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ORIGINAL ARTICLE

Outbreak of aspergillosis infections among lung transplant recipients

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Summary

Aspergillus infections have been associated with building constructions. We reported, for the first time, an outbreak of aspergillosis in lung transplant recipients exposed to heavy building construction work during hospitalization. We reviewed the files of 115 patients who underwent lung transplantation between May 1994 and June 2005. Patients operated on from May 1994 to December 2003 (group 1) were compared with those operated on between January 2004 and June 2005 (group 2) for findings of aspergillosis on follow up. Thirty-six transplant recipients (31%) had evidence of *Aspergillus* colonization, including six of the 64 patients (9.4%) operated on from 1994 to 2003 and 30 of the 51 patients (59%) operated on in 2004–2005 ($P = 0.0001$). Eight had aspergillosis, in all group 2 ($P = 0.001$) compared with group 1. All infections occurred within the first 4 month after the transplantation. On comparison of the two groups for background and medical factors, the only difference found was the initiation of building construction at the hospital, close to the transplant ward, in early 2004. We concluded that lung transplant recipients are prone to *Aspergillus* colonization following exposure to building construction work, despite prophylactic treatment. Established guidelines for the prevention of aspergillosis should be implemented and enforced during construction activities in hospitals.

Introduction

Colonization and infection by *Aspergillus* species is a well-known complication of lung transplantation (LTX). According to current estimates, airway colonization occurs in about 29% of all lung transplant recipients, airway disease in 5%, and invasive aspergillosis in 5–8% [1,2]. The infection occurs in the relatively avascular area of the bronchial anastomosis and is often associated with severe sequelae, and including anastomotic stenosis, bronchomalacia, anastomotic dehiscence, even fatal hemorrhage [3,4]. Invasive disease occurs predominantly as invasive pulmonary aspergillosis (IPA), but may also manifest as emphysema, arthritis, osteomyelitis [5], necrotizing choroiditis-retinitis [6], and endophthalmitis [7–10].

About 2 years ago, we have observed a sharp increase in the cases of *Aspergillus* colonization, local infection, and invasive disease in patients after LTX. We suspected that these findings may be related to new building construction in the proximity of the lung institute.

The aim of this article was to characterize the possible association between aspergillosis and building construction work in lung transplant recipients, and to define the natural history of such colonization according to the medical literature.

Patients and methods

One hundred and eleven patients underwent LTX at the Lung Institute of Rabin Medical Center, Beilinson

Campus between May 1994 and June 2005. The immunosuppression regimen before 1998 included a combination of prednisone, azathioprine and cyclosporine, and after November 1998, a combination of prednisone, mycophenolate mofetil and tacrolimus. Blood levels of tacrolimus were measured in whole blood (IMX immunoassay, Abbott, Abbott Park, IL,USA), and the dose was adjusted to achieve the desired trough blood levels. All transplant recipients received prophylactic itraconazole, 200 mg b.i.d., for the first 6 months following surgery. Trimethoprim–sulfamethoxazole was administered for *Pneumocystis jirovecii* pneumonia prophylaxis. Patients receiving organs from cytomegalovirus seropositive donors were given prophylactic ganciclovir.

All patients were routinely followed at least every 4 weeks at the outpatient clinic with complete blood count, blood chemistry, drug level measurements, chest radiographs, pulmonary function tests, and surveillance bronchoscopy. Additional diagnostic bronchoscopies were performed to investigate clinical, radiographic or spirometric abnormalities. All bronchoscopy specimens were submitted for fungal staining and culture.

For purposes of the study, the patients were divided into: (i) those operated on from May 1994 to December 2003 (group 1); and (ii) those operated on during the period of hospital construction work, from January 2004 to June 2005 (group 2). The groups were compared for the findings of *Aspergillus* colonization and infection during follow up. The study was approved by the Ethics Committee of Rabin Medical Center.

Samples and diagnosis

The laboratory records were reviewed for all *Aspergillus*-positive samples from sputum, bronchoalveolar lavage (BAL) fluid, biopsy, and vitreous, which were collected on follow up from the patients who underwent LTX during the study period. All fungi recovered were routinely subcultured on Sabouraud agar and identified by standard phenotypical techniques. Galactomannan serum antigen (ELISA) test (Platelia *Aspergillus*; Sanofi Diagnostics Pasteur, Marnes La-Coquette, France) was performed when aspergillosis was considered a potential cause of the clinical symptoms. Two positive findings of circulating galactomannan (with an OD of 1.0) and histopathologic evidence of invasion suggest invasive disease. No medications were noted in both the groups that may interact with the galactomannan tests. Local infection was defined as a finding of endobronchial lesions with *Aspergillus* involvement, a positive culture for *Aspergillus*, and no evidence of invasion [8]. Invasive aspergillosis was defined as a symptomatic involvement of an organ far from the airways and a positive *Aspergillus* culture of a sample from the involved organ [8].

