American Journal of Transplantation 2004; 4: 636–643
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2004.00390.x

Risk Factors of Invasive Aspergillosis after Heart Transplantation: Protective Role of Oral Itraconazole Prophylaxis

Patricia Muñoz[a,*], Claudia Rodríguez[a], Emilio Bouza[a], Jesús Palomo[b], Juan F. Yañez[b], María Jesús Domínguez[a] and Manuel Desco[c]

Departments Of [a] Clinical Microbiology-Infectious Diseases, [b] Cardiology and [c] Experimental Medicine, Hospital General Universitario 'Gregorio Marañón', Madrid, Spain
**Corresponding author: Patricia Muñoz, pmunoz@micro.hggm.es*

The study was designed to identify a subset of heart transplant (HT) recipients who could benefit from the administration of targeted antifungal prophylaxis and to evaluate the efficacy of oral itraconazole as the preventive drug. We have analyzed the risk factors for invasive aspergillosis (IA) in our entire population of HT recipients (1988–2002) and also the role of oral itraconazole prophylaxis that was provided to all patients since 1995 [400 mg q.d. of itraconazole oral (PO) for 3–6 months]. There were 24 cases of IA. Our main results indicate that the independent risk factors for IA after heart transplantation are: re-operation (RR 5.8; 95% CI 1.8–18, p = 0.002), cytomegalovirus (CMV) disease (RR 5.2; 95% CI 2–13.9, p = 0.001), post-transplant hemodialysis (RR 4.9; 95% CI 1.2–18, p = 0.02), and the existence of an episode of IA in the HT program 2 months before or after the transplantation date (RR 4.6; 95% CI 1.5–14.4, p = 0.007). Itraconazole prophylaxis showed an independent protective value against developing IA (RR 0.2; 95% CI 0.07–0.9, p = 0.03) and also determined a significantly prolonged 1-year survival (RR 0.5; 95% CI 0.3–0.8, p = 0.01). We believe that antifungal prophylaxis in heart transplant patients should be offered at least to patients with one or more of these predisposing conditions.

Key words: Antifungal prophylaxis, cardiac transplantation, CMV disease, early and late outcome analysis, heart transplantation, hemodialysis, invasive aspergillosis, Itraconazole, odds ratio, prophylaxis, risk assessment modeling, risk factors for infection

Received 26 September 2003, revised and accepted for publication 15 December 2003

Introduction

Invasive aspergillosis (IA) is the most common invasive mycosis in heart transplant recipients (HT), affecting 1–14% of these patients (1–9). Early clinical recognition of this complication is difficult and laboratory data are not specific nor sensitive enough (5,10). Accordingly, antifungal therapy is frequently initiated too late and some cases are only diagnosed after death. The mortality rate of IA in this population ranges from 53% to 78% (11,12).

The current lack of evidence-based guidelines on preventive measures against aspergillosis in solid organ transplant recipients and the emergence of resistant fungal pathogens represents an ongoing challenge (13–16). Presently, prophylaxis against *Aspergillus* is recommended in high-risk liver transplant recipients, usually with itraconazole or amphotericin B (17,18). However, this is not a common practice after HT (6), besides, risk factors for aspergillosis have not been clearly defined in HT recipients.

Between 1988 and 1995 no anti-*Aspergillus* prophylaxis was provided in our HT program. Since October 1994 we have used oral itraconazole as a universal prophylaxis for *Aspergillus* in heart transplant recipients (19). The objective of this study is to evaluate the efficacy of this strategy by analyzing the risk factors of aspergillosis in the HT population.

Patients and Methods

Institution and patient population

Our institution is a 1750-bed tertiary teaching hospital serving a population of approximately 650 000 inhabitants. HEPA filtered air is not universally provided in all areas used by the HT program.

In our hospital, 307 patients underwent heart transplantation between August 1988 and December 2002. Two hundred and seventy-eight patients who survived more than 7 days after transplantation have been included in the study [230 men (81.8%); mean age: 53 years (range: 18–69)]. Underlying heart diseases included: ischemic heart disease 140 (50.3%), dilated myocardiopathy 94(34%), valvular diseases 29 (10.5%) and others 15 (congenital 4, myocarditis 1, late re-transplantation 10). Since October 1994 we administered oral itraconazole prophylaxis to all patients who received

heart transplantation at our center (n = 93). No antifungal prophylaxis was administered to the remaining 185 patients.

Immunosuppression and antimicrobial prophylaxis

General methodology of the transplant procedure was standard and has been described elsewhere (5,20,21). Briefly, the immunosuppressive regimen consisted of induction with OKT3 (first 18 patients) or 3–5 doses of antithymocyte globulin (ATG) and maintenance with cyclosporine A (CsA), azathioprine and corticosteroids until 1998. Since then, mycophenolate mophetil and tacrolimus were also used. Doses of cyclosporine were set to achieve a plasma level of 200–400 ng/mL (determined by immuno-assay) during the first month after transplantation. Thereafter, levels of 100–200 ng/mL were targeted. Rejection episodes were diagnosed by endomyocardial biopsy and treated with i.v. boluses of methylprednisolone (250–500 mg/day) for 3 d. Antithymocyte globulin was administered when a steroid refractory acute rejection episode was observed and in patients with severe allograft dysfunction. The number of treated rejection episodes was quantified in each patient before developing *Aspergillus* disease or in patients without aspergillosis in the first 3 months after transplantation.

