ELSEVIER

The Journal of Heart and Lung Transplantation

http://www.jhltonline.org

Invasive aspergillosis among heart transplant recipients: A 24-year perspective

Patricia Muñoz, MD, PhD,[a,b,c,d] Ines Cerón, MD,[a,b] Maricela Valerio, MD,[a,b] Jesús Palomo, MD,[e] Adolfo Villa, MD, PhD,[e] Alia Eworo, MD,[a,b] Juan Fernández-Yáñez, MD,[e] Jesús Guinea, MD, PhD,[a,b,c,d] and Emilio Bouza, MD, PhD,[a,b,c,d]

From the [a]Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Madrid; [b]Department of Medicine, Facultad de Medicina, Universidad Complutense, Madrid; [c]CIBER de Enfermedades Respiratorias (CIBERES CD06/06/0058), Palma de Mallorca; [d]Red Española para la Investigación en Patología Infecciosa (RD06/0008/1025), Madrid; and the [e]Cardiology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain.

KEYWORDS:
invasive aspergillosis;
heart transplantation;
anti-fungal prophylaxis;
Aspergillus spp

BACKGROUND: Invasive aspergillosis is a well-known complication in severely immunosuppressed patients, including heart transplant recipients, and associated mortality is high. Despite the severity of the disease in this population, few recent series with secular trends have addressed the problem.
METHODS: We performed a descriptive study of 479 consecutive heart transplant recipients from 1988 to 2011 in a single institution.
RESULTS: Overall invasive aspergillosis incidence in heart transplant recipients was 6.5% (31 of 479). Incidence decreased from 8.7% (24 of 277) in the period 1988 to 2000 (historical cohort) to 3.5% (7 of 202) afterward ($p = 0.02$); 4 of the 7 cases were in the context of an outbreak. The most common presentation was lung infection, but episodes occurring >3 months after transplantation (late aspergillosis) showed a higher frequency of disseminated disease and involvement of the central nervous system and of atypical sites compared with early (first 3 months) episodes. Related mortality was 36%, with a significant decrease between the historical cohort and the present cohort: 46% vs 0% ($p = 0.04$) and a trend toward lower related death in early vs late cases (26% vs 63%, $p = 0.09$).
CONCLUSIONS: In our series, both incidence and mortality associated with invasive aspergillosis in heart transplant recipients showed a decrease in recent years. Careful environmental management and targeted anti-fungal prophylaxis may minimize the incidence of invasive aspergillosis in this setting.
J Heart Lung Transplant 2014;33:278–288

Invasive aspergillosis (IA) is a well-known complication of severe immunosuppression. IA is reported in 1.6% to 14% of heart transplant recipients[1–7] and is associated with high morbidity and mortality.[8] Information regarding IA in this population is scarce, mainly single case reports or small series collected over short periods. Most data are extrapolated from general series of fungal infections in all types of solid-organ transplant (SOT) recipients,[2,3,9–11] and data from very recent series do not allow us to assess secular trends in heart transplant recipients (Table 1). During the last 12 years, the most important improvements in the management of these patients include more effective immunosuppressive therapy, improved prophylaxis and greater protection against environmental exposure.

The aim of this large, descriptive study was to determine the incidence, epidemiologic characteristics, clinical and radiologic manifestations and outcome among heart transplant recipients with IA. We describe insights into current clinical presentation and diagnostic practices and the characteristics associated with aspergillosis mortality in heart transplant recipients.

Reprint requests: Patricia Muñoz MD, PhD, Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón, Doctor Esquerdo 46, 28007 Madrid, Spain. Telephone: +34-91-5866725. Fax: +34-91-5044906.
E-mail address: pmunoz@micro.hggm.es

1053-2498/$ - see front matter
http://dx.doi.org/10.1016/j.healun.2013.11.003

Table 1 Studies of Invasive Aspergillosis in Heart Transplant Recipients in the Literature

Investigators (year)	No. of patients	Design	Period of recruitment	Incidence of IA	Anti-fungal prophylaxis	Presentation	Early IA	Simultaneous CMV disease	Overall/ related death
Grossi et al[3] (2000)	30	Multicenter retrospective	1985–1997	1.6%	NS	IPA 93.4%, IEPA 6.6%	NS	NS	NS
Montoya et al[6] (2003)	56	Prospective follow-up	1980–1998	6.6%	10.7% (inhaled amphotericin B)	IPA 44.6%, IEPA 55.4%	IPA 72%, IEPA NS	35.7 %	NS/35.7%
Gavalda et al[19] (2005)	47	Multicenter retrospective	1990–2001	2.4%	NS	IPA 63.8%, disseminated 36.2%	68%	NS	65.9%/NS
Pappas et al[20] (2010)	23	Prospective surveillance	2001–2006	2%	NS	NS	NS	NS	NS
Shields et al[21] (2012)	8	Retrospective	2000–2011	1.7%	None	IPA 100%, disseminated 12.5%, sinus 12.5%, septic shock 75%	62.5%	50 %	NS/87.5%
Present report	31	Prospective follow-up	1988–2011	6.5%	25.8%	IPA 64.5%, disseminated 25.8%, IEPA 9.7%	74.2%	48.4 %	58.1%/32.3%

CMV, cytomegalovirus; IA, invasive aspergillosis; IEPA, invasive extrapulmonary aspergillosis; IPA, invasive pulmonary aspergillosis; NS, none specified.

Methods

Design

In this study we describe all heart transplant patients during the period from August 1988 to August 2011 in a single institution. As our study period comprised 24 years, we divided the population into two 12-year periods, pre-2000 (historical cohort) and post-2000 (present cohort). One patient was lost after 7 years and 1 was transferred to another center. Demographic and clinical variables were recorded on a structured, pre-coded data form. The local ethics committee approved the investigation. Specific informed consent was not considered necessary by the ethics committee.

