Invasive Aspergillosis in the Setting of Cardiac Transplantation

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Among patients undergoing heart transplantation, *Aspergillus* is the opportunistic pathogen with the highest attributable mortality. The median time of onset from transplantation for invasive pulmonary aspergillosis (IPA) was 46 days, but the median time to first positive culture result was 104 days among patients with *Aspergillus* colonization but no invasive disease. Most patients with IPA presented with fever and cough within the first 90 days of transplantation and with single or multiple pulmonary nodules. None of the heart transplant recipients with either IPA or invasive extrapulmonary aspergillosis (IEPA) had associated neutropenia. Human leukocyte antigen A1 locus was found significantly more frequently among patients colonized with *Aspergillus* than among patients with IPA ($P < .006$) or IEPA ($P < .001$). Even in the absence of neutropenia, IPA should be suspected for heart transplant recipients who have fever and respiratory symptoms within the first 3 months of transplantation, have a positive result of culture of respiratory secretions, and have abnormal radiological findings (particularly nodules).

Invasive fungal infections are important causes of morbidity and mortality in patients who received solid organ or bone marrow transplants [1–3]. In this patient population, infection with *Aspergillus* species can result in a variety of clinical syndromes, including sinusitis, tracheobronchitis, pneumonia, necrotizing cellulitis, brain abscess, and disseminated disease [4]. Among heart transplant recipients, *Aspergillus* most frequently causes pneumonia and is the opportunistic pathogen with the highest attributable mortality [5–9].

Although invasive aspergillosis is a serious disease in heart transplant recipients, little is known regarding the natural history of this infection in this patient population. The epidemiological, clinical, and radiological characteristics of patients undergoing heart transplantation who have invasive pulmonary aspergillosis (IPA) have been described in isolated case reports, but a study summarizing the natural history of IPA is lacking. The aim of our study was to assess the risk factors, clinical presentation, laboratory and radiological findings, and outcome of IPA in a consecutive series of 844 heart transplant recipients treated at Stanford University Medical Center during an 18-year period.

PATIENTS, MATERIALS, AND METHODS

Study population. Eight hundred forty-four patients who had undergone heart transplantation at Stanford University Medical Center (Stanford, CA) between 1 January 1980 and 31 December 1998 were studied. This study did not include patients who had undergone heart-lung or lung transplantation.

Patients with IPA were identified by review of medical records from the Stanford Transplant Database and the Stanford Clinical Laboratory Information System

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(SCLIS). Demographic and clinical variables were abstracted onto a structured, precoded data form. These variables included patient age and sex; diagnosis; date of first transplantation; previously suggested variables as risk factors for invasive aspergillosis [10], including rejection, cytomegalovirus (CMV) infection and disease, and receipt of immunosuppressive drugs (prednisone, azathioprine, cyclosporine, mycophenolate mofetil–tacrolimus, and OKT); radiographic features; prophylaxis; follow-up; and outcome. For each documented episode, available radiographs obtained before and during the clinical event were assessed by a faculty radiologist (A.N.L.). Culture of sputum was usually done daily until patients underwent extubation. Follow-up data were obtained for all patients for a minimum period of 2 years after transplantation.

Operative techniques did not change significantly during the study period [11]. Selection criteria for recipients and donors have been described elsewhere [11–13] and remained fairly constant over the period of the study, except for the age of the recipients; the initial upper age limit of 50 years has been extended over time for otherwise suitable potential recipients.

Information regarding presence or absence of invasive aspergillosis, site of disease, and its case fatality rate among patients undergoing heart transplantation between December 1980 and December 1984 at Stanford University was published by Hofflin et al. [7]. Information regarding presence or absence of invasive aspergillosis, site of disease, and role of inhaled amphotericin B prophylaxis in patients undergoing transplantation between 1990 and 1995 was published by Reichenspurner et al. [14]. Information regarding presence or absence of invasive aspergillosis, site of disease, its attributable mortality, and the impact of prophylactic agents such as ganciclovir and inhaled amphotericin B on longitudinal actuarial incidence of disease in patients undergoing heart transplantation between 1980 and 1996 was published by Montoya et al. [8]. However, this is the first report from Stanford University (or any other institution) that details the clinical, laboratory, and radiological information for heart transplant recipients with IPA or invasive extrapulmonary aspergillosis (IEPA) and for those colonized with the fungus who did not develop invasive disease

Analysis of isolates. Between 1 December 1985 and 31 July 1998, we found 4453 consecutive *Aspergillus* isolates in the SCLIS (figure 1). Of these, 245 isolates (5.5%) were excluded from the analysis because they were environmental. Of the remaining 4208 patient isolates, 72 (2%) were obtained from heart transplant recipients. Among the 72 *Aspergillus* isolates from heart transplant recipients, 15 were from patients with IPA and 57 from patients without IPA. Among the 57 heart transplant recipients without IPA, 31 had IEPA and 26 had colonization with *Aspergillus* species without disease. In addition to the 15 patients with *Aspergillus* isolates and IPA identified through the SCLIS, 10 more patients with IPA were identified. Six who underwent transplantation before 1985 were identified by use of the Stanford Transplant Database, and 4 had had the *Aspergillus* isolate identified while in an outside hospital. Thus, a total of 25 heart transplant recipients with IPA were identified. Routine fungal susceptibility studies of the *Aspergillus* isolates were not done.