All patients received the pneumococcal vaccine before transplantation, surgical prophylaxis with cefazolin and a short course of post-surgical gut decontamination with norfloxacin. Cotrimoxazole prophylaxis (double strength, 3 d a week during the first year) was also provided to these patients (22), as well as prophylaxis against tuberculosis when indicated (20). Prophylaxis against cytomegalovirus (CMV) was administered to all patients at risk of primary infection (seronegative recipient and seropositive donor) with hyperimmune gammaglobulin (CMVIG) plus i.v. ganciclovir (5 mg/kg every 12 h for the first 15 d after transplantation). CMVIG was administered according to the following regimen: 150 mg/kg administered within 72 h after transplantation and 100 mg/kg administered at weeks 1, 2, 4, 6, 8 and 12. Since June 1999, universal prophylaxis with i.v. ganciclovir has also been administered to seropositive recipients in the early post-operative period (5 mg/kg b.i.d. during 14 d). No significant structural or environmental changes were performed in the heart transplantation ward during the study period.

Itraconazole prophylaxis

From the 278 patients studied, oral itraconazole was administered in 93 cases (33.4%). No important differences were observed between these two populations. Itraconazole was administered from day five after transplantation at a loading dose of 600 mg/d for the first 3 d and subsequently at 400 mg q.d. oral (PO) for an average period of 110 ± 49 d. Patients without rejection episodes received 3 months of prophylaxis and patients with rejection episodes 6 months. Itraconazole capsules were administered with food and a cola drink to lower the gastric pH and increase absorption. Serum levels were measured by the bioassay method. Median trough level of itraconazole was 2.9 μg/mL (0–11.9).

The dose of CsA was adjusted as previously mentioned. The required dosage of cyclosporine, in order to keep the target CsA level, was reduced by 50% while itraconazole was being administrated [median Cyclosporin A dose during itraconazole therapy: 100 mg/d (50–200), and median CsA dose required by the same population after itraconazole withdrawal: 150 mg/d (75–250)].

Itraconazole was well tolerated and it was only withdrawn in three patients because of a reversible increase in liver enzymes, one with CMV hepatitis and one receiving isoniazid.

There was one case of *Scedosporium prolificans* disease in a patient receiving itraconazole and no cases with invasive *Candida* infection.

Disease definitions

All patients were prospectively followed up by an ID physician who collaborated in the diagnosis and treatment of all invasive aspergillosis episodes and prospectively filled a pre-established data entry sheet recording basal data of the patient and characteristics of every infectious episode. Pre-transplantation active infections were those which were still on treatment when the patient received HT.

Invasive aspergillosis episodes were defined according to the Mycoses Study Group criteria (23). Only definite and probable cases were included. Definite IA required histological evidence of fungal infection either by autopsy or biopsy. Probable infections were those with suggestive clinical and radiological features and culture evidence of *Aspergillus* from a significant sample (BAL, lung biopsy or transthoracic fluoroscopic or computed tomography-guided needle aspiration). Biopsy and autopsy records were also included for our study. For analysis purposes, both definitive and probable IA were grouped.

Only the aspergillosis episodes occurring in the first year after transplantation were included in the study, as late aspergillosis is known to have different risk factors. The two cases which were diagnosed after the first year of transplantation (both occurred in patients with neoplastic disease) were therefore excluded. One was diagnosed on day 533 after HT during the therapy of a central nervous system (CNS) lymphoma and the other on day 2383 in a patient with laryngeal malignancy. This latter case was only diagnosed during the autopsy.

Post-operatively, chest radiographs were routinely performed at least once per week during the first month and then at each follow-up or when clinically indicated.

Specimens for culture were obtained when clinically indicated and no surveillance was routinely performed. Specimens were processed according to standard microbiological recommendations and were stained and cultured for viruses, bacteria, fungi, mycobacteria and parasites. *Aspergillus* spp. was identified according to standard guidelines. All specimens were cultured both in fungal and conventional media. Tubes containing Saboraud-dextrose with chloramphenicol agar and brain–heart infusion agar with antibacterial agents were used as fungal culture media, and sheep-blood and chocolate agar were used as conventional media. Species were identified using culture characteristics and morphology of conidiophores and conidia.

CMV infection and CMV disease definitions were standard (24). CMV infection was based on the isolation or detection of the virus from any body fluids by shell vial assay or anti-genemia. CMV disease consisted of the detection of signs or symptoms attributable to this microorganism and included viral syndrome and CMV focal disease.

All patients were prospectively followed by an ID physician who filled a pre-established data entry sheet. Risk factors for aspergillosis identified in the literature for other types of transplantation recipients were recorded. Hemodialysis included patients requiring renal replacement therapy (hemodialysis or continuous venovenous hemodialysis). We tried to show the potential influence of environmental factors by recording the occurrence of any other episode of IA in the HT program 2 months before or after the transplantation date of each patient. Patients were followed for a median of 5 years (1834 ± 1542 d). No patient was lost and 152 (54.7%) were alive at the end of the follow-up.

Statistic analysis

Pre-operative, operative and post-operative potential risk factors were analyzed for their association with invasive aspergillosis. For univariate

statistics, the chi-square test, Student's *t*-test and Mann–Whitney's *U*-tests were used: the chi-square test was used for categorical variables; Mann–Whitney's *U*-test for continuous variables that were not normally distributed and analysis of variance or the *t*-test to compare means of approximately normal continuous variables. Stepwise logistic and Cox regression models were used in the multivariate analysis to control for potential confounders of risk factors for invasive aspergillosis. The influence of itraconazole prophylaxis on patient survival was assessed by means of Cox regression analysis.