Immunosuppression and anti-microbial prophylaxis

The general methodology of the transplant procedure was standard and has been described elsewhere.[1,12,13] We recorded the immunosuppressive therapy that each patient was receiving during their IA episode. Before transplantation, patients received the pneumococcal and influenza vaccine; seronegative patients also received the hepatitis B vaccine. After transplantation patients received prophylaxis with trimethoprim and sulfamethoxazole during the first year, against tuberculosis when indicated,[13] and against cytomegalovirus (CMV). Patients at risk of primary infection received hyperimmune gammaglobulin (CMVIg) plus intravenous ganciclovir for 15 days and then oral ganciclovir or valganciclovir for 3 months. Since June 1999, a 14-day regimen of universal prophylaxis with ganciclovir or valganciclovir has also been administered to seropositive recipients.

Anti-fungal prophylaxis

Three sequential anti-fungal prophylaxis strategies were used during the study period. Until 1994, no anti-fungal prophylaxis was administered. From 1994 to 2000, we administered universal prophylaxis with oral itraconazole, as previously described.[14,15] From 2001, based on previous data,[7] targeted anti-fungal prophylaxis was only administered to patients with at least one of the following: CMV disease; hemodialysis; re-operation; and a further case of IA within 2 months of the transplantation date. The anti-fungal agents were maintained for approximately 20 days after resolution of the risk factors for IA.[14]

Disease definitions

Episodes of IA were defined according to the criteria of the European Organization for Research and Treatment of Cancer/ Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG).[16] Only proven and probable cases were included. Proven IA required demonstration of fungal elements in diseased tissue using histopathologic, cytologic or microscopic examination of a specimen obtained by needle aspiration or biopsy or using culture of a specimen obtained with a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process. Probable infections were those with the presence of at least one predisposing host factor [transplant recipients], one clinical criterion and one mycologic criterion, such as evidence of infection by direct

test (cytology, microscopy or culture from non-sterile samples) or indirect test (galactomannan antigen). Episodes occurring during the first 3 months after transplantation were considered early IA.

Specimens were obtained for culture when clinically indicated. *Aspergillus* sp was identified following standard guidelines. Detection of galactomannan in serum and bronchoalveolar lavage was used from 2000. In 2006, *Aspergillus*-specific polymerase chain reaction (PCR) was also implemented, according to methodology proposed by Loeffler et al.[17]

Definitions of CMV infection and CMV disease were standard.[18] No specific threshold for antigenemia or PCR was required. CMV disease was defined as the detection of signs or symptoms attributable to this microorganism and included viral syndrome and CMV focal disease.

Statistical analysis

In the univariate analysis, Fisher's exact test was used for categorical variables and the Mann–Whitney test for non-normally distributed continuous variables, and the *t* test to compare the means of normally distributed continuous variables. Backward stepwise logistic model was used in the multivariate analysis. Variables with $p < 0.1$ in the univariate analysis were included in the multivariate model. Differences were considered significant at $p < 0.05$. Statistical analysis was performed with SPSS, version 17.0 (SPSS, Inc., Chicago, IL).

Results

During the 24-year study period of study (1988 to 2011), IA was diagnosed in 6.5% (31 of 479) of heart transplant recipients (Figure 1), among whom 87% (27) had proven IA and 13% (4) probable IA. A substantial decrease in the incidence of aspergillosis was recorded between the historical cohort and the present cohort: pre-2000, 8.7% (24 of 277) of cases; and post-2000, 3.5% (7 of 202) of cases ($p = 0.02$). IA occurring during the first 3 months after transplantation (early IA) accounted for 23 cases (median 35 [19 to 88] days after transplantation); in the remaining 8 cases, IA occurred a median of 125.5 (91 to 301) days after transplantation (late IA), with no differences between the historical cohort and the present cohort. The proportion of early episodes in the historical cohort and present cohort was 71% (17 of 24) and 86% (6 of 7) ($p = 0.64$), respectively.

The baseline clinical characteristics and the peri- and post-operative variables are summarized in Table 2. Significant differences were recorded in the immunosuppression regimens used between the historical cohort and the present cohort, with greater use of anti-thymocyte globulin, cyclosporine and azathioprine before 2000. Also remarkable is the higher incidence of CMV infection in the 3 months before the IA episode in the historical cohort (71% vs 14%, $p = 0.01$).

No significant differences were observed between early IA and late IA episodes, with the exception of a trend toward greater frequency of CMV infection (48% vs 88%, $p = 0.09$) (Table 3) among late episodes.

As for clinical presentation, 94% (29) of episodes of IA involved the lung: 20 had only lung infection; 5 had disseminated infection with central nervous system (CNS) involvement; and 4 had disseminated infection without CNS involvement. The 2 remaining patients had only extrapulmonary involvement (prostate 1, mediastinum 1). Table 3 summarizes the main clinical and radiologic features of early and late episodes. Late episodes involved more atypical sites of infection (skin, prostate, paranasal sinuses, gastrointestinal tract) and a trend toward greater frequency of disseminated disease with CNS involvement. Clinical manifestations were more severe in late infections with a higher frequency of neurologic symptoms and a trend toward higher mortality.