Diagnostic criteria. Cases were identified according to the National Institute of Allergy and Infectious Diseases Mycoses Study Group and the European Organization for Research and Treatment of Cancer definitions for invasive aspergillosis [15]. A case was considered definite if a patient had positive results of histology and positive results of culture of a sample from the same site or negative results of histology (or none done) but positive culture results of a sample obtained by protocol-specified invasive techniques (bronchoalveolar lavage, washings, brushings, or needle aspiration). A case was considered probable if a heart transplant recipient had unexplained respiratory symptoms, had abnormal pulmonary radiological findings, and, although an invasive procedure was contraindicated, had either 2 positive results of culture of sputum or throat samples or 1 positive result of a culture or a smear of a bronchoscopy specimen, or met criteria for definite invasive aspergillosis in another organ system [15].

Immunosuppression, rejection, and prophylaxis. Each patient underwent diagnosis and management of rejection as previously described [11, 16]. After 1 January 1980, several modifications in the immunosuppressive regimens and new antimicrobial prophylactic drugs were introduced (table 1). Each patient received prednisone, azathioprine, and cyclosporine as part of their regimen. OKT3 was introduced in June 1987 and mycophenolate mofetil–tacrolimus in February 1994. Ganciclovir was introduced in January 1987. Since July 1993, in an attempt to prevent invasive aspergillosis, aerosolized deoxycholate amphotericin B, 20 mg in sterile water t.i.d., has been administered throughout the posttransplantation hospital stay.

Statistical analysis. Data from case forms were extracted, entered into a central database, and analyzed. Statistical analysis was done with Epi-Info (Centers for Disease Control and Prevention; Atlanta, GA). $P < .05$ was used to define statistical significance; the Student's t test was used for continuous variables and χ^2 was used for differences in categorical variables.

RESULTS

During the 18-year study period (1980–1998), a total of 82 heart transplant recipients with *Aspergillus* species isolated were identified. In this group, 25 patients presented with IPA, 31 with invasive extrapulmonary disease, and 26 without disease (i.e., colonization of any body site) (figure 1). Of the 25 heart transplant recipients with IPA, 21 had lung histopathology com-

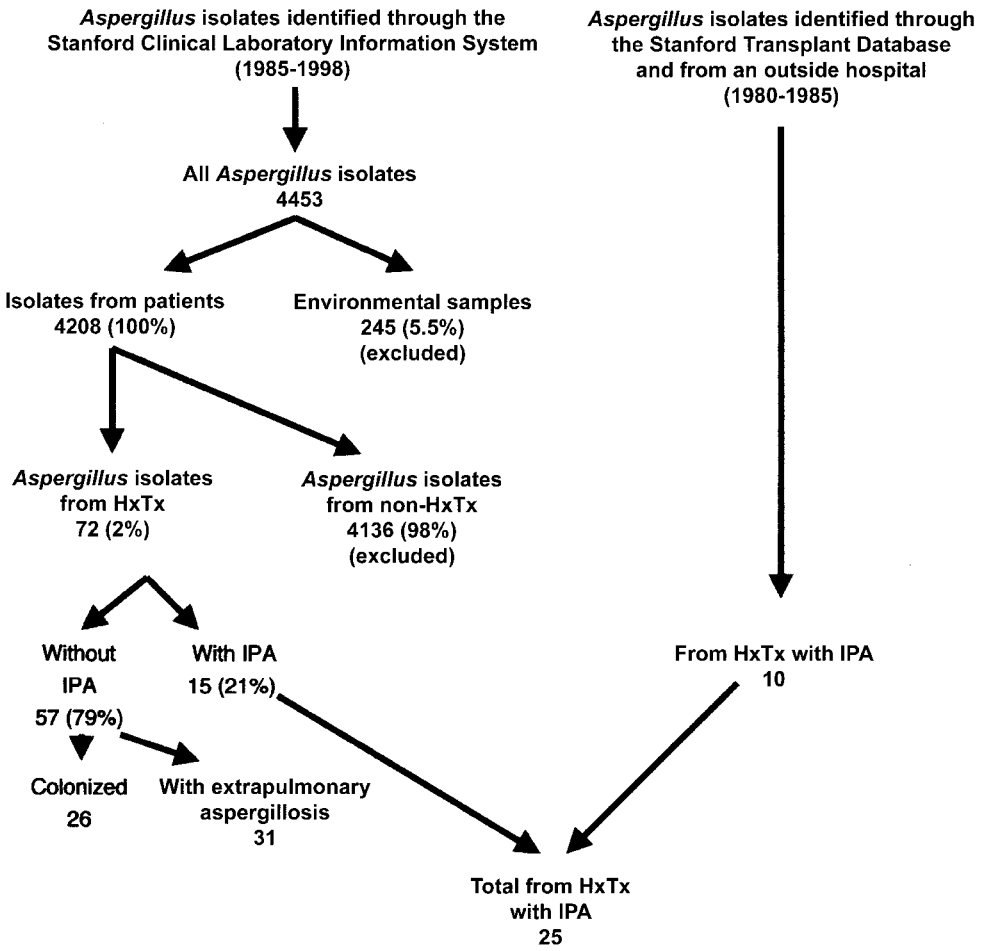

Figure 1. Algorithm used to identify *Aspergillus* isolates at Stanford University Medical Center from 1980 to 1998. Data are no. or no. (%) of isolates. HxTx, heart transplant recipient; IPA, invasive pulmonary aspergillosis.

patible with *Aspergillus* species and were classified as having definite cases, and 4 were defined as having probable cases. *Aspergillus* was isolated from each of our study patients.

IPA. Of the 25 patients with IPA, 21 (84%) were male. The mean age of the 25 patients was 43 years, and coronary artery disease (32% of patients) was the most common indication for heart transplantation (table 2). Fever was the most common IPA symptom (52% of patients), and dyspnea and cough were next most common (12% each).