Variables with a p-value < 0.1 in the univariate analysis were included in the multivariate models. Differences were considered to be significant for p-values less than 0.05. Statistical analysis was performed with the SPSSWIN 11 package (SPSS Inc., Chicago, IL, USA).

Results

Invasive aspergillosis was diagnosed in 24/278 patients (8.6%) in the first year after transplantation (a median of 50 ± 63 days after transplantation). All isolates corresponded to *Aspergillus fumigatus*. There were 18 *Aspergillus* pneumonias and 6 disseminated aspergillosis. Six of the patients were in the intensive care unit (ICU).

Patients with and without invasive aspergillosis were compared, and factors for IA found in the univariate analysis are given in Table 1.

Pre-operative and early post-surgical risk factors found were: active infection before HT (11% vs. 16.7%, p = 0.03), COPD (5.5% vs. 12.5%, p = 0.02), high bilirubin level before HT (>2 mg/dL) (40.2% vs. 62.5%, p = 0.03), transfusion after HT (66.5% vs. 87.5%, p = 0.03), prolonged mechanical ventilation (2.9 vs. 5.4 days, p = 0.05), and use of OKT3 in the induction (5.5% vs. 16.7%, p = 0.05). The post-surgical risk factors for IA found in the univariate analysis were: hemodialysis (5.1% vs. 20.8%, p = 0.01), diabetes mellitus (25.6% vs. 50%, p = 0.01), a higher number of bacterial infections in the first 3 months (0.6 vs. 1.1, p = 0.01), viral infection in the first 3 months (46.9% vs. 75%, p = 0.01), CMV disease in the first 3 months (14.6% vs. 45.8%, p = 0.001), and a single case of IA during the HT program in the 2 months before or after transplantation date category (47.2% vs. 75%, p = 0.01). Itraconazole prophylaxis was a protective factor (35.4% vs. 8.3%, p = 0.01).

The multivariate analysis showed that the independent risk factors for invasive aspergillosis after heart transplantation are: re-operation (RR 5.8; 95% CI 1.8–18, p = 0.002), CMV disease (RR 5.2; 95% CI 2–13.9, p = 0.001), post-transplant hemodialysis (RR 4.9; 95% CI 1.2–18, p = 0.02), and the existence of any episode of IA in the HT program 2 months before or after HT date (RR 4.6; 95% CI 1.5–14.4, p = 0.007) (Table 2). Itraconazole prophylaxis had an independent protective value (RR 0.2; 95% CI 0.07–0.9, p = 0.03) (Figure 1). A Cox regression probability analysis showed that 1-year survival was better in patients receiving itraconazole prophylaxis (RR 0.5 95% CI 0.3–0.8, p = 0.01) (Figure 2).

Three patients who received itraconazole prophylaxis developed IA. In two cases itraconazole prophylaxis had been stopped for more than a month and the third case had very low levels of itraconazole, at the time the patient had proton pump inhibitor therapy. Two of these patients were in the ICU.

Discussion

Although the overall incidence of invasive aspergillosis in HT patients has decreased over the past few years, having reached 25% before the introduction of CsA, it is now estimated that the infection may occur in about 5% of HT recipients (11,13). Average mortality of invasive aspergillosis in heart transplant recipients is at least 50% (25) and risk factors in this population have not been properly identified (13).

Our study was designed to identify a subset of heart transplant recipients who could benefit from the administration of targeted antifungal prophylaxis and to evaluate the efficacy of oral itraconazole as a preventive drug. We analyzed the risk factors for IA occurring in the first year after transplantation, as invasive aspergillosis is usually a relatively early complication. In our experience, the episodes were diagnosed as a median of 50 ± 63 d after HT, and in Cisneros' series a median of 36 d (19–139) after HT (1).

Our main results indicate that post-operative hemodialysis, CMV disease, re-operation and other episodes of aspergillosis in the ward close to the transplantation date are major risk factors for IA in this population. The use of oral itraconazole is an effective way of preventing this infection. The only case of aspergillosis occurring in a patient with none of these four risk factors corresponded to a patient who was exposed to a fungal overload while in the ICU after transplantation, due to the malfunctioning of the ventilation system. He was in a single room, so no other cases were detected. Both the environmental and the patient's strains had a similar random amplified polymorphic DNA (RAPD) pattern.

We have found that post-transplant hemodialysis after HT is an independent risk factor for invasive aspergillosis. Renal dysfunction has also been found to be an important risk factor for invasive fungal infection after liver transplantation (26–29). Singh et al. reported that patients requiring hemodialysis had a 14% incidence of IA, and this risk was completely abolished when prophylaxis, with a lipid preparation of amphotericin B, was administered during the time renal replacement therapy was required. However, no impact on the mortality rate was achieved (18). We have reproduced the analysis of Singh's study relating to the impact of antifungal prophylaxis on the survival of patients requiring renal replacement therapy and we replicated her

Table 1: Univariate analysis of factors associated with a higher risk of suffering an episode of invasive aspergillosis in the first year after heart transplantation (Tx)