Treatment

Aspergillus colonization was treated with itraconazole, 200 mg b.i.d. for 6–12 months, depending on clinical response, if the patient has already stopped taking it. In case of positive colonization in LTX already on itraconazole prophylaxis, he continues to take it for at least 6 months following the LTX.

In cases of persistent isolation of *Aspergillus* and/or severe infection despite itraconazloe treatment, we changed the treatment to voriconazole 200 mg b.i.d. or IV-amphotericin B (nonlipid), 1.0–1.5 mg/kg/day.

Building construction work

In 2004, heavy renovation work began in our hospital, with extensive wall destruction, air-conditioning replacement, and new construction. All the instructions were carried out according to the international guideline in order to prevent infections [8].

Statistical analysis

Continuous variables were shown as mean ± standard deviations. Pearson correlation coefficients (r) and the significance for it (P) were calculated between the variables. In order to analyze the differences of continuous parameters during and after itraconazole treatment, paired t-test was performed. P-value less or equal to 0.05 was considered statistically significant.

Results

Aspergillus colonization and infection

Aspergillus colonization was identified in 36 of the 115 transplant recipients operated on during the study period (31%). Table 1 summarizes their clinical characteristics. LTX was performed for: idiopathic pulmonary fibrosis in 14; chronic obstructive pulmonary disease in 11; bronchiectasis in six; bronchiolitis obliterans in three; primary pulmonary hypertension in one; and lymphangioleiomyomatosis in one. The mean interval between LTX and colonization was 13.2 ± 6 months. The affected patients were six of the 64 patients (9.4%) who underwent LTX from 1994 to 2003, and 30 of the 51 patients (59%) who underwent LTX from 2004 to 2005. This difference was statistically significant ($P = 0.0001$).

Table 2 describes the incidence of *Aspergillus* colonization during the study period. In the patients operated on from 1994 to 2003, the species identified were *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus terreus*. After January, 2004 (group 2), colonization with *Aspergillus*

Table 1. Clinical characteristics of the patients without *Aspergillus* colonization ($n = 79$) and the patients with *Aspergillus* colonization ($n = 36$).

Parameter	Patients without *Aspergillus* colonization	Patients with *Aspergillus* colonization	*P*-value
Age (years), mean ± SD	54 ± 15	50 ± 11	NS
Male	55 (69)	22 (61)	NS
Lung disease			
Emphysema	26 (33)	11 (31)	NS
IPF	33 (42)	14 (39)	NS
Bronchiectasis	15 (19)	6 (17)	NS
Others	5 (6)	5 (13)	NS
Type of LTX			
Single LTX	58 (73)	24 (67)	NS
Double LTX	17 (21)	10 (28)	NS
Heart–lung TX	4 (5)	2 (5)	NS
Immunosuppression	FK/MMF/CS	FK/MMF/CS	NS
H2 blockers/PPI	65 (82)	30 (83)	NS
CMV complications	0	0	NS
Acute rejection episodes	77	59	NS
Mean time from LTX to colonization	16.5 ± 7	13.2 ± 6	NS

All data are *n* (%), unless otherwise indicated.
CS, corticosteroids; DLT, double lung transplantation; IPF, idiopathic pulmonary fibrosis; FK, tacrolimus (FK 506); LTX, lung transplantation; MMF, mycophenolate mofetil; PPI, proton pump inhibitors; PPH, primary pulmonary hypertension; SLT, single lung transplantation.

Table 2. The incidence of *Aspergillus* colonization in lung transplant recipients during 1994–2005 ($n = 115$).

Parameter	1994–2003 ($n = 64$)	2004–2005 ($n = 51$)	*P*-value
Aspergillus colonizations	6	30	0.0001
Aspergillus species			
A. flavus	2	7	0.475
A. fumigatus	1	11	0.331
A. terreus	4	6	0.143
A. niger	0	11	0.118
Mean time since LTX (month, %)	13.1 ± 7	4.9 ± 8	0.223
Aspergillus infection	0	8	0.001
Disseminated aspergillosis	0	1	0.143

niger was also noted. Two patients showed simultaneous colonization by two different species. No statistical significance between-group differences were noted in the *Aspergillus* species grown. Aspergillosis occurred in eight of the 36 patients (22%), all from group 2 ($P = 0.001$ compared with group 1). Seven had local disease and one had disseminated disease. Table 3 summarizes their clinical characteristics. All infections occurred in the first 4 months after LTX. Bronchial stenosis developed in six patients, all of them were treated with itraconazole. Laser surgery was also performed in these cases to open the stenotic areas, and five patients underwent stent insertion (Fig. 1a and b). One of the six died. Two patients failed to respond to itraconazole and were switched to voriconazole, with good response.

