Nebulized Amphotericin B Prophylaxis for Aspergillus Infection in Lung Transplantation: Study of Risk Factors

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Background: Aspergillus infection remains a major cause of morbidity and mortality after lung transplantation. Therefore, some strategies have been attempted, one of which is nebulized amphotericin B (nAB); however, the efficacy of this prophylaxis has not been shown clearly. The aim is to study whether nAB can protect against Aspergillus infection in lung transplant recipients.

Patients and Methods: A study of risk factors was conducted in 55 consecutive lung allograft recipients. Twenty-three potential risk factors were analyzed. In 44 (80%) patients, nAB was indicated as prophylaxis. Multivariate analysis using logistic regression was performed.

Results: Eighteen of the 55 patients (33%) developed infection due to Aspergillus spp. Multivariate analysis showed nAB to be a preventive factor (odds ratio: 0.13; 95% confidence interval [CI] 0.02–0.69; p < 0.05) and cytomegalovirus (CMV) disease was an independent risk factor for developing Aspergillus infection (odds ratio: 5.1; 95% CI 1.35–19.17; p < 0.05). Only 1 patient required withdrawal of the prophylaxis owing to bronchospasm. nAB was well-tolerated in the remaining patients with only a few, mild, easily controlled side effects.

Conclusions: The present results show that nAB prophylaxis may be efficient and safe in preventing Aspergillus infection in lung-transplanted patients, and CMV disease increases the probability of Aspergillus infection. J Heart Lung Transplant 2001; 20:1274–1281.

Lung transplantation has become an accepted treatment for end-stage pulmonary diseases. This group of solid organ transplant recipients appears to be particularly susceptible to infections because the lung is the only transplanted organ that has continuous contact with the environment. Pneumocystis carinii pneumonia was virtually eliminated with prophylaxis. At present, other bacterial and viral infections can be prevented. However, fungal infection continues to be an important cause of morbidity and
mortality in this population. Of fungal infections, the most prevalent and important in lung-transplanted patients is caused by *Aspergillus* spp., and despite early diagnosis and aggressive treatment, related mortality remains high. Nebulized amphotericin B (nAB) has been used successfully in the rat model of invasive aspergillosis, but human data from neutropenic and other immunodepressed patients remain controversial. To date few studies exist on nAB prophylaxis against this infection in recipients of lung transplantation. These 2 studies do not sufficiently answer the question of the role of nAB in lung transplantation. Since April 1993 we have used nAB as prophylaxis for Aspergillus infections in all patients receiving a lung allograft. The purpose of this study was to evaluate the efficacy of this strategy by analyzing the risk factors of aspergillosis in our lung-transplant population.

**PATIENTS AND METHODS**

**Patient Population**

From August 1990 to September 1997, 72 patients underwent lung transplantation in our institution. The follow-up period ended in February 1998. Fifty-five patients (32 men, 23 women; mean age: 43.1 years; range: 15–67) who survived more than 7 days after transplantation were included in the study. Underlying diseases were idiopathic pulmonary fibrosis in 16 cases; emphysema in 13; bronchiectasis in 11; cystic fibrosis in 4; lymphangioleiomyomatosis in 3; primary pulmonary hypertension in 4; and silicosis, asbestosis, histiocytosis X, and bronchiolitis obliterans 1 patient each. Twenty-five single lung transplants and 30 sequential double lung transplants were performed. Surgical procedure was essentially similar over time in single and bilateral sequential lung transplantation. From April 1993 we started to administer nAB prophylaxis to all patients who received a lung allograft at our center (n = 44). Eleven lung recipients before this date did not receive any fungal prophylaxis. Mean post-operative follow-up was 14 months (range: 0.3–62 months).