	No IA (n = 254)	IA (n = 24)	p
Pre-operative factors			
Recipient age (mean ± SD) (years)	53 ± 9.7	55 ± 8.6	0.6
Male	213 (84%)	22 (92%)	0.24
NYHA IV	152 (59.8%)	17 (70.8%)	0.3
Urgent transplantation	49 (19%)	4 (16.7%)	1
ICU before transplantation	36 (14%)	3 (12.5%)	1
Active infection before Tx	28 (11%)	4 (16.7%)	0.03
Prostaglandins use	4 (1.61%)	2 (8.3%)	0.08
Reason for transplantation			0.6
Ischemic	125 (49.2%)	15 (62.5%)	
Hypertrophic	89 (35%)	5 (20.8%)	
Valvular	25 (9.8%)	4 (16.7%)	
Others	15 (%)	–	
Previous heart surgery	101 (39.8%)	10 (41.7%)	0.3
COPD	14 (5.5%)	3 (12.5%)	0.02
Previous smoker	179 (70.5%)	20 (83.3%)	0.2
Diabetes before Tx	16 (6.3%)	2 (8.3%)	0.06
High bilirubin level (>2 mg/dL)	102 (40.2%)	15 (62.5%)	0.03
Renal insufficiency before Tx	35 (13.8%)	4 (16.7%)	0.7
Hemodialysis before Tx	3 (1.2%)	1 (4.2%)	0.3
CMV mismatch	14 (5.5%)	2 (8.3%)	0.6
Pretransplant hospital stay	32 ± 45	30 ± 31	0.8
Days on the waiting list	43 ± 61	28 ± 32	0.5
Peri-operative factors			
Extracorporeal circulation time (min)	112 ± 32.6	112 ± 36	0.9
Time of ischemia	175 ± 61	173 ± 58	0.8
Transfusion after TX	169 (66.5%)	21 (87.5%)	0.03
Days in the ICU (d)	9 ± 8.7	16 ± 16	0.5
Days on mechanical ventilation	2.9 ± 6	5.4 ± 8	0.05
Re-operation*	25 (9.8%)	7 (29.2%)	0.01
Re-transplantation	17 (6.7%)	–	0.2
Post-operative factors			
Days of induction therapy	4.2 ± 2.6	4.5 ± 3.3	0.6
OKT3	14 (5.5%)	4 (16.7%)	0.05
ATG	209 (82%)	17 (74%)	0.4
Monoclonal antibodies	7 (3%)	–	
Immunosuppressive agents			
Cyclosporine A	227 (89%)	22 (96%)	0.5
Azathioprine	186 (73%)	20 (87%)	0.2
Tacrolimus	24 (9.4%)	1 (4.3%)	0.7
Mycophenolate	68 (27%)	3 (13%)	0.2
Hypogammaglobulinemia	6 (2.4%)	1 (4.2%)	0.4
Renal insufficiency after Tx	73 (28.7%)	10 (41.7%)	0.2
Hemodialysis after Tx	13 (5.1%)	5 (20.8%)	0.01
Diabetes after TX	65 (25.6%)	12 (50%)	0.01
Rejection episode in the first 3 months	104 (40.9%)	5 (20.8%)	0.07
No. of rejectionsin the first 3 months (mean ± SD)	1.26 ± 0.9	2 ± 1	0.08
No. of bacterial infections in the first 3 months	0.6 ± 0.9	1.1 ± 0.8	<0.01
Any viral infection (first 3 months)	119 (46.9%)	18 (75%)	0.01
Herpes infection	51 (20.1%)	7 (29.2%)	0.2
Asymptomatic CMV infection	36 (14.2%)	1 (4.2%)	0.2
CMV disease†	37 (14.6%)	11 (45.8%)	0.001
Lung	12 (4.7%)	5 (21.7%)	0.01
GI tract	5 (2%)	6 (26%)	0.01
CMV syndrome	24 (9.4%)	4 (17.4%)	0.2
Allograft dysfunction	53 (21%)	3 (13%)	0.3

Table 1: Continued.

	No IA (n = 254)	IA (n = 24)	p
Other case of invasive aspergillosis‡	119 (47.2%)	18 (75%)	0.01
Received itraconazole prophylaxis	90 (35.4%)	3 (8.3%)	0.01
Ongoing itraconazole prophylaxis	0	2	
Follow-up (months)	63.4 ± 51	35.6 ± 51	0.01
Mortality	110 (43.3%)	16 (66.7%)	0.03

TX: transplantation.
*The operations consisted of: *Aspergillus* group (7): three surgical wound drainages, two pericardial bleeding, 1 gastric bleeding suture and 1 intra-abdominal abscess drainage. Non-*Aspergillus* group (25): 14 pericardial or mediastinal bleeding, five re-transplantations, four surgical wound drainages, three ventricular assistance devices implantation, one surgical removal of a biopsy lead. Two patients required two separated procedures.
†Patients may have more than one clinical manifestation of CMV disease.
‡Other case of IA in the HT program 2 months before or after transplantation date.