Chest radiologic findings were diverse and had more than one pattern in many episodes; the most common finding was

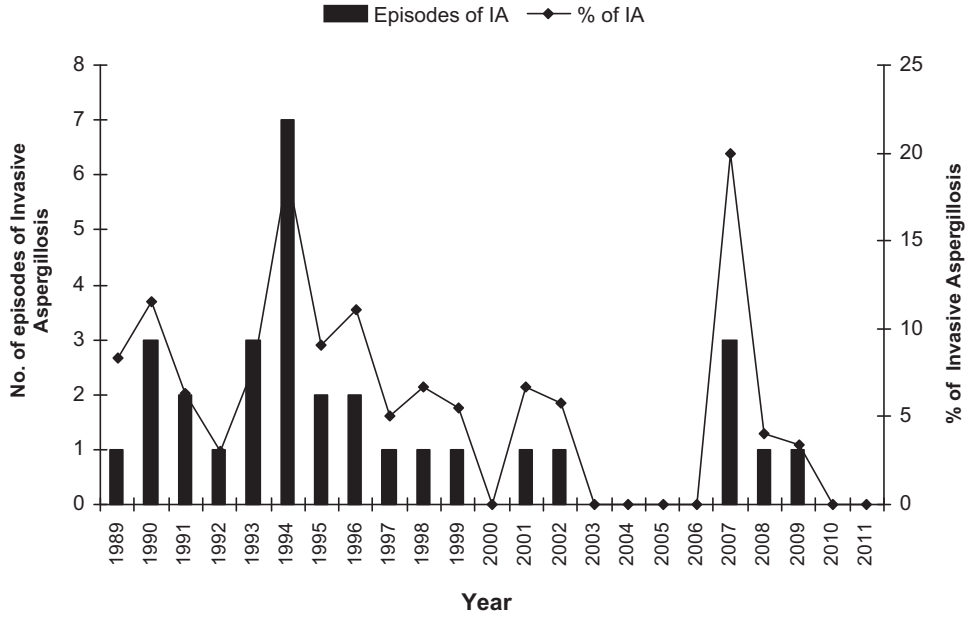

Figure 1 Annual distribution of invasive aspergillosis cases in heart transplant recipients.

Table 2 Comparison of Clinical Characteristics and Peri- and Post-operative Variables of Heart Transplant Recipients With Invasive Aspergillosis in Two Time Periods[a]

Variable	Historical cohort, 1988–1999 ($n = 24$)	Present cohort, 2000–2011 ($n = 7$)	p
Age, years (mean ± SD)	53 ± 8.2	57.1 ± 5	0.22
Male gender	21 (87.5)	5 (71.4)	0.56
Reason for transplantation			
Ischemic	15 (62.5)	6 (85.7)	0.37
Valvular	5 (20.8)	1 (14.3)	1
Dilated cardiomyopathy	4 (16.7)	0 (0)	0.54
New York Heart Association classification			
III	16 (66.7)	2 (28.6)	0.09
IV	7 (29.2)	5 (71.4)	0.07
Previous heart surgery	9 (37.5)	6 (85.7)	0.03
Other comorbidities			
Diabetes before transplantation	9 (37.5)	1 (14.3)	0.37
COPD	1 (4.2)	1 (14.3)	0.40
Renal insufficiency before transplantation	2 (8.4)	0 (0)	1
Hepatic disease	2 (8.4)	0 (0)	1
Modified Charlson score [median (range)]	3 (1–7)	4 (2–5)	0.91
Respiratory colonization by *Aspergillus* spp[b]	1 (4.2)	1 (14.3)	0.40
Peri-operative variables			
Pre-transplantation hospital stay, days [median (range)]	29.5 (0–117)	9 (3–29)	0.21
Pre-transplantation MV	4 (16.7)	4 (57.1)	0.05
Days on pre-transplantation MV [median (range)]	5.5 (1–19)	9 (1–19)	0.42
CMV mismatch	3 (12.5)	1 (14.3)	1
Days on the waiting list [median (range)]	20 (0–125)	2 (1–24)	0.11
Emergency transplantation	6 (25)	6 (85.7)	<0.01
Time of ischemia (range)	189 (60–240)	170 (127–250)	0.31
Post-operative variables			
Days in ICU [median (range)]	8.5 (1–55)	11 (8–80)	0.34
Days on post-transplantation MV [median (range)]	1 (1–41)	9 (1–66)	0.07
Re-operation	10 (41.7)	2 (28.6)	0.67
Induction immunosuppression			
OKT3	3 (12.5)	0 (0)	1
ATG	19 (79.2)	1 (14.3)	<0.01
Daclizumab	1 (4.2)	6 (85.7)	<0.01
Immunosuppressive agents			
Cyclosporine	23 (95.8)	2 (28.6)	<0.01
Azathioprine	23 (95.8)	1 (14.3)	<0.01
Tacrolimus	0 (0)	4 (57.1)	<0.01
Mycophenolate mofetil	2 (8.4)	5 (71.4)	<0.01
Hemodialysis after transplantation	3 (10.2)	3 (42.9)	0.11
Diabetes after transplantation	12 (50)	2 (28.6)	0.41
Other cases of IA in the program[c]	15 (62.5)	3 (42.9)	0.41
Rejection episode in the 3 months before the diagnosis of IA	11 (45.8)	1 (14.3)	0.2
Number of rejection episodes (previous 3 months) [median (range)]	3 (1–3)	1	0.43
Neutrophil count <500 cells/μl	2 (8.3)	0 (0)	1
Bacteremia in the 3 months before the IA episode	6 (25)	0 (0)	0.29
CMV infection in the 3 months before the IA episode	17 (70.8)	1 (14.3)	0.01
Other simultaneous invasive fungal infection[d]	1 (4.2)	0 (0)	1
Anti-fungal prophylaxis	7 (29.1)	2 (28.6)	1
With itraconazole	7 (100)	1 (50)	0.61
With caspofungin	0 (0)	1 (50)	0.22

ATG anti-thymocyte globulin; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; IA, invasive aspergillosis; ICU, intensive care unit; MV mechanical ventilation.

[a]Values are shown as number (%) unless otherwise indicated.

[b]In the 6 months prior to transplantation.

[c]Two months before or after date of transplantation.

[d]One with mucormycosis.

presence of nodules (65%). Late episodes had a higher frequency of cavitated nodules (32% vs 86%, $p = 0.04$) than early episodes. Both lungs were affected in 76% of the IA episodes. With regard to the 17 heart transplant recipients with IA who simultaneously underwent chest X-ray and computed tomography (CT), findings were similar for both techniques in 7 patients (41%); the chest CT provided significant additional information in 7 patients (41%) (mainly greater spread of the disease); and, more importantly, only the chest CT showed abnormalities (normal chest X-ray) in the remaining 3 patients (18%).