Complications

Besides bronchial anastomotic stenosis (six patients), complications included deterioration in pulmonary function and pulmonary infection. The mean decrease in forced expiratory volume at 1 s (FEV1) was 15 ± 10% of predicted (range: 8–22%). Pneumonia developed in two patients at 1 month and 3 weeks after aspergillosis. The first responded to IV antibiotics, but the second (patient no. 3) died of respiratory failure.

One patient (patient no. 8) developed endophthalmitis 1 month after bilateral LTX for cystic fibrosis. The presenting symptom was blurred vision. Cultures from vitreous tap were positive for *A. terreus*. No involvement of other organs, including the lung, was observed. Because the patient was already receiving itraconazole, he was treated with intravenous voriconazole and with immunosuppressive modulation (stopped of steroids and mycophenolate mofetil and lowered tacrolimus level). Recovery was good, with no visual impairment.

Building construction work

Comparison of the groups for background and medical parameters yielded no significant differences. The only outstanding risk factor we were able to identify in 2004–2005 that could explain the high rate of *Aspergillus* colonization during that period was, as expected, the building construction work. The isolation of fungi from the

Table 3. Clinical characteristics of the patients with *Aspergillus* infections ($n = 8$).

No.	Sex /age	Diagnosis	*Aspergillus* species	Time since LTX	Complication	Antibiotics	Airway management	Response to antifungal treatment	Survival
1	F/43	LAM	*A. terreus* *A. flavus*	4	Bronchial stenosis	Itraconazole	Laser Stent	Good	Alive
2	F/30	CF	*A. fumigatus*	4	Bronchial stenosis	Itraconazole	Laser Stent	Good	Alive
3	M/56	IPF	*A. terreus*	4	Bronchial stenosis	Itraconazole	Laser	No response	Died
4	M/56	IPF	*A. fumigatus*	3	Bronchial stenosis	Itraconazole	Laser Stent	Good	Alive
5	M/40	IPF	*A. terreus* *A. niger*	1	Bronchial stenosis	Voriconazole	Laser Stent	Good	Alive
6	F/61	COPD	*A. niger*	4	Bronchial stenosis	Itraconazole	Laser Stent	Good	Alive
7	M/62	COPD	*A. fumigatus*	1	No	Voriconazole	–	Good	Alive
8	M/22	CF	*A. terreus*	1	Endophthalmitis	Voriconazole	–	Good	Alive

LTX, lung transplantation; LAM, lymphanigolomyomatosis; CF, cystic fibrosis; IPF, idiopathic pulmonary fibrosis; COPD, chronic obstructive pulmonary disease.

air-conditioning system in the lung institute corroborated this assumption.

Discussion

Outbreaks of *Aspergillus* infection have been reported in bone marrow transplant and renal transplant recipients [12,13]. However, to the best of our knowledge, this is the first report in lung transplant recipients. Our findings revealed a significantly higher rate of *Aspergillus* airway colonization in lung transplant recipients in our center from January 2004 to June 2005 (59%) than from May 1994 to December 2003 (9.4%). In their review, Mehard *et al.* [1] estimated the incidence of *Aspergillus* colonization from 26% to 29%, with clinical infection rates of 4–5% and invasive or disseminated infection rates of 5–8%. None of the patients in our series operated on in 1994–2003 had either a local infection or invasive disease, whereas among the group operated later, eight patients had clinical infections, of eight, seven had local disease and one had disseminated disease.

Lung transplant patients are characterized by at least three parameters that can trigger *Aspergillus* colonization: a large lung surface area with connection to air; a bronchial anastomosis, which, in the early post-transplant period, is relatively ischemic and prone to the growth of epithelial tissue that invades the airway lumen, with secondary *Aspergillus* colonization [4]; and immunosuppression, namely corticosteroid suppression of macrophage function, cyclosporine and tacrolimus inhibition of gamma interferon (which activates macrophages), and mycophenolate mofetil or azathioprine inhibition of lymphocytic activity [4].