Table 2: Multivariate analysis (regression logistic model) of independent risk factors for invasive episode in the first year after a heart transplantation (Tx)

	RR	95% CI	p
Re-operation	5.8	1.8–18	0.002
CMV disease	5.2	2–13.9	0.001
Post-transplant hemodyalisis	4.9	1.2–18	0.02
Other case of IA in the HT program 2 months before or after Tx date	4.6	1.5–14.4	0.007
Itraconazole prophylaxis	0.2	0.07–0.9	0.03

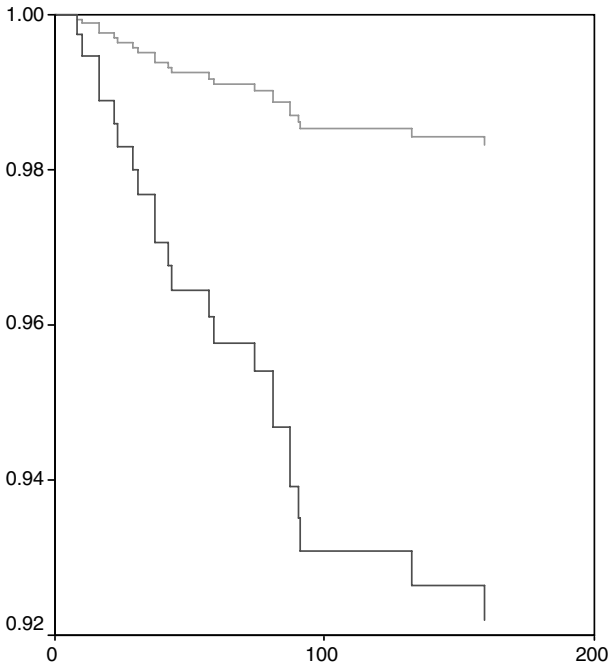

Figure 1: Cox regression probability graph showing time free of invasive aspergillosis (RR 0.2, 95% CI 0.04–0.9, p = 0.03) in heart transplant recipients with (grey) and without (black) itraconazole prophylaxis.

results by not finding a significant difference of survival in this subset of patients (77% in patients not receiving prophylaxis vs. 60% on those on itraconazole).

The pathogenesis of this increased risk is not clear. It has been suggested that toxic levels of CsA may increase the risk of IA by reducing the anti-fungicidal capacity of granulocytes, especially when administered with steroids (30) but also patients with chronic renal failure have been shown to have a wide spectrum of immune deficiencies (31). In Singh's study, hemodialysis increased the risk of IA independently of the time post-transplantation at which it was required (38% of IA after early onset and 33% after late-onset dialysis) (18). The median time to onset of fungal infection after renal replacement therapy was 13 days (2–191 days). This special population merits special attention considering that chronic renal failure after solid organ transplantation is a growing problem (32).

The need for re-operation increased the risk of IA in our series of HT, similar to what has been described after liver transplantation (33–35), probably reflecting a more severe condition and longer stay of the patients in the ICU.

CMV disease has also been identified as an independent risk factor for aspergillosis after heart transplantation in our series. CMV is an immunomodulating virus that renders the patient more susceptible to suffer opportunistic infections, by dysregulation of cytokines. Previous CMV disease and a CMV positive donor were also shown to have an independent relationship with the development of invasive fungal disease after liver (36–38) and lung transplantation (39). Despite advances in prophylaxis, the incidence of CMV infection preceding invasive aspergillosis seems to remain stable (40). The use of prophylactic ganciclovir reduced the incidence of fungal infections in heart transplant patients, but mainly late-onset infections (41). In our opinion, solid organ transplant recipients with CMV disease should be offered antifungal prophylaxis.

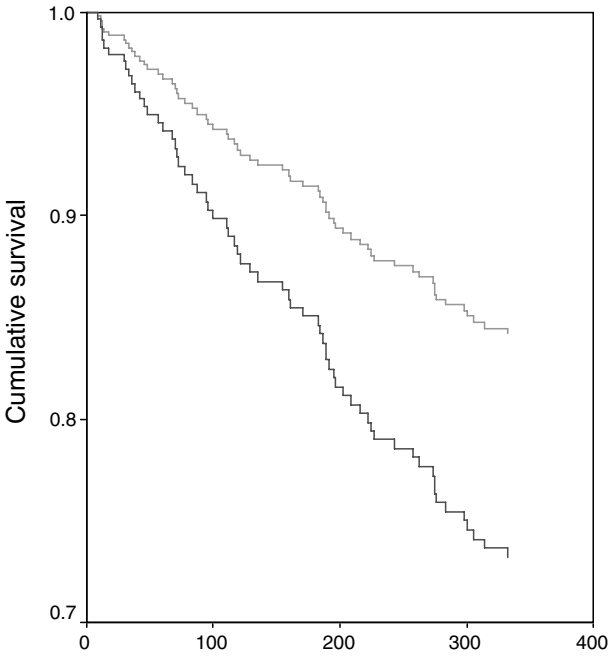

Figure 2: Cox regression probability graph showing 1-year survival in heart transplant recipients with (grey) and without (black) itraconazole prophylaxis (RR 0.5 95% CI 0.3–0.8, p = 0.01).

Increased environmental contamination is one of the most important risk factors for IA, and outbreaks have been attributed to the contamination of air ducts. It is very difficult to maintain all the areas where transplant recipients are cared for or in transit free of fungal spores. Constant environmental surveillance is a difficult goal as exposure may occur at different sites of the hospital or in the community. That is why we considered that the presence of an episode of IA in the HT program close to the transplantation date of each patient could reflect a high load of environmental spores. In fact, this variable showed an independent predictive value in our model (RR 4.6). We recommend that the diagnosis of any episode of aspergillosis shortly before or after the transplantation date should be considered an alarm risk and appropriate action taken, such as obtaining environmental cultures and providing prophylaxis to the potentially exposed patients (42).