The diagnosis of CNS involvement was made using CT in 3 patients (hemorrhagic infarction 1, multiple brain abscesses 1, multiple hypodense cerebral lesions 1) and clinically in 2 cases. The diagnosis of 1 of the patients was confirmed by autopsy. Table 4 summarizes the clinical presentation, diagnosis and outcome of the 31 patients with IA.

The most common species isolated was *Aspergillus fumigatus* (71%). Two patients had 2 simultaneous fungal species and, in 7 cases, it was not possible to identify the *Aspergillus* species (in 4 because the diagnosis was made at autopsy and in 3 because species data were not available). In 9 cases (29%), the infection was polymicrobial, with other classes of microorganisms, including fungus (*Mucorales* in 1 case) CMV (5 cases) and different species of bacteria (5 cases).

Table 3 Clinical Characteristics of Invasive Aspergillosis in Heart Transplant Recipients: Early (First 3 Months Post-transplantation) and Late (>3 Months Post-transplantation) Episodes Compared[a]

Variable	Early episodes ($n = 23$)	Late episodes ($n = 8$)	p
Risk factors			
Hemodialysis after transplantation	6 (26.1)	0 (0)	0.29
Other cases of IA in the program[b]	13 (56.5)	5 (62.5)	1
Rejection episode in the previous 3 months	7 (30.4)	5 (62.5)	0.09
Median number of rejections episode (range)	1.5 (1–3)	3 (1–3)	0.2
CMV infection in the previous 3 months	11 (47.8)	7 (87.5)	0.09
Site of disease			
Lung	22 (95.7)	7 (87.5)	0.45
Central nervous system	2 (8.7)	3 (37.5)	0.09
Myocardium	2 (8.7)	0 (0)	1
Mediastinum	2 (8.7)	0 (0)	1
Other sites[c]	2 (8.7)	4 (50)	0.02
Symptoms			
Asymptomatic	5 (21.7)	1 (12.5)	1
Fever	10 (43.5)	5 (62.5)	0.43
Dyspnea	7 (30.4)	5 (62.5)	0.20
Cough	6 (26)	4 (50)	0.38
Expectoration	4 (17)	1 (12.5)	1
Chest pain	5 (21.7)	1 (12.5)	1
Neurologic symptoms[d]	1 (4.3)	3 (37.5)	0.04
Findings in chest radiology[e]			
Without alterations	1 (4.5)	0 (0)	1
Nodules	5 (22.7)	1 (14.3)	1
Nodules number [median (range)]	2 (1–5)	4 (1–5)	0.45
Cavitated nodules	7 (31.8)	6 (85.7)	0.04
Alveolar infiltrate	6 (27.3)	2 (28.6)	1
Pleural fluid	11 (50)	1 (14.3)	0.10
Halo sign	1 (4.5)	0 (0)	1
Mass	1 (4.5)	0 (0)	1
Extension of pulmonary involvement			
Bilateral	16 (72.7)	6 (85.7)	1
Systemic involvement			
Need for mechanical ventilation	11 (47.8)	4 (50)	1
Renal failure	9 (39.1)	1 (12.5)	0.22
Hepatic failure	2 (8.7)	1 (12.5)	1
Anemia	9 (39.1)	2 (25)	0.67
Thrombocytopenia	13 (56.5)	2 (25)	0.21
Mortality	12 (52.2)	5 (62.5)	0.7
Related mortality	6 (26%)	5 (63%)	0.09

[a]Values are shown as number (%) unless otherwise indicated.
[b]Two months before or after date of transplantation.
[c]Skin, prostate, digestive tract and paranasal sinuses.
[d]Seizures, quadriparesis, coma and focal neurologic deficit.
[e]Percent was calculated considering only patients with lung involvement in each group: early IA, $n = 22$; and late IA, $n = 7$.

Table 4 Summary of Clinical Presentation, Diagnosis, and Outcome of the 31 Patients with IA After Heart Transplantation

Gender/ age year IA	Time from Tx to IA Dg (days)	Anti-fungal prophylaxis	Symptoms	Extent of involvement	Chest-x ray findings	Diagnostic methods	Co-infection	Treatment	Mortality risk factor	Outcome
M, 54 y	36	None	Fever	Lung	Alveolar infiltrate	Autopsy	CMV, *E aerogenes*	None	CMV, AI	Unrelated death
M, 59 y	91	None	Fever, hemoptysis, coma	Lung CNS	Nodules, alveolar infiltrate	Culture, radiography	CMV	Amph B	CMV, AI	Related death
M, 58	98	None	Fever, cough, dyspnea, coma, focal neurologic deficit	Lung CNS	Cavitated nodules	Culture, radiography	None	Amph B	None	Related death
M, 54 y	80	None	Dyspnea	Lung	Alveolar infiltrate, pleural fluid	Culture, radiography	None	Amph B	CMV, AI	Related death
M, 51 y	158	None	Fever, cough, dyspnea, chest pain, expectoration	Lung	Cavitated nodules	Culture, radiography	None	Amph B Flucytosine	None	Cured
M, 63 y	54	None	Fever, cough, dyspnea, chest pain	Lung	Nodules	Culture, radiography	None	L-AMB	None	Cured
M, 56 y	30	None	Fever, chest pain	Lung	Cavitated nodules, pleural fluid	Culture, histology, radiography	None	Amph B → L-AMB + surgery	CMV	Cured
M, 38 y	32	None	Fever	Lung	Pleural fluid	Autopsy[b]	None	None	CMV	Unrelated death
M, 53 y	36	None	Fever, cough, dyspnea	Lung CNS	Cavitated nodules	Culture, histology, radiography	None	L-AMB + surgery	CMV	Related death
M, 61 y	58	None	Asymptomatic	Lung	No images available	Autopsy	None	None	CMV	Unrelated death
M, 35 y	24	None	Asymptomatic	Lung	Nodules, pleural fluid	Culture, radiography	None	Amph B → L-AMB	CMV	Cured
F, 61 y	86	None	Dyspnea	Lung	Alveolar infiltrate, pleural fluid	Culture	None	Amph-B→L-AMB + flucytosine	CMV, AI	Related death
M, 48 y	73	None	Cough	Lung	Cavitated nodules, pleural fluid	Culture, histology, radiography	None	Amph-B→L-AMB + surgery	CMV	Unrelated death
M, 52 y	132	None	Asymptomatic	Lung	Cavitated nodules	Culture, radiography	None	L-AMB	None	Cured
M, 51 y	34	None	Fever, cough, dyspnea, expectoration	Lung	Nodules, pleural fluid	Culture, radiography	*H influenzae*	L-AMB	None	Cured
M, 59 y	16	None	Expectoration	Lung	Alveolar infiltrate, pleural fluid	Culture, radiography	*P aeruginosa*	L-AMB	AI	Related death
M, 56 y	22	None	Cough, expectoration	Lung	Cavitated nodules	Culture, radiography	*Cunninghamella*	L-AMB + surgery	None	Unrelated death