**Immunosuppression and Anti-microbial Prophylaxis**

All patients were under the same protocol based on triple therapy with cyclosporine, azathioprine, and corticosteroids. Cyclosporine was started on day 1 with dose adjusted by trough blood levels between 200 to 300 ng/ml by immunoassay with monoclonal specific antibody. In the last 3 years, Neoral (Novartis, East Hanover, NJ) has replaced classic cyclosporine in all patients. Azathioprine was started in the second post-operative week at a dose between 1 to 3 mg/kg/day depending on white cell count and avoiding a total leucocyte count less than 4.0 × 10^9/liter. Methylprednisolone was started in the operating room at a dose of 10 mg/kg before graft reperfusion, followed by 375 mg/day the first day, approximately 0.5 mg/kg/day the first 3 months, and a maintenance dose approximately 0.1 to 0.2 mg/kg/day for life. In recent years, cyclosporine was replaced by tacrolimus as rescue therapy in chronic and recurrent acute rejection in some patients. Occasionally, mycophenolate mophetil 2 g/day or methotrexate 5 to 10 mg/week substituted azathioprine for the same indication. When tacrolimus was used, the dose was adjusted at a trough level between 5 and 15 ng/ml by immunoassay with monoclonal non-specific antibody (MEIA, Imx autoanalyzer, Abbott Laboratories, Abbott Park, IL). Acute rejection was treated with pulse intravenous (IV) administration of methylprednisolone at a dose of 10 mg/kg/day for 3 days. OKT3 was occasionally used when no response to standard treatment for acute rejection occurred. Some selected patients with non-resolved rejection underwent plasmapheresis and/or total lymphatic irradiation. Total immunosuppressive therapy was quantified in each patient before developing Aspergillus disease or until the end of the study. Quantification was made as follows: 1 point for each treatment with 3 pulse doses of methyprednisolone, 1 point for each course of OKT3, 1 point for each treatment with plasmapheresis, and 2 points if the patient underwent total lymphatic irradiation.

In the immediate post-operative period, patients without pre-operative septic disease received cefuroxime 1.5 g every 8 hours (1990–1996) or amoxicillin-clavulanate 2 g every 8 hours plus aztreonam 1 g every 8 hours (1997–1998). Prophylaxis was modified according to the microorganisms isolated from the last cultures performed in recipients with a septic underlying disease. Duration of prophylaxis was contingent on the results of recipient and donor intraoperative cultures. Since June 1993, IV ganciclovir has been used to prevent cytomegalovirus (CMV) infection and disease. Anti-viral prophylaxis consisted of IV ganciclovir at 5 mg/kg every 12 hours for the first 15 days after the procedure for all patients, followed by a course of 6 mg/kg five months.
times a week for 45 days for CMV-seropositive patients and 90 days for CMV-seronegative patients. All patients received prophylaxis with cotrimoxazol once a day (400 mg sulfametoxazol plus 80 mg trimetropim) for life, starting when oral intake was possible. Isoniazid prophylaxis was prescribed in patients with tuberculosis infection (positive PPD) and those in whom cutaneous anergy was shown when they were included on the waiting list. The isoniazid dose was 5 mg/kg/day and completion of prophylaxis was considered at 12 months.

**nAB Prophylaxis**

Fifty milligrams of nAB desoxicolate for injection (Fungizona, Bristol-Myers Squibb Co, New Brunswick, NJ) were dissolved in 10 ml of sterile water for a concentration of 5 mg/ml; this solution was then diluted in sterile water for a total volume of 50 ml with one final concentration of 1 mg/ml. Solution maintained stable for at least 30 days at 4°C, measured by high-pressure liquid chromatography. A dose of 6 ml every 8 hours of nAB was started the first post-operative day and was continued until day 120 and then 6 mg once a day for life. The nebulization technique: nAB was nebulized by one jet nebulizer with a CR60 compressor (air pressure of 27.2 psi and flow 7.3 liter/minute) equipped with a disposable bacterial exhale filter. The aerosol droplet size was measured by TSI particle size analyzer and more than 90% of particles had a size of less than 5 μm. Patients were instructed by a trained staff nurse to inhale through a mouthpiece and exhale through the nose. Two patients failed to adapt adequately to the instructions and the system was thus adapted to a facial mask. The procedure took from 15 to 20 minutes. All patients received 2 puffs of salbutamol from a metered dose inhaler before nebulization of amphotericin B. To avoid contamination, the nebulizer was washed and brushed with soap and water after each administration; once rinsed, it was submerged in a sodium hypochlorite solution at 1% until the next administration. Cultures were performed of the nebulized solution and the different parts of the system to rule out bacterial contamination. This control showed no microbial contamination.