Itraconazole prophylaxis effectively protected our patients from invasive aspergillosis, but most importantly, it was independently associated with improved 1-year survival, as has been previously shown with other antifungal prophylaxis schemes in immunosuppressed patients (43,44). In our institution we witnessed a profound decrease in invasive aspergillosis after the implantation of itraconazole prophylaxis (400 mg/q.d.) during the early months after heart transplantation. In our study, mean duration of prophylaxis was 110 d but it was more prolonged in patients with persistent increased immunosuppression as suggested by other authors (45,46). We administered prophylaxis during a 3- to 6-month period because this is the phase of greatest vulnerability to infection. In our study, itraconazole prophylaxis was associated with a decrease of nosocomial and community-acquired cases of aspergillosis.

Very few data on antifungal prophylaxis in HT recipients are available. Some authors use aerosolized amphotericin B (6,47), but there were no trials documenting that *Aspergillus* disease can be effectively prevented after heart transplantation. We decided to use itraconazole because it had shown good efficacy both in the treatment of invasive aspergillosis in patients with severe diseases, including heart transplant recipients (48–51), as well as in prophylaxis in other groups of immunosuppressed patients (52–56). Besides, its toxicity and cost were acceptable, and it contributed to the saving of CsA, as was shown with other azoles (57). The wide spectrum of the drug may be another advantage. The oral solution of itraconazole is better absorbed, which may be an important factor, as low serum levels are associated with prophylaxis failures (58), as happened in one of our patients. When administered to patients with nasogastric tubes, the dose should be increased and the levels monitored, due to impaired absorption.

The recommended administration schedule includes loading doses (600 mg daily for 4 d) and subsequent doses of at least 400 mg daily (50,59–61). Cyclosporine levels should be monitored and itraconazole serum concentrations measured within 7 d of starting therapy. When the itraconazole level is low [e.g. <1 mg/L by high-performance liquid chromatography (HPLC), or <5 mg/L by bioassay) the dose should be increased to 600 mg or 800 mg daily (by increments of 200 mg) or therapy changed altogether. Itraconazole capsules should be administered with food and an acid-based beverage, such as a cola drink. The new formulation of itraconazole in cyclodextrin should be used initially in the same dose (62).

As mentioned, itraconazole prophylaxis may also be a cost-effective measure. The administration of itraconazole enabled us to reduce the dose of CsA by 50% maintaining adequate serum levels. Compliance was good and side-effects included gastrointestinal disturbances and reversible increase of liver enzymes in three patients.

Our study has some limitations. The most important is the comparison of the efficacy of the antifungal prophylaxis using a historic cohort. Considering the relatively low number of transplanted patients each year in a single institution and given the variable conditions of different centers, a non-comparative, unicenter study with historical controls design (176 patients) was selected as the basis of our study. Besides, the problem of aspergillosis is so severe, that a placebo-controlled trial was not considered ethical at the time it was initiated.

We conclude that post-transplant hemodialysis, reoperation, CMV disease and the occurrence of any episode of aspergillosis in the area of the ward are independent risk factors for IA after HT. The administration of prophylaxis with itraconazole is an independent protective factor for IA and for increased overall survival by 1 year after transplantation. Antifungal prophylaxis in HT patients should be offered at least to patients with one or more of these predisposing conditions. Issues that should be determined in multicentric comparative studies are the role of new oral and intravenous antifungal compounds, the ideal dose and duration of the prophylaxis and the potential impact on the emergence of resistant isolates.

Acknowledgments

We would like to thank Dr Luis Alcalá for his help with the design of the study and to Dr Teresa Peláez for the processing of the fungal samples. We would also like to thank the Mr Lawrence Baron for his help with the English text.

The study was supported in part by Red Española de Infección y Trasplante. (RESITRA-G03/75). ISCIII. Ministerio de Sanidad y Consumo. C.R. is supported by a grant from the Fundación Carolina. Ministerio de Asuntos Exteriores.