Continued on page 284

Table 4 *(Continued)*

Gender/ age year IA	Time from Tx to IA Dg (days)	Anti-fungal prophylaxis	Symptoms	Extent of involvement	Chest-x ray findings	Diagnostic methods	Co-infection	Treatment	Mortality risk factor	Outcome
M, 56 y	119	Itraconazole	Fever, cough, dyspnea	Lung, paranasal sinuses	Cavitated nodules	Culture, radiography histology	None	L-AMB	CMV	Related death
F, 37 y	80	Itraconazole	Chest pain	Lung	Nodules, cavitated nodules	Culture, radiography	None	L-AMB	None	Unrelated death
M, 51 y	301	Itraconazole	Cough, dyspnea, ulcerative skin lesions	Lung, skin	Cavitated nodules	Culture, autopsy, radiography	None	L-AMB	CMV	Related death
F, 44 y	88	Itraconazole	Fever, dyspnea, seizure, coma	Lung, CNS, paranasal sinuses	Alveolar infiltrate	Culture, radiography	CMV	L-AMB	CMV, AI	Related death
M 47 y	71	Itraconazole	Asymptomatic	Lung, digestive tract, myocardium	Pleural fluid	Autopsy[b]	CMV	None	CMV	Related death
M, 66 y	108	Itraconazole	Fever, dyspnea, seizures	Lung, CNS, digestive tract	Cavitated nodules, pleural fluid, alveolar infiltrate	Culture, histology, radiography	CMV	None[a]	CMV, AI	Related death
M, 62 y	11	Itraconazole	Dyspnea	Lung	Cavitated nodules	Culture, radiography	No	L-AMB	None	Cured
M, 59 y	29	Itraconazole	Cough, chest pain	Lung	Mass, halo sign	Culture, radiography	None	L-AMB	AI	Cured
M, 57 y	18	None	Asymptomatic	Lung	Nodules	Culture radiography	*N otitidis-caviar-um*	Vori	None	Cured
M, 64 y	264	None	Dysuria, urinary urgency, tenesmus	Prostate	Normal	Culture, PCR radiography	None	Vori	None	Cured
M, 51 y	16	Caspofungin	Fever, expectoration	Lung	Pleural fluid, alveolar infiltrate	Culture, PCR radiography	*S. malto-phila*	Vori + caspo	AI	Unrelated death
F, 58 y	4	None	Asymptomatic	Lung	Normal	Culture, PCR, GM	None	Vori → vori + caspo	None	Unrelated death
F, 50 y	49	None	Fever, purulent exudate on wound	Lung, myocardium, mediastinum	Cavitated nodules, pleural fluid	Culture, PCR, histology, radiography, GM	None	Vori + caspo + surgery	CMV	Cured
M, 61 y	40	None	Fever, chest pain, purulent exudate on wound	Mediastinum	Normal	Culture, radiography	None	Caspo + vori → L-AMB + vori + surgery	None	Cured

F female; M male; GM galactomannan; Amph B amphotericin B deoxycholate; L-AMB liposomal amphotericin; vori, voriconazole; caspo, caspofungin; CMV simultaneous cytomegalovirus disease; AI alveolar compromise,
[a]Diagnosis date close to death;
[b]Positive galactomannan in stored serum samples.

Table 5 Efficacy of Methods Used to Diagnose Invasive Aspergillosis

Diagnostic methods	*n*	Overall positive test (%)	Historical cohort positive/ performance test (%)	Present cohort positive/ performance test (%)	*p*
Culture					
Non-invasive samples[a]	37	20 (54.1)	16 of 29 (55.2)	4 of 8 (50)	1
Bronchoalveolar lavage	16	12 (75)	11 of 15 (73.3)	1 of 1 (100)	1
Needle biopsy/aspiration[c]	17	12 (71)	11 of 15 (73.3)	1 of 2 (50)	0.51
Surgical biopsy	3	2[b] (66.7)	1/2 (50)	1 of 1 (100)	1
Pan-*Aspergillus* PCR	4	4 (100)	NA	4 of 4 (100)	
Galactomannan	7	2 (28.6)	NA	2 of 7 (28.6)	
Histology	13	3 (23.1)	3/10 (30)	0/3 (0)	0.52

NA, not available; PCR, polymerase chain reaction.
[a]Sputum, bronchial aspirate, protected brush.
[b]Lung = 1, bone = 1.
[c]Transbronchial = 4, transthoracic = 7, transtracheal = 6, transurethral = 1.

