is initially devascularized, which may result in the sloughing of epithelial tissue into the airway lumen. Saprophytic fungal organisms are easily airborne and thus have ready access to the airway lumen and the relatively ischemic BA. As saprophytes are organisms that obtain their nourishment from nonliving organic matter, the ischemic and necrotic airway debris at the BA provides a fertile environment for their proliferation. All of the BA saprophytic fungal infections in patients in our series occurred within the first 160 days following transplantation and thus are consistent with this scenario. This interval is also consistent with those given in other reports, demonstrating that serious Aspergillus infections tend to be more common in the first 6 months following transplantation. In addition to the relative airway ischemia, other factors, including the intensity of immunosuppression in the early post-transplantation period and increased graft susceptibility following the reimplantation response, may favor the proliferation of these organisms and may result in infection.

The significance of pharmacologic immunosuppression in relation to these infections cannot be overstated. For instance, it has been suggested that the use of corticosteroids in the early postoperative period may be a risk factor for the development of saprophytic infections, especially those resulting from Aspergillus. In a rabbit model, the function of the main immunoregulatory cells (ie, macrophages and granulocytes) involved in controlling Aspergillus proliferation was shown to be altered with the use of corticosteroids, resulting in invasive Aspergillus infections. Certain groups of patients who require LTX, such as those with cystic fibrosis, may be at particular risk as their airways may be heavily colonized with Aspergillus prior to LTX. This allows for a high inoculum of organism to be present at the ischemic BA immediately following the transplant procedure and, when combined with the institution of intense immunosuppression, may make these recipients be at increased risk for anastomotic infections resulting from this organism.

A surprisingly high number of saprophytic fungal infections involving the BA (15 of 61 infections [24.6%]) occurred in our recipient population. As stated, most of these infections were secondary to Aspergillus infection and represented a substantial increase over the rates of Aspergillus infections reported in other LTX series. One possible explanation for this may be the geographic location of our center. The southeastern portion of the United States is known to be a highly endemic region for airborne spores, mold, and fungi. For example, data analysis has demonstrated high rates of airway colonization with Aspergillus and allergic bronchopulmonary aspergillosis in patients with cystic fibrosis who were living in the southeastern United States. Although unproven, construction underway in the vicinity of the medical center may have increased the burden of airborne fungi. Another potential explanation for the high prevalence of fungal infections relative to other LTX programs may involve the employed prophylactic pharmacologic regimen. The standard prophylactic dosing (200 mg per day) of itraconazole that was used in our program may not be optimal. It has been suggested that a dose of 400 mg per day may result in superior serum drug levels and, therefore, could potentially affect the prevalence of this infection. A third possible contributing factor may be the aforementioned early use of therapy with corticosteroids, which is standard in most programs. In addition to the high number of infections in the 61 study recipients, we have experienced three fatalities (4.9%) from saprophytic BA infections. This fatality rate is slightly higher than that experienced at other centers reporting similar infections.

While other series have reported hemorrhage, tracheobronchitis, and pneumonia as consequences of posttransplantation saprophytic fungal infections, our series is unique in that it addressed additional complications arising specifically at the BA. While we report one fatal hemorrhage, the remaining complications of BA infections in our series were symptomatic bronchial stenosis and/or bronchomalacia. All complications were considered to be substantial and to require intervention, thus denoting significant associated morbidity. Some recipients required multiple attempts at balloon dilatation or laser debridement. One such attempt at dilatation with bronchial stent placement resulted in a disruption of the bronchus, which did not heal and contributed directly to the recipient’s death.

An analysis of our data suggested not only a positive correlation between a saprophytic fungal infection of the BA and additional complications, but also revealed a high RR of developing such a complication once an infection was experienced. Expressed somewhat differently, the odds in favor of developing a complication of the BA for those...
recipients with a saprophytic fungal anastomotic infection was eight times that of developing a similar complication in those recipients without such an infection. And while a mechanism has been proposed by which Aspergillus may become angioinvasive, resulting in hemorrhage at the anastomotic site, the mechanism by which bronchomalacia and bronchial stenosis may arise remains speculative. Each of the infections in our series was shown not to be limited to only the luminal epithelium but rather to invade directly into the bronchial wall. Thus, a loss of bronchial wall integrity and cartilaginous support resulting in significant airway collapse at the site of the BA might not be unexpected. Furthermore, as blood flow to the BA improves following the initial ischemic episode, reparative mechanisms may be initiated that promote collagen deposition at the site. Assuming that the recipient’s saprophytic infection is adequately treated, the proliferation of granulation tissue and collagen deposition could encroach on the airway lumen and result in airway stenosis.

There are potential sources of error in our data that deserve mention. First, since anastomotic infections were initially suspected during visual inspection of the airway at the time of bronchoscopy, some degree of interobserver variability may exist. Since sampling was performed only for those anastomotic sites that were visually suspicious for infection, what was assessed to be normal by one bronchoscopist may have appeared to be abnormal to another. A more statistically compelling approach would have been to routinely sample each anastomotic site, whether or not it appeared to be abnormal. Second, the routine use of itraconazole prophylaxis in our recipient population may have affected the prevalence of fungal infections since most of our BA infections were caused by Aspergillus. However, while some data have suggested that the use of itraconazole may decrease the prevalence of disseminated Aspergillus infections, its use may not affect the development of anastomotic Aspergillus infections. Last, as alluded to previously, differences in the use of immunosuppressive medications may have affected the development of saprophytic fungal infections. These differences mostly involve the timing of corticosteroid initiation, but the use of other immunosuppressive agents (i.e., cyclosporine vs tacrolimus, or azathioprine vs mycophenolate mofetil) and dosing practices also may have had an impact.

In summary, our data suggest that lung transplant recipients who experience infections of the BA caused by saprophytic fungal organisms are at risk for developing subsequent complications of the anastomotic site which include hemorrhage, bronchomalacia, and bronchial stenosis. Vigilant postoperative inspection, with biopsy and culture of the BA when visually suspect, is mandatory. With regard to such complications, the role of antimicrobial prophylaxis and treatment, as well as the optimal utilization of immunosuppressive medications, is unclear.

References