Aspergillus infection in lung transplant recipients affects mainly the tracheobronchus or bronchial anastomosis [18]. There are two types of disseminated infection, IPA and aspergillosis of an organ not directly connected to the airways. The latter is associated with significant morbidity and mortality [1–6]; complications include bronchomalacia, bronchial stenosis, anastomosis dehiscence, and fatal bleeding [6]. IPA is associated with a 68–100% mortality rate in solid organ recipients, and an even higher rate in neutropenic patients, such those after bone marrow transplantation. Treatment may warrant procedures, such as laser, countercoagulation, and balloon dilatation, which themselves have risk factors. Stent insertion is used as a last resort, and may also have serious sequelae.

Only one of our affected patients (patient no. 8) had disseminated disease, namely endophthalmitis, with isolation of the fungus from the vitreous. This young man had cystic fibrosis, and positive sputum cultures were found already before transplantation. The fungal disease developed 1 month after surgery. The patient responded well to conversion therapy with voriconazole and repeated vitreous synthesis.

Previously described outbreaks of IPA in hospitals were all associated with activities that increased the count of airborne spores, such as construction work [14,15]. Nevertheless, such outbreaks are rare, and the majority of cases of IPA occur sporadically.

All our lung transplant recipients received itraconazole as prophylaxis starting from the third postoperative day. The effectiveness of the broadly accepted protocol is supported by some reports [16,17]. In our study, during the construction work the prophylaxis regimen

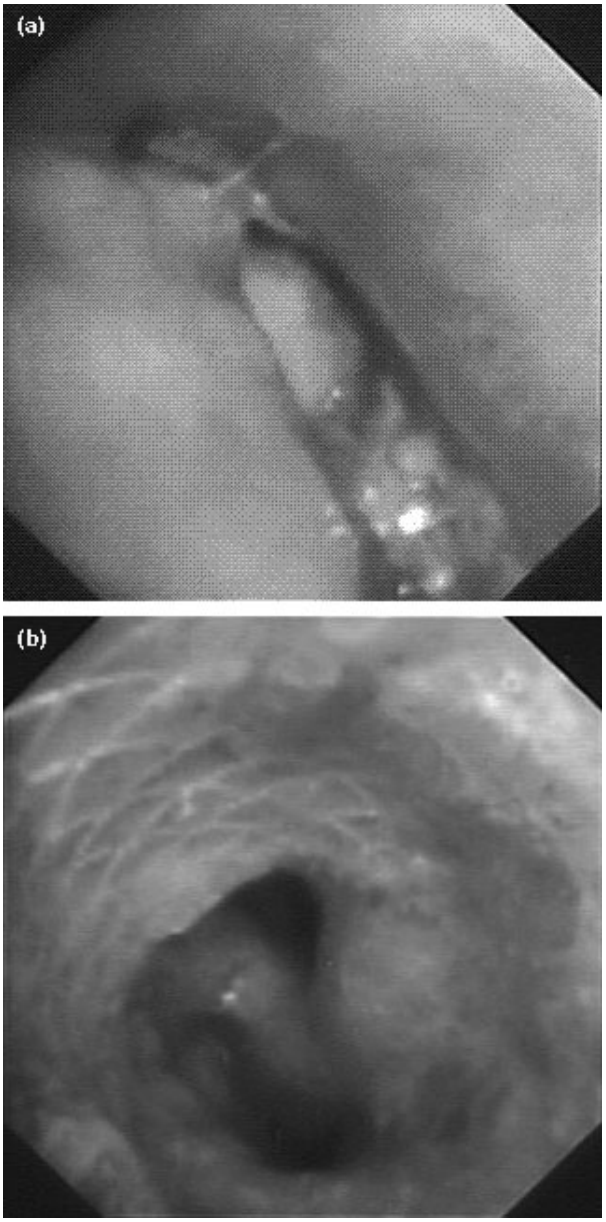

Figure 1 (a) Severe bronchial stenosis in the anastomotic area in lung transplant recipient with *Aspergillus* infection. (b) The same patient after laser therapy and stent insertion.

was continued with the occurrence of 30 patients out of 50 colonized with *Aspergillus* of whom six had an infection and disease. Thus, the prophylaxis does not seem to be effective. However, itraconazole serum levels were not measured in our study. As already mentioned, invasive aspergillosis occurs in 5–8% of the lung transplant recipients [1,2], so six out of 50 patients (12%) are certainly higher. The change in the type of *Aspergillus* species isolated during the construction period is also noteworthy, in addition to the finding of a simultaneous infection by more than two species in two patients. These may be at least partly explained by the high load of organisms released to the air during building construction.