Table 5 summarizes the efficacy of the different diagnostic methods. It is remarkable that, in 9 patients (29%), the diagnosis was possible only after obtaining an invasive sample (bronchoalveolar lavage or biopsy/aspiration). Eight of these patients belonged to the historical cohort. No differences were observed between the early and late IA episodes or between the historical cohort and the present cohort with regard to efficacy of diagnostic methods.

Six patients (19%) underwent surgery, including 3 lung, 2 mediastinum and 1 CNS IA. Five patients did not receive anti-fungal therapy, as the diagnosis was made post-mortem (4 patients) or peri-mortem (1 patient). Initial monotherapy was used in 71% of patients (with amphotericin B deoxycholate or liposomal amphotericin in the historical cohort and with voriconazole in the present cohort). Combined therapy was used in 6 patients (19%) (as a rescue strategy in 2). Itraconazole was used as maintenance therapy in 9 patients during 1989 to 1999 (median 55 [7 to 1,250] days) and voriconazole in 4 patients after 2000 (median 84.5 [14 to 193] days).

Overall mortality was 61% and related mortality was 36%, with a significant reduction in recent years (46% [11 of 24] in the historical cohort and 0% in the present cohort, $p = 0.04$). Related mortality was lower in early episodes (26% [6 of 23]) than in late episodes (63% [5 of 8]) ($p = 0.09$). Candidate variables examined as clinical characteristics associated with mortality are shown in Table 6. Multivariate analysis showed that alveolar infiltrates and CMV disease during the IA episode were independent clinical characteristics associated with mortality.

Discussion

Secular trends in this large series of heart transplant patients with IA show an overall incidence of 6.5% during the 24-year study period, with a significant decrease in incidence in recent years. Up to 26% of episodes occurred >3 months after transplantation. Better immunosuppression, environmental prevention and early anti-fungal therapy are among the reasons for the reduction in IA incidence.

Since 2000, the incidence of IA in heart transplant recipients at our institution has decreased from 8.7% to 3.5%, probably owing to a series of improvements, including implementation of improved anti-fungal prophylaxis

Table 6 Risk Factors for Mortality Due to Invasive Aspergillosis After Heart Transplantation

Variable	Dead ($n = 19$)	Alive ($n = 12$)	*p* (univariate analysis)	OR (95% CI) multivariate analysis	*p*
Male gender	78.9%	91.7%	0.62		
Age, mean (SD)	53.2 (7.7)	55.1 (8)	0.52		
Need of hospital admission before the Tx	79%	75%	1		
Mean days of hospital stay before Tx (SD)	48.4 (30.1)	19.4 (8.8)	<0.01		
Need for pre-Tx MV	15.8%	41.7%	0.2		
Emergency transplantation	26.3%	58.3%	0.13		
OKT3 induction	16.7%	0%	0.25		
Need for hemodialysis after transplantation	26.3%	8.3%	0.36		
Simultaneous bacterial infection	68.4%	33.3%	0.07		
Simultaneous CMV disease	68.4%	25%	0.02	2.76 (1.00-7.59)	0.04
CNS involvement	26.3%	0%	0.12		
Alveolar involvement	42.1%	8.3%	0.04	4.44 (1.52-12.97)	<0.01
Anti-fungals other than voriconazole	89.5%	66.7%	0.17		

CI, confidence interval; CMV, cytomegalovirus; CNS, central nervous system; MV, mechanical ventilation; OR, odds ratio; SD, standard deviation; Tx, transplantation.

programs. Other series described an incidence of IA ranging from 1.6% to 6.6%.[3,6,19–21] Since we began to prescribe targeted prophylaxis with echinocandins or voriconazole in the at-risk population, we had only 6 cases of IA, all but 1 related, in one way or another, to breaks in our protocol. One patient had very late prostatic aspergillosis, with no indication for prophylaxis, which resolved with anti-fungal therapy.[22] Another case involved underdosing of prophylactic caspofungin in a patient with liver insufficiency, who developed pulmonary aspergillosis on Day 16 after transplantation and died with bacterial ventilator-associated pneumonia. The remaining 4 cases occurred in the context of an outbreak of IA in the major heart surgery unit linked to peaks of abnormally high *A fumigatus* airborne conidia levels; the outbreak was due to a breakdown in the air-conditioning system and has been reported in detail.[23,24] In summary, 7 patients (3 heart transplant recipients) developed IA in the major heart surgery intensive care unit (6 IPA, 2 mediastinitis). During the outbreak period, the occurrence of the cases was linked to peaks of abnormally high airborne conidia levels in the unit. Matches between *A fumigatus* genotypes collected from the air and from representative clinical samples were found in 3 of the 6 patients with *A fumigatus*. The outbreak abated when high-efficiency particulate air filters were installed in the affected areas and no other case of IA in heart transplant recipients has been diagnosed in recent years.

Of particular interest is the relatively high proportion of episodes occurring >90 days after transplantation (late episodes), with risk factors different from those of the early episodes.[3,6,21] Consistent with the risk factors for late IA described by Gavalda et al,[19] age was the main risk factor in our series, as all patients were >50 years of age.

We have shown that imaging-based diagnostic and microbiologic results of IA obtained in patients with hematologic malignancy cannot be applied systematically to heart transplant recipients.[25,26] Non-specific radiographic findings were found in 69% of patients with lung involvement. Park et al[27] observed that SOT recipients were more likely to show peri-bronchial consolidation or ground-glass opacity and less likely to have macro-nodules, mass-like consolidation, halo signs and air-crescent signs.