References

1. Cisneros JM, Muñoz P, Torre-Cisneros J et al. Pneumonia after heart transplantation: a multi-institutional study. Spanish Transplantation Infection Study Group. Clin Infect Dis 1998; 27: 324–331.
2. Denning DW, Stevens DA. Antifungal and surgical treatment of invasive aspergillosis: review of 2,121 published cases [published erratum appears in Rev Infect Dis 1991; 13: 345]. Rev Infect Dis 1990; 12: 1147–1201.
3. Grossi P, Farina C, Fiocchi R, Dalla Gasperina D. Prevalence and outcome of invasive fungal infections in 1,963 thoracic organ transplant recipients: a multicenter retrospective study. Italian Study Group of Fungal Infections in Thoracic Organ Transplant Recipients. Transplantation 2000; 70: 112–116.
4. Muñoz P, Torre-Cisneros J, Bouza E et al. Invasive aspergillosis in transplant recipients. A large multicentric study. In: Program and Abstracts of the 36th ICAAC (New Orleans), abstract 1416. Washington DC: American Society for Microbiology, 1996.
5. Muñoz P, Alcalá L, Sánchez 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: 1–4.
6. Montoya JG, Giraldo LF, Efron B et al. Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. Clin Infect Dis 2001; 33: 629–640.
7. Hofflin JM, Potasman I, Baldwin JC, Oyer PE, Stinson EB, Remington JS. Infectious complications in heart transplant recipients receiving cyclosporine and corticosteroids. Ann Intern Med 1987; 106: 209–216.
8. Hummel M, Thalmann U, Jautzke G, Staib F, Seibold M, Hetzer R. Fungal infections following heart transplantation. Mycoses 1992; 35: 23–34.
9. Schulman LL, Smith CR, Drusin R, Rose EA, Enson Y, Reemtsma K. Respiratory complications of cardiac transplantation. Am J Med Sci 1988; 296: 1–10.
10. Kaiser L, Huguenin T, Lew PD, Chapuis B, Pittet D. Invasive aspergillosis. Clinical features of 35 proven cases at a single institution. Medicine (Baltimore) 1998; 77: 188–194.
11. Muñoz P. Invasive aspergillosis in heart transplantation. http://www.aspergillus.man.ac.uk. 1999; Section of Treatment. Invasiveaspergillosis.
12. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. Medicine (Baltimore) 1999; 78: 123–138.
13. Singh N. Antifungal prophylaxis for solid organ transplant recipients: seeking clarity amidst controversy. Clin Infect Dis 2000; 31: 545–553.
14. Patterson JE. Epidemiology of fungal infections in solid organ transplant patients. Transpl Infect Dis 1999; 1: 229–236.
15. Hibberd PL, Rubin RH. Clinical aspects of fungal infection in organ transplant recipients. Clin Infect Dis 1994; 19 (Suppl. 1): S33–S40.
16. Alexander BD. Prophylaxis of invasive mycoses in solid organ transplantation. Curr Opin Infect Dis 2002; 15: 583–589.
17. Utili R, Zampino R, De Vivo F et al. Improved outcome of pulmonary aspergillosis in heart transplant recipients with early diagnosis and itraconazole treatment. Clin Transplant 2000; 14: 282–286.
18. Singh N, Paterson DL, Gayowski T, Wagener MM, Marino IR. Preemptive prophylaxis with a lipid preparation of amphotericin B for invasive fungal infections in liver transplant recipients requiring renal replacement therapy. Transplantation 2001; 71: 910–913.
19. Muñoz P, Palomo J, Yañez J et al. Primary prophylaxis with itraconazole of invasive aspergillosis in heart transplant patients. In: Program and Abstracts of the 39th ICAAC (San Francisco), abstract 1416. Washington DC: American Society for Microbiology, 1999: 566.
20. Muñoz P, Palomo J, Muñoz R, Rodríguez Creixéms M, Pelaez T, Bouza E. Tuberculosis in heart transplant recipients. Clin Infect Dis 1995; 21: 398–402.
21. Muñoz P, Palomo J, Guembe P, Rodriguez-Creixems M, Gijon P, Bouza E. Lung nodular lesions in heart transplant recipients. J Heart Lung Transplant 2000; 19: 660–667.
22. Muñoz P, Muñoz RM, Palomo J, Rodriguez-Creixems M, Muñoz R, Bouza E. *Pneumocystis carinii* infection in heart transplant recipients. Efficacy of a weekend prophylaxis schedule. Medicine (Baltimore) 1997; 76: 415–422.
23. Patterson TF, Kirkpatrick WR, White M et al. Invasive aspergillosis. Disease spectrum, treatment practices, and outcomes. I3 Aspergillus Study Group. Medicine (Baltimore) 2000; 79: 250–260.
24. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002; 34: 1094–1097.
25. Denning DW. Therapeutic outcome in invasive aspergillosis. Clin Infect Dis 1996; 23: 608–615.
26. Briegel J, Forst H, Spill B et al. Risk factors for systemic fungal infections in liver transplant recipients. Eur J Clin Microbiol Infect Dis 1995; 14: 375–382.
27. Collins LA, Samore MH, Roberts MS et al. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. J Infect Dis 1994; 170: 644–652.
28. Kusne S, Torre-Cisneros J, Manez R et al. Factors associated with invasive lung aspergillosis and the significance of positive Aspergillus culture after liver transplantation. J Infect Dis 1992; 166: 1379–1383.
29. Rogers J, Rohal S, Carrigan DR et al. Human herpesvirus-6 in liver transplant recipients: role in pathogenesis of fungal infections, neurologic complications, and outcome. Transplantation 2000; 69: 2566–2573.

