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# The incidence of invasive aspergillosis among solid organ transplant recipients and implications for prophylaxis in lung transplants

**Key words:**
invasive aspergillosis; solid organ transplantation

**Abstract:** *Background.* Invasive aspergillosis (IA) is associated with significant morbidity and mortality in solid organ transplant recipients but data on the incidence rates stratified by type of solid organ are limited. *Objective.* To describe the attack rates and incidence of IA in solid organ transplant recipients, and the impact of universal *Aspergillus* prophylaxis (aerosolized amphotericin B or oral itraconazole) in lung transplant recipients. *Patients.* The 2046 patients who received solid organ transplants at the Cleveland Clinic Foundation from January 1990 through 1999 were studied. *Methods.* Cases were ascertained through computerized records of microbiology, cytology, and pathology reports. Definite IA was defined as a positive culture and pathology showing septate hyphae. Probable IA was clinical disease and either a positive culture or histopathology. Disseminated IA was defined as involvement of two or more noncontiguous anatomic sites. *Results.* We identified 33 cases of IA (28% disseminated) in 2046 patients (attack rate = 1.6%) for an incidence of 4.8 cases per 1000 patient-years (33 cases/6813 pt-years). Both the attack and the incidence rates were significantly higher for lung transplant recipients vs. other transplant recipients: lung 12.8% (24 cases/188 patients) or 40.5 cases/1000-pt year vs. heart 0.4% (3/686) or 1.4 per 1000-pt year vs. liver 0.7% (3/439) or 2.1 per 1000-pt year vs. renal 0.4% (3/733) or 1.2 per 1000-pt year ($P < 0.01$). The incidence of IA was highest during the first year after transplantation for all categories, but cases occurred after the first year of transplantation only in lung transplant recipients. The attack rate of IA in lung transplant recipients was significantly lower after institution of routine *Aspergillus* prophylaxis (4.9% vs. 18.2%, $P < 0.05$). *Conclusions.* The highest incidence and attack rate of invasive aspergillosis among solid organ transplant recipients occurs in lung transplant recipients and supports the routine use of *Aspergillus* prophylaxis for at least one year after transplantation in this group.

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Invasive aspergillosis (IA) remains a major cause of morbidity and mortality in solid organ transplant recipients. The diagnosis is frequently delayed or made postmortem, with mortality approaching 100% in patients with disseminated disease (1, 2). A recent review of IA in solid organ recipients found the highest attack rates among lung recipients (8.4%), followed by heart (6.2%), liver (1.7%), and kidney (0.7%) recipients (3).

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However, most series of IA in solid organ transplant recipients did not include all types of organ transplants or report incidence rates. The primary objective of our study was to describe the attack and incidence rates of IA in solid organ recipients at the Cleveland Clinic Foundation (CCF) over a 10-year period and the impact of universal *Aspergillus* prophylaxis in lung transplant recipients.

## Materials and methods

A total of 2046 patients received solid organ transplants at CCF between January 1990 and December 1999. Transplant recipients included 695 kidney, 686 heart, 439 liver, 183 lung, 38 kidney-pancreas, and 5 heart-lung transplant recipients. Among lung transplant recipients, single lung transplantation was performed in 112 cases.

Patients were routinely started on 3 mg/kg/day cyclosporine via continuous infusion, adjusted to maintain monoclonal fluorescence concentration polarization immunoassay concentrations of 310–425 ng/mL. Methylprednisolone (1 g) was given intravenously immediately prior to transplantation followed by 125 mg IV every 8 h for 48 h, and 2 mg/kg of azathioprine IV. Once oral intake was started, cyclosporine (8–12 mg/kg/day) was continued with azathrioprine (2 mg/kg/day) with prednisone (40 mg/day).

## Case ascertainment

Cases of invasive aspergillosis during the study period were obtained from three sources: (i) a line listing of cases prospectively collected by the department of infection control and epidemiology, (ii) review of a database of histopathology reporting "septate hyphae consistent with aspergillosis," and (iii) review of the microbiology database for all positive cultures of "*Aspergillus* species." The medical records of solid organ transplant patients identified in these groups were obtained for review.

## Case definitions

We used standard definitions for IA in transplant recipients (3). Definite invasive aspergillosis was defined as either a positive culture for *Aspergillus* species and histologic evidence of tissue invasion (the presence of septate hyphae with dichotomous branching at 45-degree angles within tissue), or a positive culture for *Aspergillus* species from a specimen collected from a normally sterile body site in the presence of clinical disease. Probable IA was defined as a positive culture for *Aspergillus* species or pathology consistent with *Aspergillus* plus clinical findings: either a chest radiograph suggestive of IA (nodular or cavitary lesions and/or crescent or "halo" signs) and/or respiratory symptoms of cough and dyspnea with a noncharacteristic pulmonary infiltrate. Disseminated IA was defined as involvement of $>1$ noncontiguous anatomic sites. Patients who had only a culture positive for *Aspergillus* species in respiratory secretions and were not treated, or received an empiric short course ($<10$ days) of anti-*Aspergillus* therapy for respiratory symptoms attributed later to rejection, were rejected. Patients with ulcerative *Aspergillus* bronchitis were identified based on a previously proposed definition (4).