Each of the 25 patients had abnormalities described by report on their chest radiographs, but radiographic images were available for review for only 8 patients, 5 of whom also had CT done. Nodules were identified by either chest radiography or CT for all 8 patients (table 3); the mean number of nodules per patient was 4.4 (range, 1–14 nodules). A peripheral consolidative region resembling a parenchymal infarct was seen as an associated finding in 2 patients. One patient presented radiographically with a 5.5-cm mass and 2 additional <1-cm nodules, which were detectable only on CT. In 4 of 5 cases, CT demonstrated a higher number of nodules than observed on the corresponding chest radiograph. A CT halo sign (figure 2) [17] was identified in 1 patient; cavitation was present on the initial ($n = 1$) or follow-up ($n = 3$) studies for 4 patients (figure 3). Associated pleural effusions were present in 4 of 8 patients.

The median time from appearance of the radiological abnormality to laboratory diagnosis was 1 day (range, 8 days before to 1 day after). In the 6 patients who had follow-up studies done, the median time to observation of radiological improvement was 17 days (range, 8–37 days) (figure 3). Resolution of radiographic findings occurred at a median time of 4 months (range, 2–10 months) for the 4 patients who had complete radiographic follow-up (figure 4).

Among patients with IPA, the median time to first isolation of *Aspergillus* species after transplantation was 46 days (72% within 90 days), and the median time of onset of IPA after transplantation was 46 days. In contrast, the median time to first isolation of *Aspergillus* species after transplantation in pa-

Table 1. Immunosuppressive therapy periods and rates of aspergillosis in heart transplant recipients at Stanford University Medical Center between 1980 and 1998.

Period	Medications (n = 844)	Invasive aspergillosis[a] (n = 56)	IPA (n = 25)
1 Jan 1980–31 Dec 1986	Prd, Aza, Cysp (n = 287)	19 (6.6)	12 (4.2)
1 Jan 1987–31 May 1987	Prd, Aza, Cysp, Gcv[b] (n = 19)	2 (10.5)	2 (10.5)
1 Jun 1987–30 Jun 1993	Prd, Aza, Cysp, Gcv, OKT3[c] (n = 324)	29 (9.0)	7 (2.2)
1 Jul 1993–4 Feb 1994	Prd, Aza, Cysp, Gcv, AmB[d] (n = 24)	1 (4.2)	1 (4.2)
5 Feb 1994–31 Dec 1998	Prd, Aza, Cysp, Gcv, AmB, mycophenolate mofetil–tacrolimus[e] (n = 190)	5 (2.6)	3 (1.6)

NOTE. Data are no. of patients (%). AmB, amphotericin B; Aza, azathioprine; Cysp, cyclosporine; Gcv, ganciclovir; IPA, invasive pulmonary aspergillosis; OKT3, Muromonab/CD3; Prd, prednisone.

[a] Includes IPA and pulmonary.
[b] Ganciclovir was begun in January 1987.
[c] OKT3 was begun in June 1987.
[d] Amphotericin B prophylaxis was begun in July 1993.
[e] Mycophenolate mofetil–tacrolimus was begun in February 1994.

tients with only aspergillus colonization was 104 days. In the month prior to IPA diagnosis, no patients had absolute neutrophil counts of <500 cells/μL, (mean WBC count, 7608 cells/μL; range, 2100–13,900 cells/μL; mean number of polymorphonuclear cells, 5574 cells/μL; range, 966–12,420 cells/μL). Twenty-two patients (88%) received steroid therapy, 11 (44%) developed rejection, and 9 (36%) presented with CMV disease at some time after transplantation and after IPA (median onset for CMV disease after transplantation was 65 days).

Aspergillus fumigatus was isolated from 24 patients (96%; 1 patient had coinfection with *Aspergillus niger* and *A. fumigatus*) and *Aspergillus flavus* from 1 patient (4%). Of the 25 patients with IPA, 21 (84%) did not receive inhaled amphotericin B. The mortality among those who did not receive amphotericin B prophylaxis was 29% (6 patients). Of the 4 patients who received prophylaxis with inhaled amphotericin B, 3 (75%) developed IPA and died with signs of persistent infection. A total of 21 patients (84%) received treatment with systemic amphotericin B, and 3 patients (12%) also received itraconazole. The case fatality rate for patients with IPA was 36%.

***Comparison of patients with IPA with patients colonized with* Aspergillus.** Patients with IPA were compared with heart transplant recipients who had a positive result of culture of sputum for *Aspergillus* species but did not develop invasive disease (colonized group) (table 2).

Age and sex were not significantly different between patients with IPA and colonized patients. Coronary artery disease was the most common underlying diagnosis before heart transplantation among both groups (8 patients in the IPA group and 14 patients in the colonized group). The mean WBC count in the month before diagnosis was 7608 cells/μL (range, 2100–13,900 cells/μL) for patients with IPA and 7349 cells/μL (range, 2988–16,100 cells/μL) for colonized patients. There were no significant differences in the duration of stay in the intensive care unit after transplantation.

Important clinical differences were found between patients with IPA and colonized patients. Fever was the predominant symptom in the IPA group (present in 13 patients [52%]), but not in the colonized group (2 patients [8%]). The sensitivity and specificity of fever, among patients with a positive result of sputum culture for *Aspergillus* species, for a diagnosis of IPA were 52% and 92%, respectively (positive predictive value, 86%). Although the frequency of cough alone was not statistically different between the 2 groups, when it was present and associated with fever, patients were more likely to have IPA. The sensitivity and specificity of the presence of fever and cough, among patients with a positive result of culture of sputum for *Aspergillus* species, for a diagnosis of IPA were 36% and 96%, respectively (positive predictive value for both fever and cough, 90%).