30. Roilides E, Robinson T, Sein T, Pizzo PA, Walsh TJ. In vitro and ex vivo effects of cyclosporin A on phagocytic host defenses against Aspergillus fumigatus. Antimicrob Agents Chemother 1994; 38: 2883–2888.
31. Ori Y, Korzets A, Malachi T, Gafter U, Breitbart H. Impaired lymphocyte calcium metabolism in end-stage renal disease: enhanced influx, decreased efflux, and reduced response to mitogen. J Lab Clin Med 1999; 133: 391–400.
32. Magee C, Pascual M. The growing problem of chronic renal failure after transplantation of a nonrenal organ. N Engl J Med 2003; 349: 994–996.
33. Rabkin JM, Oroloff SL, Corless CL et al. Association of fungal infection and increased mortality in liver transplant recipients. Am J Surg 2000; 179: 426–430.
34. Dockrell DH, Mendez JC, Jones M et al. Human herpesvirus 6 seronegativity before transplantation predicts the occurrence of fungal infection in liver transplant recipients. Transplantation 1999; 67: 399–403.
35. Lorf T, Braun F, Ruchel R, Muller A, Sattler B, Ringe B. Systemic mycoses during prophylactical use of liposomal amphotericin B (Ambisome) after liver transplantation. Mycoses 1999; 42: 47–53.
36. Singh N, Gayowski T, Wagener MM. Posttransplantation dialysis-associated infections: morbidity and impact on outcome in liver transplant recipients. Liver Transpl 2001; 7: 100–105.
37. George MJ, Snydman DR, Werner BG et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. Am J Med 1997; 103: 106–113.
38. Fortún J, Martín-Dávila P, Moreno S et al. Risk factors for invasive aspergillosis in liver transplant recipients. Liver Transpl 2002; 8: 1065–1070.
39. Monforte V, Roman A, Gavalda J et al. Nebulized amphotericin B prophylaxis for Aspergillus infection in lung transplantation: study of risk factors. J Heart Lung Transplant 2001; 20: 1274–1281.
40. Singh N, Avery RK, Muñoz P et al. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. Clin Infect Dis 2003; 36: 46–52.
41. Wagner JA, Ross H, Hunt S et al. Prophylactic ganciclovir treatment reduces fungal as well as cytomegalovirus infections after heart transplantation. Transplantation 1995; 60: 1473–1477.
42. Patterson JE, Peters J, Calhoon JH et al. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. Transpl Infect Dis 2000; 2: 22–28.
43. Slavin MA, Osborne B, Adams R et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation – a prospective, randomized, double-blind study. J Infect Dis 1995; 171: 1545–1552.
44. Hansen JA, Gooley TA, Martin PJ et al. Bone marrow transplants from unrelated donor for patients with chronic myeloid leukemia. N Engl J Med 1998; 338: 962–968.
45. Rubin RH. The therapeutic prescription for the transplant patient. Transpl Infect Dis 2002; 4: 1–2.
46. Singh N, Husain S. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. J Heart Lung Transplant 2003; 22: 258–266.
47. Reichenspurner H, Gamberg P, Nitschke M et al. Significant reduction in the number of fungal infections after lung-, heart-lung, and heart transplantation using aerosolized amphotericin B prophylaxis. Transplant Proc 1997; 29: 627–628.
48. Viviani MA, Tortorano AM, Langer M et al. Experience with itraconazole in cryptococcosis and aspergillosis. J Infect 1989; 18: 151–165.
49. Faggian G, Livi U, Bortolotti U et al. Itraconazole therapy for acute invasive pulmonary aspergillosis in heart transplantation. Transplant Proc 1989; 21: 2506–2507.
50. Denning DW, Lee JY, Hostetler JS et al. NIAID Mycoses Study Group Multicenter Trial of Oral Itraconazole Therapy for Invasive Aspergillosis. Am J Med 1994; 97: 135–144.
51. Speich R, van der Bij W. Epidemiology and management of infections after lung transplantation. Clin Infect Dis 2001; 33 (Suppl. 1): S58–S65.
52. Thunnissen PL, Sizoo W, Hendriks WD. Safety and efficacy of itraconazole in prevention of fungal infections in neutropenic patients. Neth J Med 1991; 39: 84–91.
53. Colby WD, Sharpe MD, Ghent CN et al. Efficacy of itraconazole prophylaxis against systemic fungal infection in liver transplant recipients. In: 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco), abstract 1650. Washington, DC: American Society for Microbiology, 1999: 129.
54. Grossi P, De Maria R, Caroli A, Zaina MS, Minoli L. Infections in heart transplant recipients: the experience of the Italian heart transplantation program. Italian Study Group on Infections in Heart Transplantation. J Heart Lung Transplant 1992; 11: 847–866.
55. Bohme A, Just Nuebling G, Bergmann L, Shah PM, Stille W, Hoelzer D. Itraconazole for prophylaxis of systemic mycoses in neutropenic patients with haematological malignancies. J Antimicrob Chemother 1996; 38: 953–961.
56. Mouy R, Veber F, Blanche S et al. Long-term itraconazole prophylaxis against Aspergillus infections in thirty-two patients with chronic granulomatous disease. J Pediatr 1994; 125: 998–1003.
57. Keogh A, Spratt P, McCosker C, Macdonald P, Mundy J, Kaan A. Ketoconazole to reduce the need for cyclosporine after cardiac transplantation. N Engl J Med 1995; 333: 628–633.
58. Menichetti F, Del Favero A, Martino P et al. Itraconazole oral solution as prophylaxis for fungal infections in neutropenic patients with hematologic malignancies: a randomized, placebo-controlled, double-blind, multicenter trial. GIMEMA Infection Program. Gruppo Italiano Malattie Ematologiche dell' Adulto. Clin Infect Dis 1999; 28: 250–255.
59. Denning DW, Stepan DE, Blume KG, Stevens DA. Control of invasive pulmonary aspergillosis with oral itraconazole in a bone marrow transplant patient. J Infect 1992; 24: 73–79.
60. Caillot D, Casasnovas O, Bernard A et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. J Clin Oncol 1997; 15: 139–147.
61. Patterson TF, Peters J, Levine SM et al. Systemic availability of itraconazole in lung transplantation. Antimicrob Agents Chemother 1996; 40: 2217–2220.
62. Prentice AG, Donnelly P. Oral antifungals as prophylaxis in haematological malignancy. Blood Rev 2001; 15: 1–8.