**Follow-up**

Before transplantation, respiratory samples from the recipient lungs were cultured for bacteria, mycobacterium, and fungi. On the day of the operation, respiratory samples from the donor and recipient were cultured in the same way. After discharge, patients were followed at our outpatient clinic on a regular basis with maximum intervals of 6 weeks. Respiratory samples were obtained and cultured for bacteria, micobacteria, and fungi when the patient had sputum production or bronchoscopy was indicated. Compliance and possible adverse effects of immunosuppressive treatment and prophylaxis, including nAB, were routinely investigated. Every patient underwent surveillance bronchoscopy examinations at 2 and 4 weeks after transplantation and again at 3-month intervals the first year. In addition, bronchoscopy was indicated when clinical deterioration was present. Samples obtained by bronchoscopy included bronchoaspirate and bronchoalveolar lavage (BAL) for cellular examination; Gram and acid-fast bacilli stain; bacterial, fungal, mycobacterial and Legionella spp. cultures; and transbronchial biopsies for histopathologic assessment and immunohistochemical stains. Fungal cultures were plated on 3 different media: brain-heart infusion agar, inhibitory mold agar, which has gentamicin and chloramphenicol, and inhibitory mold agar with 10% sheep blood that has gentamicin, chloramphenicol, and cycloheximidine. Cultures were maintained for 4 weeks, and for 8 weeks when infection with dimorphic fungi was suspected.

**Disease Definitions**

Colonization by *Aspergillus* spp. was defined as positive respiratory culture samples with no clinical signs of disease. Aspergillus infection was considered when the patient had clinical symptoms, 2 or more respiratory samples were positive for *Aspergillus* spp., and at least 1 of these was obtained by bronchoscopy. Aspergillus infection was categorized as follows: Aspergillus tracheobronchitis diagnosed by clinical symptoms with purulent sputum production plus bronchoscopy findings with red edematous mucosa and mucus plugging; ulcerative tracheobronchitis diagnosed by bronchial biopsy and/or bronchoscopy findings with necrotic ulcers in the anastomosis or in the tracheobronchial tree that disappeared after treatment; and invasive pulmonary aspergillosis diagnosed when *Aspergillus* spp. was found on lung histopathology or radiologic evidence of invasion was observed.

Diagnosis of CMV infection was based on isolation or detection of the virus from any body fluid or tissue specimen by shell vial assay or anti-genemia. CMV disease included CMV viral syndrome and CMV focal diseases such as pneumonitis, hepatitis, or gastrointestinal disease. CMV viral syndrome was
defined as persistent fever, with or without leukopenia and thrombocytopenia in patients with positive blood culture or anti-genemia for CMV, in the absence of other causes. CMV focal disease was defined as the isolation of CMV from any tissue or body fluid plus consistent histologic findings. Diagnosis of acute rejection was based on clinical signs and symptoms and chest X-ray findings with or without lung biopsy. Chronic rejection was defined as a persistent drop in FEV₁ of 20% or more compared with baseline with or without histologic findings of bronchiolitis obliterans when other causes of pulmonary dysfunction were ruled out.

Data Analysis

A total of 23 potential risk factors, pre-operative, intraoperative, and post-operative, were analyzed for their association with Aspergillus infection (Table I). Univariate analysis was performed using chi-square test for categorical variables; Wilcoxon’s rank sum test was used to compare continuous variables that were not normally distributed, and analysis of variance or t-test was used to compare means of approximately normal continuous variables. Logistic models were developed with the use of forward stepwise regression to assess the risk factors for Aspergillus infection. Differences were considered to be significant at p values less than 0.05. Statistical analysis was performed with the SPSSWIN 9.0 package.

RESULTS

Fifty-five patients who underwent lung transplantation were studied; nAB was indicated in 44 (80%). Patients were followed for a mean of 14 months (range: 0.3–46 months). Thirty-two of the 55 (58%) patients were followed for a mean of 14 months (range: 0.3–46 months). Thirty-two of the 55 (58%) patients were followed for a mean of 14 months (range: 0.3–46 months).
patients studied were alive at the end of follow-up. Eighteen of the 55 (33%) developed Aspergillus infection. Mean time elapsed between transplantation and Aspergillus infection was 8.8 months (range: 0.3–41 months). Fourteen episodes (78%) were diagnosed 2 months post-transplantation. The cumulative rate of Aspergillus infection after 6 and 18 months of follow-up was 26% and 32%, respectively. Crude univariate analysis showed that 91.9% of patients who did not suffer from Aspergillus infection and 55.6% of patients suffering from it were under nAB prophylaxis, respectively. Moreover, in the group of patients who did not develop Aspergillus infection, 24.3% of the patients had CMV disease, and in the group of patients who developed Aspergillus infection, 61.1% of them had CMV disease ($p < 0.05$) (Table I). Results of multivariate analysis confirmed the importance of nAB as an independent factor to decrease Aspergillus infection (odds ratio: 0.13; 95% confidence interval [CI] 0.02–0.69; $p < 0.05$) and maintained CMV disease as an independent risk factor of Aspergillus infection after the procedure (odds ratio: 5.1; 95% CI 1.35–19.17; $p < 0.05$).