The isolation of *A fumigatus* from any type of respiratory tract sample in a heart transplant recipient is highly suggestive of IA. During a 10-year study period, *Aspergillus* spp were recovered from 10.5% of heart transplant recipients and had a positive predictive value (PPV) of 60% to 70% for IPA. However, when the species was *A fumigatus* the PPV increased to 78% to 91%, and when the respiratory sample was different from sputum to 88% to 100%.[1]

Since 2000, we have been using serum galactomannan for microbiologic diagnosis, with low effectiveness (28.6%); this technique has proven useful in patients with hematologic malignancies and profound immunosuppression.[28–30] Some studies in SOT recipients showed low sensitivity and specificity.[31–34] These findings could explain the low diagnostic effectiveness observed in our population (28.6%). Pan-*Aspergillus* PCR in respiratory or tissue samples is a very promising tool for the diagnosis of IA.[35–39] The results are better when the technique is performed on bronchoalveolar lavage specimens, with sensitivities and specificities of pan-*Aspergillus* PCR reaching 100% (95% CI 79% to 100%) and 88% (95% CI 79% to 92%), respectively.[40,41] *Aspergillus* PCR showed a positive result in the 4 patients studied who had invasive aspergillosis. It should be noted that the specificity of PCR performed on lower respiratory tract samples is moderate, as a positive result only indicates the presence of *Aspergillus*, and does not allow distinguishing between colonization and infection.[42]

Our series also illustrates the change in therapeutic approaches to IA. Since 2003, following the recommendations of the Infectious Disease Society of America,[43] the treatment of choice has been voriconazole, in monotherapy or in combination with caspofungin or liposomal amphotericin, followed by maintenance with oral voriconazole. Combination therapy is not recommended, although some data suggest its utility in high-risk SOT recipients, such as those with renal failure or those infected with *A fumigatus*.[44,45]

Related mortality was 36%, although this figure decreased significantly in the present cohort. We have recorded no deaths attributable to IA since 2000, probably owing to the emergence of more active and safer anti-fungal agents, the availability of more tools that enable an earlier diagnosis, better support and overall management of patients in the intensive care unit, and changes in immunosuppressive therapy. For example, OKT3, which was once associated with an increased risk of life-threatening opportunistic infections, is no longer prescribed.[46–48] Consistent with findings by Montoya et al,[6] all patients with disseminated IA and CNS involvement in our series died. In the recent series by Shields and colleagues, mortality was very high, but 75% of patients presented with concomitant septic shock or multiple-organ failure.[21] In our study, the only clinical characteristic associated with mortality was concomitant CMV infection during IA. The negative influence of CMV disease on the prognosis of IA has also been described in liver recipients[49] and could reflect intensive immunosuppressive therapy or deeper immunosuppression. Previous series indicated CMV co-infection in 25% to 35.7% of IA episodes.[3,6,21]

The main limitations of our study are its descriptive nature, its single-center design, and the necessary assumption of the changes in diagnosis, therapy and prevention to adapt to progress and improvements in patient management during the study period. Finally, there were 2 patients only diagnosed with autopsy, although they occurred simultaneously with a peak of IA in the program.

In conclusion, the incidence of IA in our cohort has decreased in recent years and this trend was accompanied by a significant reduction in IA-related mortality. Transplantation centers must emphasize prevention of IA through correct identification of at-risk patients and optimization of environmental management, especially in the first 3 months after transplantation and in elderly patients. The combination of targeted anti-fungal prophylaxis and optimal environmental management may minimize the incidence of IA cases in heart transplant programs.

Disclosure statement

The authors have no conflicts of interest to disclose.

This study was supported in part by the Red Española de Infección y Trasplante (RESITRA-G03/75), ISCIII, Ministerio de Sanidad y Consumo; Ministerio de Asuntos Exteriores, Spain (REIPI); and the PROMULGA Project, Instituto de Salud Carlos III (PI1002868).