The time of diagnosis was defined as the date of initiating therapy for IA or death for patients with postmortem diagnosis. The time of onset of IA was the interval from transplantation to the time of diagnosis; we used the date of the last transplantation in patients who had undergone retransplantation. We considered IA to be suspected clinically if treatment was started before the report of positive pathology or culture of a specimen collected by an invasive procedure.

Cytomegalovirus (CMV) infection was defined as viremia and tissue-invasive CMV disease was defined as the presence of cytomegalic inclusion bodies in a tissue specimen and considered associated with opportunistic mold infection if occurring within 30 days of diagnosis of IA. Quantitative CMV–DNA by Hybrid Capture (Digene Corporation, Silver Spring, Maryland, USA) has been used at our institution since 1998.

Results of *Aspergillus* serology using standard complement fixation and immunodiffusion assays were reviewed, if tests were performed within 30 days prior to diagnosis. A course of $\geq 1.5$ g of parenteral methylprednisolone was considered as steroid pulse therapy.

## Prophylaxis of lung transplant recipients

Universal anti-aspergillosis prophylaxis was instituted for all lung transplant recipients in the post-transplant period (*ad infinitum*) at our institution in 1997 because the attack rate of invasive aspergillosis was 14% (5). Patients received inhaled aerosolized amphotericin (5–10 mg bid) in the immediate post-transplant period and were then converted to 200 mg itraconazole capsules (with meal and carbonated beverage), or solution (on an empty stomach) as soon as oral intake could be tolerated. The duration of aerosolized amphotericin was variable among patients and, in some patients, the aerosolized amphotericin overlapped itraconazole for up to 2 weeks. Itraconazole levels were routinely monitored to assure absorption (itraconazole levels $>50$ ng/mL by HPLC assay) (6).

## Statistical analysis

All data were entered into a computer database for analysis (7). The attack rate of invasive aspergillosis for each group of transplant patients was determined by the number of cases divided by the total number of transplant patients. The incidence rate was determined using total patient transplant-years as denominator calculated through December 1999. The Student *t*-test and the Fisher two-tailed exact test were used for univariate analyses of the significance of associations. Differences were considered statistically significant at a *P*-value of 0.05 or less.

## Results

The attack rate of invasive aspergillosis was 1.6% (33 cases/2046 patients) during the 10-year study period, an incidence of 4.8 cases per 1000 transplant patient-years (Table 1). Lung transplant recipients had the highest attack and incidence rates. The median interval from transplantation to diagnosis was 128 days (range: 15–1824 days). A total of 79% of cases occurred within the first year following transplantation. Only lung transplant recipients developed IA after one year. The shortest interval to diagnosis of IA after transplantation (15 days) occurred in a recipient of a bilateral lung transplant in which cultured specimens of the donor bronchus and tissue grew *Aspergillus flavus*. The patient died after 11 days of itraconazole followed by 3 days of amphotericin B. Postmortem examination showed ulcerative bronchitis and dehiscence at the bronchial anastomosis sites and fungal hyphae invading underlying soft tissue and cartilage, and erosion into the pulmonary artery on the right side. Lungs showed scattered acute thrombi of the right lung. Fungal abscesses were found in the posterior parietal cortex, thyroid, and both kidneys. Another case of ulcerative bronchitis was documented by bronchoscopy and endobronchial biopsy; there was no evidence of fungal organisms on transbronchial biopsy.

IA involved the lungs in all 24 lung transplant recipients and in 67% (6/9) of the other cases. In the 13 cases of IA among single-lung transplant recipients, aspergillosis involved both lungs in 69%, the graft in 16%, and the native lung in 15%. Extrapulmonary sites without lung involvement occurred in three patients: isolated cerebral aspergillosis in a heart transplant recipient, vertebral osteomyelitis with perirectal abscess in a kidney transplant, and peritoneal dissemination with graft involvement in a kidney transplant.

Selected characteristics of the 33 cases of IA stratified by type of solid organ transplant are shown in Table 2. Seventeen (52%) patients were smokers, 27% had diabetes mellitus, and only one patient had transient neutropenia. A total of 26 (79%) patients received intense immunosuppression prior to diagnosis. Steroid pulse therapy was given for either suspected (10 cases) or documented (11 cases) acute rejection, and muromonab-CD3 (OKT3, 5 mg/kg/day for 7–14 days) was administered for steroid-resistant or severe rejection in 5 patients. Bronchiolitis obliterans was documented in 42% of the 24 lung transplant recipients within 3 months of diagnosis of IA (three of whom were undergoing photopheresis in addition to one patient with post-transplant lymphoproliferative disorder). CMV infection occurred within 30 days of diagnosis of IA in 48% of patients (16/33) and tissue invasive CMV disease was documented in four of these patients (three with pneumonitis and one with hepatitis). Of the 13 patients with serum IgG levels measured, 77% (10) had levels $< 400$ mg/dL.