Each of the patients in the colonized group had episodes of rejection during the postoperative period, but only 11 patients in the IPA group (44%) developed rejection at any time after transplantation. However, the numbers of rejection episodes before diagnosis of IPA or first positive culture result were not significantly different between the 2 patient groups.

The median time to first positive result of culture for *Aspergillus* species after transplantation was 46 days for the IPA group and 104 days for the colonized group. This difference was not statistically significant, but 73% of patients with IPA received their diagnosis before a positive culture result occurred in the colonized group.

Results of human leukocyte antigen (HLA) typing for the A locus were available for 23 patients in the IPA group and 24 patients in the colonized group. A higher frequency of HLA-A1 was found in the colonized group (in 50% of patients) than

Table 2. Comparison of clinical and laboratory findings for patients undergoing heart transplantation who have invasive pulmonary aspergillosis (IPA) versus those colonized with *Aspergillus* species.

Variable	IPA (n = 25)	Colonized[a] (n = 26)	P
Age, years, mean	43	48	.5
Sex			
Male	21 (84)	23 (88)	.95
Female	4 (16)	3 (12)	
Underlying diagnosis prior to heart transplantation			
Coronary artery disease	8 (32)	14 (54)	.1
Cardiomyopathy	4 (16)	8 (31)	.2
Congenital disease	2 (8)	0 (0)	
Valvular disease	1 (4)	1 (4)	
Other	0	3 (12)	.2
Symptoms			
Fever	13 (52)	2 (8)	<.001
Dyspnea	3 (12)	0	.2
Cough alone	3 (12)	3 (12)	.7
Asymptomatic	1 (4)	4 (15)	.3
Cough plus fever	9 (36)	1 (4)	.01
Diagnostic method			
Culture	25 (100)	26 (100)	
Pathology	21 (84)	1 (4)	<.001
Time to first positive culture result for *Aspergillus* species, days after transplantation, median	46	104	.2
WBC count, cells/μL, mean (range)	7608 (2100–13,900)	7349 (2988–16,100)	.9
Neutrophil count, cells/μL, mean (range)	5574 (966–12,420)	6940 (2790–15,636)	.9
Risk factor			
Corticosteroid therapy	22 (88)	26 (100)	.2
Rejection	11 (44)	26 (100)	<.001
No. of rejections	1.7	2.1	.6
CMV coinfection (serology only)	9 (36)	17 (65)	.03
CMV disease (symptomatic)	9 (36)	10 (38)	.8
R+/D−	9 (36)	17 (65)	.03
R+/D+	8 (32)	7 (27)	.7
R−/D−	10 (40)	9 (35)	.7
R−/D+	2 (8)	3 (12)	1
OKT3	12 (48)	17 (65)	.2
Aza	25 (100)	25 (96)	
Aza dose, mg	82.7	152.8	.004
ICU, days	8	8.7	.6
Inhaled amphotericin B prophylaxis after July 1993	4 (16)	2 (8)	.6
Treatment			
Amphotericin B	21 (84)	1 (4)	<.001
No treatment	4 (16)	25 (96)	
Itraconazole	3 (12)	0	
Mortality due to *Aspergillus*	9 (36)	0	.002
Duration of follow-up from time of first positive result of culture for *Aspergillus,* days after transplantation, mean	1291	1605	.4

NOTE. Data are no. of patients (%), unless otherwise indicated. Aza, azathioprine; CMV, cytomegalovirus; D+, donor positive for CMV-specific IgG (before transplantation); D−, donor negative for CMV-specific IgG (before transplantation); ICU, intensive care unit; IgG, immunoglobulin G; R+, recipient positive for CMV-specific IgG (before transplantation); R−, recipient negative for CMV-specific IgG (before transplantation).

[a] Colonization of any body site (mostly the respiratory tract).

Table 3. Radiological findings for 8 patients with invasive pulmonary aspergillosis.

Patient age, sex	No. of nodules		Mass	Effusion	Cavitation	Halo sign	Days to improvement (days of treatment)
	Chest radiography	CT					
55, M	1	ND	No	No	No	ND	17 (16)
50, F	0	2	Yes	No	Yes	No	11 (10)
70, M	1	ND	No	Yes	No	ND	17 (16)
36, M	6	ND	No	No	Yes	ND	10 (11)
38, M	1	1	No	Yes	No	No	ND
56, M	3	8	No	No	No	No	8 (6)
49, M	1	14	No	Yes	Yes	Yes	37 (29)
56, M	2	3	No	Yes	Yes	No	ND
No. of patients with result	7	5	1	4	4	1	ND

NOTE. F, female; M, male; ND, not done.

in the IPA group (13%) ($P = .006$). Information available for HLA B locus indicated no significant differences between the 2 groups.

IEPA. Of the 31 patients with IEPA, 27 were male. The mean age of the 31 patients was 43 years, and coronary artery disease was the most common indication for heart transplantation (table 4). Fever was the most common symptom, followed by cough. No patients had absolute neutrophil counts of <500 cells/μL at the time of diagnosis of IEPA. Thirty patients received steroid therapy, 26 developed rejection, 18 were coinfected with CMV, and 11 developed symptomatic CMV disease. After a follow-up of 1308 days from time of first positive culture result for *Aspergillus* species, the attributable mortality rate for patients with IEPA was 35%.