References

1. Muñoz P, Alcala L, Sanchez Conde M, et al. The isolation of *Aspergillus fumigatus* from respiratory tract specimens in heart transplant recipients is highly predictive of invasive aspergillosis. Transplantation 2003;75:326-9.
2. Grossi P, De Maria R, Caroli A, et al. Infections in heart transplant recipients: the experience of the Italian heart transplantation program. J Heart Lung Transplant 1992;11:847-66.
3. Grossi P, Farina C, Fiocchi R, et al. Prevalence and outcome of invasive fungal infections in 1,963 thoracic organ transplant recipients: a multicenter retrospective study. Transplantation 2000;70:112-6.
4. Hofflin JM, Potasman I, Baldwin JC, et al. Infectious complications in heart transplant recipients receiving cyclosporine and corticosteroids. Ann Intern Med 1987;106:209-16.
5. Hummel M, Thalmann U, Jautzke G, et al. Fungal infections following heart transplantation. Mycoses 1992;35:23-34.
6. Montoya JG, Chaparro SV, Celis D, et al. Invasive aspergillosis in the setting of cardiac transplantation. Clin Infect Dis 2003;37(suppl 3): S281-92.
7. Muñoz P, Rodriguez C, Bouza E, et al. Risk factors of invasive aspergillosis after heart transplantation: protective role of oral itraconazole prophylaxis. Am J Transplant 2004;4:636-43.
8. Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. Clin Microbiol Rev 2005;18:44-69.
9. Paya CV. Fungal infections in solid-organ transplantation. Clin Infect Dis 1993;16:677-88.
10. Guillemain R, Lavarde V, Amrein C, et al. Invasive aspergillosis after transplantation. Transplant Proc 1995;27:1307-9.
11. Muñoz P, Torre J, Bouza E, et al. A large multicentric study invasive aspergillosis in transplant recipients. 36th Conference on Antimicrobial Agents and Chemotherapy (New Orleans), Washington, DC: American Society for Microbiology; 1996.
12. Muñoz P, Palomo J, Guembe P, et al. Lung nodular lesions in heart transplant recipients. J Heart Lung Transplant 2000;19:660-7.
13. Muñoz P, Palomo J, Muñoz R, et al. Tuberculosis in heart transplant recipients. Clin Infect Dis 1995;21:398-402.
14. Muñoz P, Valerio M, Palomo J, et al. Targeted antifungal prophylaxis in heart transplant recipients. Transplantation 2013;96:664-9.
15. Muñoz P, Valerio M, Giannella M, et al. Optimal duration of selective antifungal prophylaxis in heart transplant recipients. In: 21st European Congress of Clinical Microbiology and Infectious Diseases, Milan, Italy; 2010.
16. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 2008;46:1813-21.
17. Loeffler J, Henke N, Hebart H, et al. Quantification of fungal DNA by using fluorescence resonance energy transfer and the light cycler system. J Clin Microbiol 2000;38:586-90.
18. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002;34:1094-7.
19. Gavalda J, Len O, San Juan R, et al. Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case-control study. Clin Infect Dis 2005;41:52-9.
20. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis 2010;50:1101-11.
21. Shields RK, Nguyen MH, Shullo MA, et al. Invasive aspergillosis among heart transplant recipients is rare but causes rapid death due to septic shock and multiple organ dysfunction syndrome. Scand J Infect Dis 2012;44:982-6.
22. Valerio M, Fernandez-Cruz A, Fernandez-Yanez J, et al. Prostatic aspergillosis in a heart transplant recipient: case report and review. J Heart Lung Transplant 2009;28:638-46.
23. Guinea J, Garcia de Viedma D, Pelaez T, et al. Molecular epidemiology of *Aspergillus fumigatus*: an in-depth genotypic analysis of isolates involved in an outbreak of invasive aspergillosis. J Clin Microbiol 2011;49:3498-503.
24. Pelaez T, Muñoz P, Guinea J, et al. Outbreak of invasive aspergillosis after major heart surgery caused by spores in the air of the intensive care unit. Clin Infect Dis 2012;54:e24-31.
25. Bergeron A, Porcher R, Sulahian A, et al. The strategy for the diagnosis of invasive pulmonary aspergillosis should depend on both the underlying condition and the leukocyte count of patients with hematologic malignancies. Blood 2012;119:1831-7.
26. Franquet T, Muller NL, Gimenez A, et al. Spectrum of pulmonary aspergillosis: histologic, clinical, and radiologic findings. Radiographics 2001;21:825-37.
27. Park SY, Kim SH, Choi SH, et al. Clinical and radiological features of invasive pulmonary aspergillosis in transplant recipients and neutropenic patients. Transpl Infect Dis 2010;12:309-15.
28. Maertens J, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood 2001;97: 1604-10.
29. Maertens J, Verhaegen J, Demuynck H, et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. J Clin Microbiol 1999;37:3223-8.
30. Ulusakarya A, Chachaty E, Vantelon JM, et al. Surveillance of *Aspergillus* galactomannan antigenemia for invasive aspergillosis by enzyme-linked immunosorbent assay in neutropenic patients treated for hematological malignancies. Hematol J 2000;1:111-6.
31. Husain S, Kwak EJ, Obman A, et al. Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. Am J Transplant 2004;4:796-802.
32. Kwak EJ, Husain S, Obman A, et al. Efficacy of galactomannan antigen in the Platelia *Aspergillus* enzyme immunoassay for diagnosis of invasive aspergillosis in liver transplant recipients. J Clin Microbiol 2004;42:435-8.
33. Fortun J, Martin-Davila P, Alvarez ME, et al. *Aspergillus* antigenemia sandwich-enzyme immunoassay test as a serodiagnostic method for invasive aspergillosis in liver transplant recipients. Transplantation 2001;71:145-9.
34. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis 2006; 42:1417-27.
35. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. J Clin Microbiol 2004;42:2733-41.
36. Costa C, Costa JM, Desterke C, et al. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. J Clin Microbiol 2002;40:2224-7.
37. Loeffler J, Kloepfer K, Hebart H, et al. Polymerase chain reaction detection of *Aspergillus* DNA in experimental models of invasive aspergillosis. J Infect Dis 2002;185:1203-6.
38. Raad I, Hanna H, Sumoza D, et al. Polymerase chain reaction on blood for the diagnosis of invasive pulmonary aspergillosis in cancer patients. Cancer 2002;94:1032-6.

39. Mengoli C, Cruciani M, Barnes RA, et al. Use of PCR for diagnosis of invasive aspergillosis: systematic review and meta-analysis. Lancet Infect Dis 2009;9:89-96.
40. Musher B, Fredricks D, Leisenring W, et al. *Aspergillus* galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. J Clin Microbiol 2004;42:5517-22.
41. Luong ML, Clancy CJ, Vadnerkar A, et al. Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalvelolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. Clin Infect Dis 2011;52:1218-26.
42. Guinea J, Padilla C, Escribano P, et al. Evaluation of MycAssay *Aspergillus* for diagnosis of invasive pulmonary aspergillosis in patients without hematological cancer. PLoS One 2013;8:e61545.
43. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2008;46:327-60.
44. Singh N, Pursell KJ. Combination therapeutic approaches for the management of invasive aspergillosis in organ transplant recipients. Mycoses 2008;51:99-108.
45. Singh N, Limaye AP, Forrest G, et al. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. Transplantation 2006;81:320-6.
46. Segovia J, Rodriguez-Lambert JL, Crespo-Leiro MG, et al. A randomized multicenter comparison of basiliximab and muromonab (OKT3) in heart transplantation: SIMCOR study. Transplantation 2006;81:1542-8.
47. Lindenfeld J, Miller GG, Shakar SF, et al. Drug therapy in the heart transplant recipient: part I: cardiac rejection and immunosuppressive drugs. Circulation 2004;110:3734-40.
48. Lindenfeld J, Miller GG, Shakar SF, et al. Drug therapy in the heart transplant recipient: part II: immunosuppressive drugs. Circulation 2004;110:3858-65.
49. Rosenhagen M, Feldhues R, Schmidt J, et al. A risk profile for invasive aspergillosis in liver transplant recipients. Infection 2009;37:313-9.