Diagnosis was suspected antemortem in 73% of cases and made postmortem in 6 cases (3 lung, 2 heart, and 1 liver). In the 30 patients with pulmonary aspergillosis, 20% had nodular infiltrates on chest radiographs and 90% (9/10) who underwent chest computed tomography (CT) scanning showed nodular lesions with or without cavitation (3 patients had "halo signs"). The histopathologic examination failed to

**Comparative rates of invasive aspergillosis (IA) in 2046 organ transplant recipients at Cleveland Clinic Foundation, 1990–1999**

| Type of transplantation | No. patients | Transplant-patient-years | Attack rate % IA (n) | Incidence* | Incidence 1st year | Incidence after 1st year |
|---|---|---|---|---|---|---|
| All categories | 2046 | 6813.5 | 1.6 (33) | 4.8 | 15.7 | 1.4 |
| Lung† | 188 | 593.1 | 12.8 (24) | 40.5 | 125.6 | 15.3 |
| Kidney‡ | 733 | 2584.2 | 0.4 (3) | 1.2 | 5.2 | 0 |
| Heart | 686 | 2171.6 | 0.4 (3) | 1.4 | 5.3 | 0 |
| Liver§ | 439 | 1464.6 | 0.7 (3) | 2.1 | 8.5 | 0 |

* Per 1000 transplant patient-years.
† Includes 5 heart-lung recipients, none of whom had IA.
‡ Includes 38 kidney–pancreas recipients, none of whom had IA.
§ Includes 1 liver-kidney recipient who had IA.

*Table 1*

**Characteristics of 33 cases of invasive aspergillosis**

| Type of transplant | Number of cases (n) | Mean age (years) | Male/ Female | Primary disease (n) | Steroid pulse (n) OKT3 (n) Atgam (n) | CMV infection concurrent with IA (n) | Definitive IA n (%) | Disseminated n (%) |
|---|---|---|---|---|---|---|---|---|
| Lung | 24 | 47 | 16/8 | Emphysema (13) | | | | |
| | | | | Cystic fibrosis (4) | 16 | 12* | | |
| | | | | Pulmonary fibrosis (2) | 2 | | 15 (63) | 3 (13) |
| | | | | Pulmonary hypertension (2) | 1 | | | |
| | | | | Other# (3) | | | | |
| Kidney | 3 | 50 | 3/0 | Renal artery occlusion† | 2 | | | |
| | | | | Polycystic renal disease | 1** | 2 | 3 (100) | 3 (100) |
| | | | | Membranopro-liferative glomer-ulonephritis | 1 | | | |
| Heart | 3 | 52 | 2/1 | Ischemic cardiomyopathy | 1 | | | |
| | | | | Rheumatic disease | 1 | 0* | 0 (0) | 1## (33) |
| | | | | Lymphocytic cardiomyopathy | 0 | | | |
| Liver | 3 | 53 | 0/3 | Wilson's disease†† | 2 | | | |
| | | | | Primary biliary cirrhosis | 1 | 2 | 2 (75) | 2 (66) |
| | | | | Autoimmune hepatitis | 0 | | | |
| Total | 33 | 48 | 21/12 | | 21 | | | |
| | | | | | 5 | 16 | 20 (61) | 9 (28) |
| | | | | | 2 | | | |

* One (LTR) and 2 (HTR) were not screened for cytomegalovirus within 1 month of IA diagnosis.
# Sarcoidosis, bronchiectasis and lymphangiomatosis, one each.
† The third kidney transplant 15 years after the second one.
** Course started 3 months prior to diagnosis. In all remaining cases, monoclonal antibody and pulse steroid were given within 6 weeks prior to diagnosis.
## One additional patient with heart transplant had isolated cerebral IA.
†† Underwent liver re-transplant for hepatitis C virus-induced cirrhosis and kidney transplant for cyclosporine related nephropathy 13 years after the first liver transplant.

***Table 2***

identify fungal elements in 19 patients with probable (10 patients) or definite (9 patients) pulmonary IA who underwent transbronchial biopsies. *Aspergillus* was isolated from sputum or tracheal aspirate cultures in 67% of patients (14/21), and isolated from bronchoalveolar lavage specimens in 83% of patients (19/23). *Aspergillus fumigatus* was the most common species, isolated in 68% (21/31) of cases, followed by *A. flavus* (2 cases), *A. niger* and *A. nodularis* (one case each). Two patients had A. *fumigatus* with either *A. flavus* or *A. niger*. Eight lung transplant recipients had at least one respiratory culture specimen positive for *Aspergillus* species prior to the diagnosis of IA (range: 3 weeks to 6 months). *Aspergillus* serology testing at the time of diagnosis of IA was negative in all nine patients tested.