All the clinical infections described in our study occurred in the first 4 months after transplantation. Similar findings were also described before [4]. It is possible that the intraoperative conditions augment the risk, added to the relative devascularization in the anastomosis and the heavy immunosuppressant and the antibiotic therapies in the early post-transplantation period.

We recommend therefore, like in other organ transplantation, compliance with proven environmental protective measures that follow the Healthcare Infection Control Practices Advisory Committee guidelines, such as appropriate placement of impermeable barriers, use of high efficiency particulate air filters in HVAC systems, N95 respirator use by patients when traveling through potentially contaminated areas, and prevention of traffic between construction and the patient care areas [11].

Following the outbreak, we applied these guidelines and observed a significant reduction in *Aspergillus* colonization rate in lung transplant recipients after June 2005.

In summary, physicians should be aware that lung transplant recipients are prone to *Aspergillus* colonization and infection following exposure to heavy construction work despite prophylactic antifungal therapy. Established guidelines for the prevention of *Aspergillus* infections should be implemented during construction activities in hospitals.

References

1. Mehard B, Pacaiocco G, Martinez FJ, Ojo TC, Iannettoni MO, Lynch JP. Spectrum of *Aspergillus* infection in lung transplant recipients: case series and review of the literature. *Chest* 2001; **119**: 169.
2. Cahill BC, Hibbs JR, Savik K, *et al*. *Aspergillus* airway colonization and invasive disease after lung transplantation. *Chest* 1997; **112**: 1160.
3. Hadajiliadis D, Howell DN, Davis RD, *et al*. Anastomotic infection in lung transplant recipients. *Ann Tranplant* 2000; **5**: 13.
4. Nunley DR, Gal AA, Rega JD, Pezlino C, Smith P, Lawrence EC. Saprophytic fungal infections and complications involving the bronchial anastomosis following human lung transplantation. *Chest* 2002; **122**: 1185.
5. Nathan SD, Shorr AF, Schmidt ME, Burton NA. *Aspergillus* and endobronchial abnormalities in lung transplant recipients. *Chest* 2000; **118**: 403.
6. Kramer MR, Denning DW, Marshall SE, *et al*. Ulcerative tracheobronchitis after lung transplantation. A new form of invasive aspergillosis. *Am Rev Respir Dis* 1991; **144**: 552.

7. Lodge BA, Ashley ED, Steele MP, Perfect JR. *Aspergillus fumigatus* enpyema, arthritis and calcaneal osteomyelitis in a lung transplant patient successfully treated with posaconazole. *J Clin Microbiol* 2004; **42**: 1376.
8. Nadal JA. *Textbook of Respiratory Medicine*, 3rd edn. Philadelphia: W.B. Saunders Co, 2005: 1131–1134.
9. Anteby I, Kramer MR, Rahav G, Benezra D. Necrotizing chorditis-retinitis as presenting symptom of disseminated aspergillosis after lung transplantation. *Eur J Ophtalmol* 1997; **7**: 294.
10. Graham DA, Kinyoun JL, George DP. Endogeneous *Aspergillus* endophthalmitis after lung transplantation. *Am J Ophtalmol* 1995; **119**: 107.
11. Panackal AA, Dahlman A, Keil KT, *et al.* Outbreak of invasive aspergillosis among renal transplant recipients. *Transplantation* 2003; **75**: 1050.
12. Rotestein C, Cummings KM, Tidings J, *et al*. An outbreak of invasive aspergillosis among allogeneic bone marrow transplants: a case-control study. *Infect Control* 1985; **6**: 347.
13. Walsh TJ, Dixon DM. Nosocomial aspergillosis: environmental microbiology, hospital epidemiology, diagnosis and treatment. *Eur J Epidemiol* 1989; **5**: 131.
14. Annaise EJ, Stratton SL, Diganani MC, *et al.* Pathogenic *Aspergillus* species recovered from a hospital water system; a three-year prospective study. *Clin Infect Dis* 2002; **34**: 780.
15. Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbial Rev* 1999; **12**: 310.
16. Gerson SL, Talbot GH, Hurwitz S, Storm BL, Lusk EJ, Cassileth PA. Prlonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann Intern Med* 1984; **100**: 345.
17. Brown RS Jr, Lake JR, Katzman BA, *et al.* Incidence and significance of *Aspergillus* cultures following liver and kidney transplantation. *Transplantation* 1996; **61**: 666.
18. Gustafson TL, Schaffner W, Lavely GB, Stratton CW, Johnson HK, Hutcheson RH Jr. Invasive aspergillosis in renal transplant recipients:correlation with corticosteroid therapy. *J Infect Dis* 1983; **148**: 130.