Fever or fever and cough were more common among patients with IPA than among patients with IEPA patients ($P < .006$ and $P < .01$, respectively; table 4). The median time to first positive result of culture for *Aspergillus* species after transplantation was

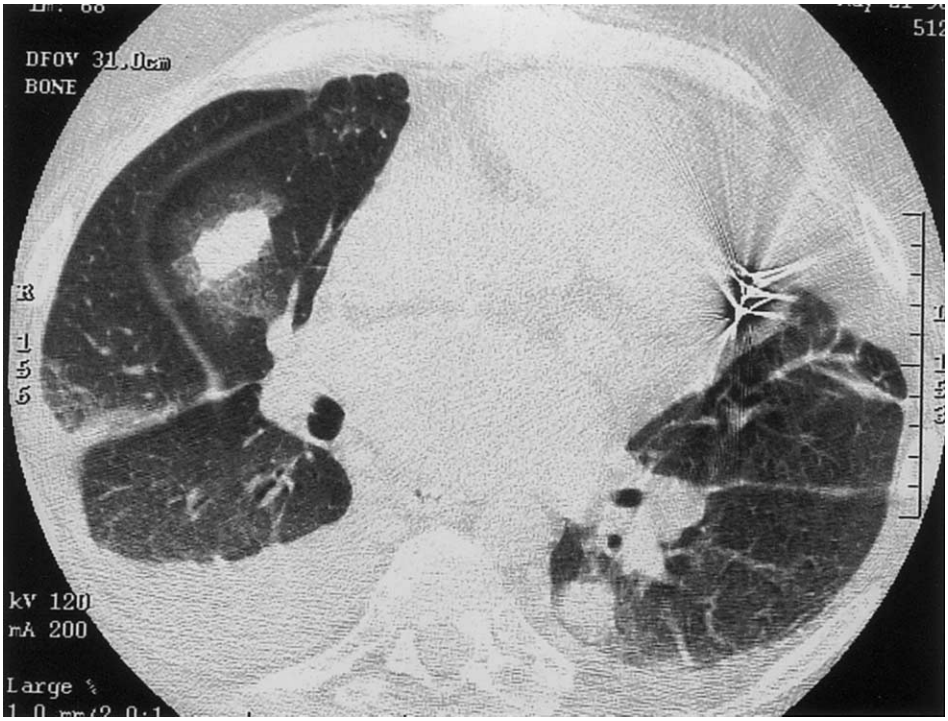

Figure 2. Thin-section CT scan of a 49-year-old cardiac transplant recipient revealing a nodule surrounded by ground-glass attenuation (i.e., CT halo sign) in the right middle lobe.

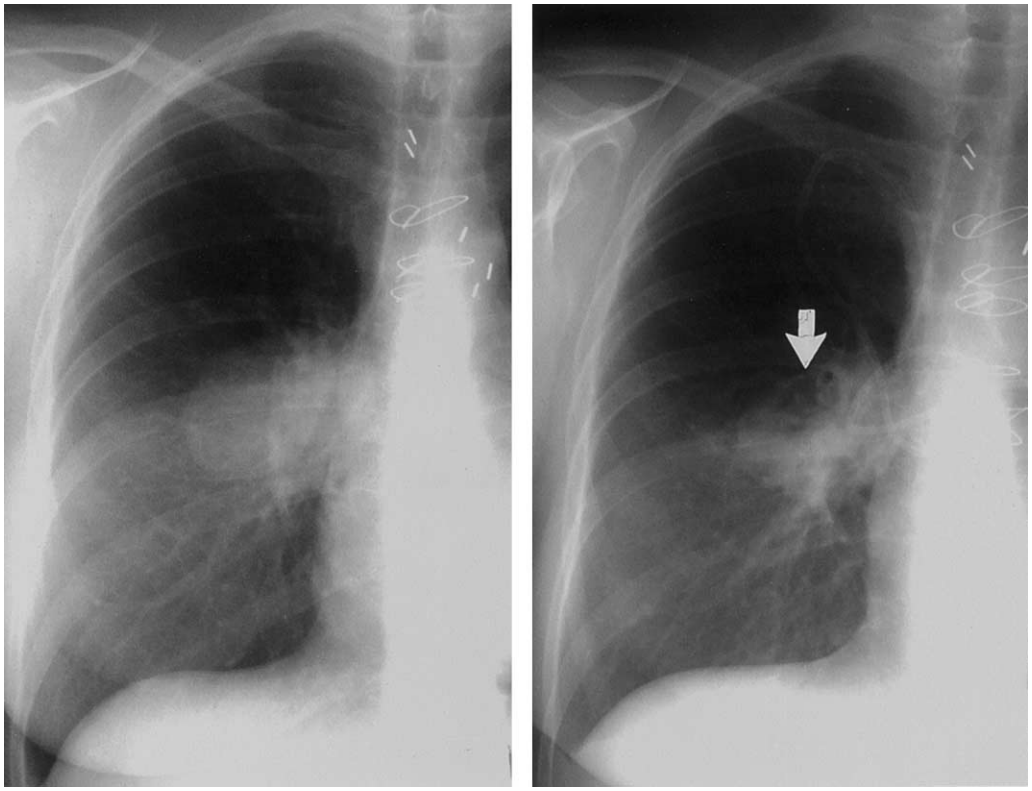

Figure 3. Development of cavitation in a 50-year-old heart transplant recipient. *A,* Radiograph obtained at the time of initial detection, showing a 5-cm mass in the right lower lobe; *B,* Radiograph obtained 11 days later, showing interval development of cavitation *(arrow).*

not significantly different from that for patients with IPA. The median time of onset of IEPA after transplantation was 56 days. Twenty-six patients (84%) with IEPA developed rejection at any time after transplantation, in contrast to 11 patients (44%) from the IPA group ($P < .001$).