The overall mortality rate for solid organ transplant recipients with IA was 61% and aspergillosis was the direct cause of death in 30%. Treatment failed in 50% of patients who died a median of 8 days after initiation of treatment with amphotericin B (range: 1–26 days). Two of three patients who underwent surgical intervention (one pneumonectomy, one diskectomy, and partial vertebrotomy) were cured; however, one patient who underwent debridement of an infected perirenal lymphocele died with disseminated peritoneal aspergillosis. The mortality rates for patients with IA were not significantly different among nonlung transplant recipients vs. lung transplant recipients (67% vs. 50%, $P = 0.13$), or for patients with extrapulmonary IA vs. pulmonary IA (80% vs. 52%, $P = 0.4$).

## Prophylaxis in lung transplant recipients

We compared the incidence and attack rates of invasive aspergillosis for lung transplant recipients who did and did not receive *Aspergillus* prophylaxis with inhaled amphotericin B and/or itraconazole in the post-transplant period. There were 16 cases of IA among the 88 lung transplant recipients without prophylaxis in 322.3 transplant-years of follow-up (attack rate = 18.2% and incidence rate = 49.7/1000 pt-years) compared with 4 cases of IA among the 81 lung transplant recipients who received prophylaxis in 126 pt-years of follow-up (attack rate = 4.9% and incidence rate = 31.6/1000 pt-years) ($P < 0.05$).

## Discussion

Our results demonstrate that IA has the highest incidence and attack rate among lung transplant recipients compared with other solid organ transplant recipients (12.8% vs. < 1% and incidence of 40.5 vs. ≤ 2.1 per 1000 pt-years). Notably, the risk of IA in lung transplant recipients remained elevated after the first year of transplantation when compared to other solid organ transplant recipients, and antifungal prophylaxis appears to have reduced the risk of IA. We found a higher rate (46%) of IA occurring after 9 months of lung transplantation than previously reported (8).

The attack rate for IA among our lung transplant recipients was comparable with other reports (9–11). The increased risk for lung transplant recipients compared with other solid organ transplant recipients may be explained by both host and environmental factors. A transplanted lung is the only allograft in continuous contact with the external environment, which may contain fungal spores. In addition, local host defenses in the transplanted lung may be impaired (12). Risk factors for IA identified in case-control studies in lung transplant patients include prior airway colonization with *Aspergillus* species (5, 9), and CMV infection (5, 11). Hypogammaglobulinemia has recently been associated with IA in lung transplant recipients at our institution (13). It remains unclear if hypogammaglobulinemia is a cause or effect of an immunosuppressed state. Humoral immunity is not traditionally thought to play a major role in the immune response to *Aspergillus* species.

The low attack rates of IA we observed in heart (0.7%) and liver (0.4%) transplant recipients when compared to some other centers (10–15%) (2, 14) may be due to uncommon utilization of OKT3 in our immunosuppressive regimens. It should also be noted that the autopsy rate for the 439 patients who died during the study period was approximately 15%, and therefore cases of IA may have been missed.

Diagnosis of IA in solid organ transplant recipients is challenging. As in other reported studies, 18% of our patients were diagnosed postmortem. Because the lung is the most common site for IA, radiographic studies, especially chest CT scans, are extremely important in the assessment of these patients. Even with transbronchial biopsies, bronchoscopy with lavage was only 83% sensitive in establishing a diagnosis of IA in our 30 patients with definite or probable pulmonary IA. These results are comparable to other studies in bone marrow, heart, and lung transplant recipients (15, 16). Serologic testing was negative in all patients tested. The high incidence of IA among lung transplant recipients led us to pursue a strategy of post-transplant prophylaxis. Beginning in 1997, patients received amphotericin B aerosols (10 mg twice daily). When patients were able to take oral medicines, itraconazole was administered with attention paid to serum levels. Other studies have demonstrated the efficacy of amphotericin B aerosols in the prevention of aspergillosis (17, 18) and of itraconazole in preventing IA in those individuals colonized with *Aspergillus* species (19). Although our study was not a randomized placebo-controlled trial, we found that universal prophylaxis was associated with a reduction in the incidence of IA in lung transplant recipients compared with historical controls. For the time being, we advocate amphotericin B aerosols followed by long-term oral itraconazole in lung transplant recipients. Multicenter clinical trials will be needed to determine the optimal strategy for *Aspergillus* prevention in these high-risk patients (8).

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