The type of Aspergillus infection was tracheobronchitis in 8 cases, ulcerative tracheobronchitis in 5, and 5 recipients were diagnosed of invasive pulmonary aspergillosis. All the patients with invasive aspergillosis died, 2 with ulcerative tracheobronchitis and none with tracheobronchitis. Aspergillus fumigatus was the etiologic agent in 14 episodes (77%); the remaining infections were due to other species of Aspergillus spp. During the study period, 12 episodes of colonization due to Aspergillus spp. were diagnosed in 12 patients. All episodes of Aspergillus colonization were resolved by increasing the dose of nAB to three times a day. One hundred and 6 episodes of colonizations by fungi other than different of Aspergillus spp. were diagnosed (Table II). Two patients developed tracheobronchitis by Blastoschizomyces capitatus and Pseudallescheria boydii, respectively. They were cured with liposomal amphotericin B. The side effects of nAB prophylaxis were cough (14/44, 32%), mild bronchospasm (4/44, 9%), and nausea (3/44, 7%). Only 1 patient had to stop the prophylaxis because of repeated bronchospasm. Four patients observed non-regular compliance and 2 abandoned prophylaxis spontaneously.

**DISCUSSION**

The aim of this work was to evaluate the efficacy of nAB as a preventive strategy against Aspergillus infection using a study of risk factors for this infection in recipients of a lung allograft at our institution. The main results show CMV infection to be an independent risk factor for developing Aspergillus infection and that the use of nAB may be useful for preventing this infection. The incidence of systemic fungal infection varies with the type of organ transplanted.\(^4\) Candida spp. is the most common cause of fungal infections in the majority of solid organ transplantations, except in lung and heart where the fungal infection is due predominantly to Aspergillus spp. The incidence of Aspergillus spp. isolation in respiratory samples of lung transplantation patients ranges from 8% to 50% and the rate of patients with disease also ranges from 5% to 20%.\(^1\)-\(^3\),\(^12\) These striking differences between groups are mostly due to differences in definitions of Aspergillus infection, because differentiation between colonization and tracheobronchitis or invasive disease may be difficult. Although the overall incidence of Aspergillus infection in our hospital was 33%, 44% were tracheobronchitis forms with good prognosis. Other factors could be responsible for this variability in the incidence of Aspergillus infection in lung-transplanted patients. Aspergillus spp. is ubiquitous and

### TABLE II  Fungal colonization in 55 lung transplantation patients

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of colonizations</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td><em>Candida guilliermondii</em></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Candida lambica</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><em>Candida pseudotropicalis</em></td>
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<td>1</td>
</tr>
<tr>
<td><em>Candida spp.</em></td>
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<td>9</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
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<td>2</td>
</tr>
<tr>
<td><em>Cephalosporium</em> sp.</td>
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<td>1</td>
</tr>
<tr>
<td><em>Fusarium</em> sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Geotrichum capitatum</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Geotrichum</em> sp.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><em>Hansenula anomala</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Mucor</em> sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Paeolomyces</em> sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Penicillium</em> sp.</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td><em>Rhodotorula glutinis</em></td>
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<td>1</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Scopulariopsis</em> sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Trichosporon capitatum</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Torulopsis</em> glabrata</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>100</td>
</tr>
</tbody>
</table>
appears to be transmitted by air; thus, one crucial influencing factor is the degree of environmental exposure. The range of Aspergillus spore numbers per mm³ of ambient air was found to be between 0.2 and 3.5 in the western world. Although no published data exist on spore concentrations in the air of Barcelona, 1 study demonstrated that spores of Aspergillus spp. were isolated in the dust of almost half the homes of healthy people. The problem could be more undercontrolled in the case of building works. A construction-associated outbreak of Aspergillus infection was reported in 1 hospital. Cases that occur within the early post-transplant weeks suggest an excess of exposure during hospitalization, whereas cases that occur later than 2 months post-lung transplantation may be acquired outside the hospital. In our study, 78% of patients who developed Aspergillus infection were diagnosed after 2 months post-transplantation, suggesting a source outside the hospital.