Of the 31 patients with IEPA, 29 (94%) did not receive inhaled amphotericin B prophylaxis. Mortality among the 29 patients was 35% (11). The 2 patients who received prophylaxis developed cutaneous aspergillosis only, at previous iv catheter sites. Each of the 31 patients with IEPA received treatment with amphotericin B, and none of these patients received itraconazole.

Results of HLA typing for the A locus were available for 29 patients, 3 of whom were HLA-A1 (10%). HLA-A1 frequency was lower than that found in the colonized group (50% of patients; $P < .001$). However, no statistically significant differences between the HLA-A1 frequencies between patients with IPA and those with IEPA ($P = .89$) were found.

Incidence and mortality of invasive aspergillosis by immunosuppressive and prophylactic protocols. The incidence of invasive (IPA and IEPA) aspergillosis in heart transplant recipients during the different therapy periods is shown in table 1. Mortality among patients with IPA treated before the use of prophylactic amphotericin B was 28%, and it increased to 75% after July 1993. At the time of the conclusion of the study period, the differences in mortality between the 2 cohorts (before and after the introduction of prophylactic amphotericin B) were not statistically significant ($P = .2$).

DISCUSSION

Invasive infection with *Aspergillus* species remains a major cause of morbidity and mortality in patients who had undergone heart transplantation. This study reports in detail the clinical, laboratory, and radiological findings of 56 heart transplant recipients with invasive aspergillosis.

In this report, the *Aspergillus* species most commonly found was *A. fumigatus;* the contribution to invasive disease by other species, such as *A. niger* or *A. flavus,* was rare. Similar findings have been reported by others [6, 18, 19].

Patients with IPA more frequently presented with fever or fever and cough within the first 3 months after transplantation. Seventy-two percent of cases were diagnosed during the first 90 days after transplantation. Similar time of onset for IPA after transplantation has been reported by at least 1 other group [20]. Sixty-five percent of the isolates recovered by Grossi et al. [20] were found in the first 3 months, and the rate of infection decreased from >0.15 episodes/patient-month in the

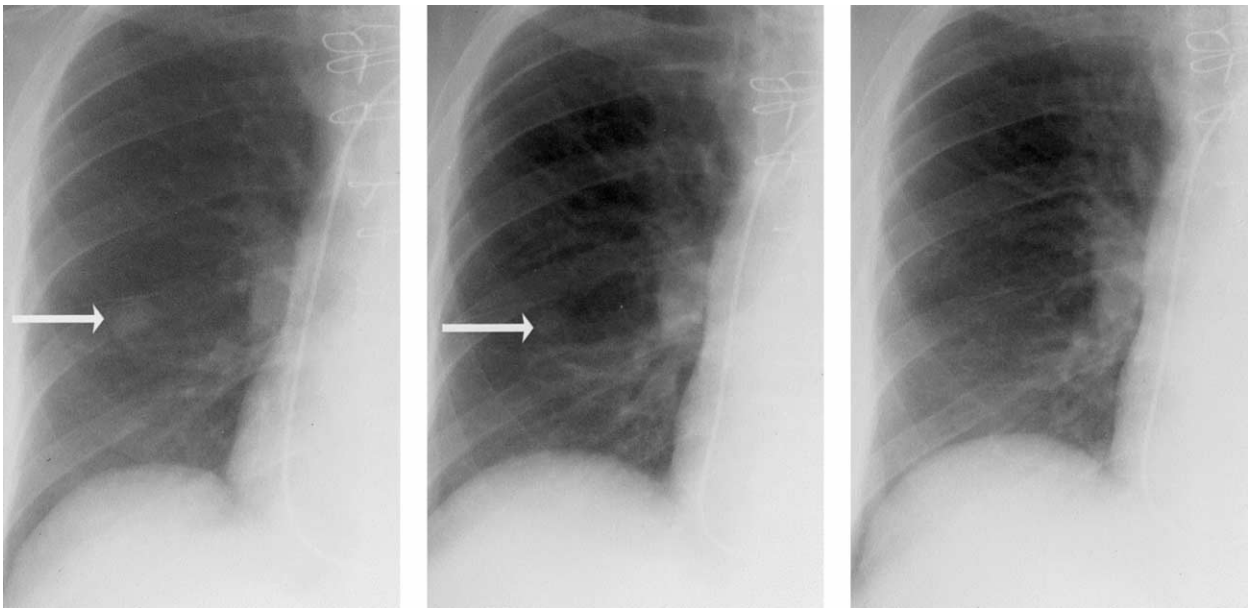

Figure 4. Evolution of treated invasive pulmonary aspergillosis in a 56-year-old cardiac transplant recipient. *A,* Radiograph obtained at the time of initial detection, showing a 1.5-cm nodule *(arrow)* in the right middle lung zone; *B,* Radiograph obtained 8 days later, showing an interval decrease in the size of the nodule *(arrow); C,* Radiograph obtained 59 days after the time of initial detection, showing resolution of the nodule.

first 3 months to <0.05 episodes/patient-month after 6 months [20].

In the present study, the presence of fever alone, and of fever plus cough, was found to be significantly more common in patients with IPA than in patients colonized with *Aspergillus.* The sensitivity of these symptoms was not high (52% for fever alone and 36% for fever and cough), but their positive predictive value (86% and 90%, respectively) might be useful in interpreting a positive culture result for *Aspergillus* species for a patient with heart transplantation, particularly within the first 3 months after transplantation.

Risk factors for invasive aspergillosis that have been reported include prolonged neutropenia [21], neutrophil function deficits [22], corticosteroid therapy [23], graft-versus-host disease, and CMV disease [10]. Prolonged neutropenia is a commonly recognized risk factor in patients with leukemia [24] and bone marrow transplantation [21]. In contrast to patients with hematologic malignancies, neutropenia does not appear to be a risk factor for IPA in heart transplant recipients. None of our patients was neutropenic (absolute neutrophil count, <500 cells/mm^3). IPA should be suspected in heart transplant recipients who present with fever and respiratory symptoms during the first 3 months after transplantation, have a positive result of culture for *Aspergillus* species, and have abnormal radiological findings (in particular, nodules) despite the absence of neutropenia.

The radiological findings of invasive aspergillosis in the 8 patients for whom results of radiological studies were available are similar to those previously described in transplant recipients [25–27] and patients with leukemia [25, 28]. Multiple pulmonary nodules are the most common radiological manifestation [25, 27–29] and were present in 63% of our patients. Cavitation, present in 50% of our patients, is a frequent associated finding and may either be present at the time of initial nodule detection or develop over the course of infection [26, 27]. Although the CT halo sign has been proposed as a useful clue in the early diagnosis of IPA, it is uncommon, occurring in only 2 of 9 patients in the original series in which the sign was first described [28] and seen in only 1 of 5 patients who underwent CT in our study. Radiographic improvement in IPA is a gradual process. Similar to results previously reported by Haramati et al. [26], the median times to observation of radiographic improvement and resolution of IPA in our series were 17 days and 4 months, respectively.

In this study, rejection was less common in patients with IPA than in the colonized group. The fact that the colonized group had more frequent episodes of rejections than the IPA group suggests the possibility of a higher immune activation or a lower efficacy or intensity of the immunosuppressive regimen in the colonized group. The end result of either explanation would be that the patients in the colonized group would be more "protected" against invasive disease caused by the *Aspergillus* organisms already present in their airways.

A higher frequency of HLA-A1 was present in patients colonized by *Aspergillus* species than in patients with IPA, suggesting the possibility of a genetic marker associated with the lower rates of invasive aspergillosis observed in the colonized group. Thus, it appears that patients with the HLA-A1 locus

Table 4. Comparison of clinical and laboratory findings for patients undergoing heart transplantation who have invasive pulmonary aspergillosis (IPA) versus those with invasive extrapulmonary aspergillosis (IEPA).

Variable	IPA (n = 25)	IEPA (n = 31)	P
Age, years, mean	43	43	.9
Sex			
Male	21 (84)	27 (87)	.9
Female	4 (16)	4 (13)	
Underlying diagnosis prior to heart transplantation			
Coronary artery disease	8 (32)	16 (52)	.14
Cardiomyopathy	4 (16)	9 (29)	.25
Congenital disease	2 (8)	0	
Valvular disease	1 (4)	0	
Other	0	6 (19)	
Symptoms			
Fever	13 (52)	6 (19)	.006
Dyspnea	3 (12)	0	
Cough alone	3 (12)	5 (16)	1
Asymptomatic	1 (4)	1 (3)	
Cough plus fever	9 (36)	2 (6)	.01
Diagnostic method			
Culture	25 (100)	31 (100)	
Pathology	21 (84)	6 (19)	<.001
Time to first positive culture result for *Aspergillus* species, days after transplantation, median	46	56	.2
WBC count, cells/μL, mean (range)	7608 (2100–13,900)	8769 (1800–21,688)	.9
Neutrophil count, cells/μL, mean (range)	5574 (966–12,420)	7256 (924–17,888)	.9
Risk factor			
Corticosteroid therapy	22 (88)	30 (97)	.45
Rejection	11 (44)	26 (84)	<.001
CMV coinfection (serology only)	9 (36)	18 (58)	.1
CMV disease (symptomatic)	9 (36)	11 (35)	1
R+/D−	9 (36)	0	
R+/D+	8 (32)	0	
R−/D−	10 (40)	0	
R−/D+	2 (8)	0	
OKT3	12 (48)	24 (77)	.15
Aza	25 (100)	30 (97)	
ICU, days	8	7	
Inhaled amphotericin B prophylaxis after July 1993			
No	21 (84)	29 (94)	.5
Yes	4 (16)	2 (6)	
Treatment			
Amphotericin B	21 (84)	31 (100)	.07
No treatment	4 (16)	0	
Mortality due to *Aspergillus*	9 (36)	11 (35)	.9
Duration of follow-up from time of first positive result of culture for *Aspergillus,* days after transplantation, mean	1291	1308	1

NOTE. Data are no. of patients (%), unless otherwise indicated. Aza, azathioprine; CMV, cytomegalovirus; D+, donor positive for CMV-specific IgG (before transplantation); D−, donor negative for CMV-specific IgG (before transplantation); ICU, intensive care unit; IgG, immunoglobulin G; R+, recipient positive for CMV-specific IgG (before transplantation); R−, recipient negative for CMV-specific IgG (before transplantation).

might have a higher net state of immune activation, which results in both higher rates of rejection episodes and lower frequency of invasive aspergillosis. The precise role of HLA-A1 in responding to antigens of *Aspergillus* species and generating a cytokine response is unknown and should be further addressed to elucidate the immunology of this disease. This finding could suggest that cellular immune responses associated with the HLA-A1 locus and likely driven by T helper type 1 (Th1) cells could be necessary to control progression of the disease after the airways have been colonized. The importance of Th1 cell defense has been shown in a murine model of invasive aspergillosis [30]. Of interest, HLA restriction studies in patients with allergic bronchopulmonary aspergillosis have revealed a statistically significant association between HLA-DR2 and HLA-DR5 and allergic bronchopulmonary aspergillosis [31–33].