Recognized risk factors for Aspergillus infection are neutropenia and chronic corticosteroid intake. In lung transplant patients, CMV pneumonitis, airway ischemia, and single lung procedure have also been recognized as possible risk factors. No relationship with rejection or augmented immunosuppression was discernible, but this possibility cannot be ruled out. In the present study, only CMV infection was found as an independent risk factor. CMV is an immunomodulating virus that induces a state of additional transient immunosuppression, rendering the lost more susceptible to suffering an opportunistic microorganism-induced infection. Duncan et al. showed that the percentage of lung-transplanted patients who had suffered bacterial or fungal pneumonia was significantly higher in those with a previous diagnosis of CMV-pneumonitis. Yeldandi et al. and Husni et al. found a greater incidence of CMV infection in patients who developed Aspergillus infection. On the other hand, other variables also recognized as possible risk factors in other reports were not found in the present study. The reason could be the efficacy of nAB prophylaxis which prevents Aspergillus spp. colonization of ischemic airways or native lung.

As the most common route of entry of Aspergillus is the respiratory tract, HEPA and laminar airflow rooms have been used to reduce the risk of exposure and subsequent development of aspergillosis in patients undergoing bone marrow transplantation. High costs are the obvious disadvantage of this strategy, which is only useful for patients who are vulnerable for limited periods of time, and the protective effect is lost if the patient is moved outside the HEPA-filtered facility, even for short periods of time. In lung transplantation, in which it is likely that many patients acquired the infection after they left hospital, this strategy may be not useful. Low-dose prophylactic IV amphotericin B desoxycholate was used in successful bone marrow transplantation during the pre-transplant and peritransplant periods. In the early era of lung transplantation, some groups used this strategy but data were not published. The main disadvantage of this approach is that in lung-transplanted recipients, the risk of infection due to Aspergillus spp. is prolonged. Itraconazol has been used by some lung transplant groups in the prophylaxis of Aspergillus infection and other fungal infections. Encouraging results have been obtained in other immunocompromised patients but no data on its efficacy in lung transplantation have been reported. Moreover, itraconazol is more expensive than nAB and its interactions with cyclosporine and other drugs may be problematic. Furthermore, this drug has low bioavailability.

Prophylaxis with nAB, the gold standard antifungal drug for the treatment of Aspergillus infection, could represent an opportunity to avoid amphotericin B systemic adverse effects with efficiency and low cost. The efficacy of nAB in other immunocompromised patients remains controversial. In lung-transplanted patients, only 2 references are available: Reichenspurner et al. showed a 2% incidence of invasive disease due to Aspergillus spp. in patients who received prophylaxis with nAB in contrast to historical controls with 12% after the first 12 post-operative months; Calvo et al. using fluconazol plus nAB found no fungal infection during the early post-operative period. The present study on nAB prophylaxis appears to prevent Aspergillus infection using a study of risk factors for Aspergillus infection. At the end of 1999, 2 years after the end of the study period, 111 patients had used nAB as fungal prophylaxis. Sixteen of these patients were diagnosed with Aspergillus infection (14.4%) but only 2 developed invasive pulmonary aspergillosis (1.8%). These figures are similar to those found by Reichenspurner et al.

The safety of nAB was acceptable in most studies with immunocompromised patients. In lung transplantation, the only side effect observed by Reichenspurner et al. with 5 mg three times a day of nAB was nausea in 7.9% of their patients, which led to discontinuation of treatment in 1.6%. The dose and concentration as used in our study
6 mg three times a day, 1 mg/ml) were also well tolerated, although side effects were more important when the dose was increased. Due to the lack of knowledge about the appropriate nAB regimen, perhaps when the bronchial anastomosis is particularly vulnerable nAB three times a day seems to be the best option, and 3 or 4 months later a more comfortable regimen of nAB once a day could be the best choice.

A further important point of note is the possibility of systemic absorption of nAB, particularly in the case on lung-transplanted patients who receive other nephrotoxic drugs such as cyclosporine or tacrolimus. Diot et al. found undetectable serum levels of amphotericin B in 3 patients with aspergillosis who received a daily dose of 5 mg of nAB. Myers et al. found significant serum levels (2 µg/ml) in 3 of 5 neutropenic patients who received a dose of 20 mg twice daily, but in none of 7 patients who received lower doses. We evaluated this point and found no levels in 5 patients with 6 mg once a day. Thus, systemic absorption is minimal and nAB-related nephrotoxicity has not been a problem.

In summary, CMV disease increases the probability of Aspergillus infection and nAB prophylaxis may be efficient and safe in preventing Aspergillus infection in lung-transplanted patients. Further studies evaluating efficacy of nAB are required to confirm this preliminary results.

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