CMV infection has been reported to be a risk factor for the development of IPA in solid organ transplant and bone marrow transplant recipients [34]. In our study, CMV infection (defined as having CMV-specific IgG but without CMV symptomatic disease) was found to be more frequent in the colonized group than in the IPA group. However, the incidence of symptomatic CMV disease was the same in both groups, further supporting our hypothesis that the patients colonized with *Aspergillus* species appear to have some degree of genetic resistance against invasive disease caused by opportunistic pathogens.

Although it has been suggested that the incidence of invasive aspergillosis has been significantly reduced in patients with heart transplantation receiving prophylactic inhaled amphotericin B [8, 14], limited success has been observed in patients with invasive disease associated with prolonged neutropenia [35]. Inhaled amphotericin B prophylaxis was instituted in our transplantation program in July 1993. Most of the patients with IPA received their diagnosis before this date, and they had not received benefit from this prophylactic approach. Only 4 patients with IPA (16%), 2 patients with IEPA (6%), and 2 colonized patients (8%) received inhaled amphotericin B prophylaxis after this date. Since the introduction of amphotericin B prophylaxis, the overall incidence of aspergillosis fell to <5%.

Although the incidence of IPA has decreased since the introduction of inhaled amphotericin B, the mortality due to IPA has increased from 28% (before July 1993) to 75%. These findings raise the possibility of invasive disease caused by resistant *Aspergillus* strains selected by the widespread use of inhaled amphotericin B or that prophylactic amphotericin B does not prevent the more aggressive forms of invasive aspergillosis. Future studies should address the possibility that exposure of the fungus to inhaled deoxycholate amphotericin B might raise the amphotericin B MIC for *Aspergillus* and therefore increase treatment failures and case fatality rates.

Despite the lack of statistical significance in mortality, we recommend routine testing for amphotericin susceptibility of isolates from patients who have received amphotericin B prophylaxis and consideration of initial combination therapy with newer agents (i.e., voriconazole plus caspofungin) or higher-dose amphotericin therapy, as may be achieved with lipid-complexed amphotericin preparations, as initial and primary therapy for IPA and IEPA.

Since the introduction of cardiac transplantation as a therapeutic modality for end-stage congestive heart failure in 1968, *Aspergillus* has been recognized as a major opportunistic pathogen in the posttransplantation period [1]. During the past 30 years, major changes have been introduced in immunosuppressive protocols, management of rejection, and treatment and prophylaxis of opportunistic infections. After the introduction of cyclosporine in 1980, there have been 5 different immunosuppressive protocols. The majority of patients who had undergone heart transplantation received prednisone, azathioprine, and cyclosporine as part of their regimen. Other immunosuppressive (OKT3 in June 1987, mycophenolate mofetil–tacrolimus in February 1994) and prophylactic drugs (ganciclovir in January 1987, inhaled amphotericin B in July 1993) were introduced. It is very likely that these changes in immunosuppressive and antimicrobial prophylactic regimens exerted an impact on the incidence of invasive aspergillosis. Before the introduction of cyclosporine, the overall incidence and mortality of IPA was reported to be as high as 27% and 60%, respectively [1]; more recently its overall incidence and mortality were reported to be 8% and 36%, respectively [8].

At Stanford University, the median onset after transplantation for invasive aspergillosis was significantly delayed (from 23 to 52 days) by the introduction of prophylactic ganciclovir in 1987 [8]. It has been proposed that the delay in median onset after transplantation for CMV disease observed after the introduction of ganciclovir contributed to the delay in onset observed for invasive aspergillosis [8]. In an attempt to prevent disease caused by *Aspergillus* species, administration of inhaled amphotericin B was instituted in July 1993 as a routine prophylactic measure. Both Reichenspurner et al. [14] and Montoya et al. [8] have shown that actuarial incidence and the linearized rate of invasive aspergillosis (including IPA) at Stanford University have been significantly reduced among patients receiving aerosolized amphotericin B prophylaxis after heart, lung, and heart-lung transplantation [8, 32]. The role of aerosolized amphotericin B in this decrease may have been partially obscured by the fact that construction of a parking lot at Stanford Hospital was finished at a date close to the time at which therapy with inhaled amphotericin B was instituted [8].

In summary, morbidity and mortality due to IPA have steadily declined for the past 20 years because of the introduction of cyclosporine and probably of inhaled amphotericin B. In addition, during this period, the mean onset after transplan-

tation for IPA has been delayed to a time when infection would occur in patients with lesser degrees of immunosuppression. Patients with IPA usually present with respiratory symptoms and fever within the first 90 days after transplantation, they are not neutropenic, and the most common radiological presentations include single or multiple pulmonary nodules. Cavitation or the halo sign in the nodules are rare in patients with IPA. It appears that patients who present with positive results of culture of respiratory secretion specimens for *Aspergillus* species after day 90 and who have the HLA-A1 locus have a very low probability of developing IPA. Although the incidence of invasive aspergillosis appears to have decreased after the introduction of aerosolized amphotericin B, the mortality from this disease seems to have increased for patients who have been exposed to and given prophylaxis with aerosolized amphotericin B. The advent of 2 new antifungals with significant activity against *Aspergillus* species, voriconazole [36] and caspofungin [37, 38], may improve the prospects of our patients with IPA.

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