MAJOR ARTICLE

Risk Factors for Invasive Aspergillosis in Solid-Organ Transplant Recipients: A Case-Control Study

J. Gavalda,[1] O. Len,[1] R. San Juan,[5] J. M. Aguado,[5] J. Fortun,[6] C. Lumbreras,[5] A. Moreno,[2] P. Munoz,[7] M. Blanes,[10] A. Ramos,[8] G. Rufi,[3] M. Gurgui,[4] J. Torre-Cisneros,[11] M. Montejo,[12] M. Cuenca-Estrella,[9] J. L. Rodriguez-Tudela,[9] and A. Pahissa,[1] for RESITRA (Spanish Network for Research on Infection in Transplantation)

[1]Hospital Vall d'Hebron, [2]Hospital Clínic, [3]Hospital de Bellvitge, and [4]Hosptial de la Santa Creu i Sant Pau, Barcelona, [5]Hospital Doce de Octubre, [6]Hospital Ramón y Cajal, [7]Hospital Gregorio Marañón, [8]Hospital Puerta de Hierro, and [9]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, [10]Hospital La Fe, Valencia, [11]Hospital Reina Sofía, Córdoba, and [12]Hospital de Cruces, Barakaldo, Spain

Background. To facilitate the design of strategies for prevention of invasive aspergillosis in solid-organ transplant recipients, this study investigates whether the development of early-onset and late-onset aspergillosis are related to different risk factors, thereby distinguishing 2 risk populations for this serious complication.

Methods. A retrospective case-control study was performed, including 156 cases of proven or probable invasive aspergillosis in patients recruited from 11 Spanish centers since the start of the centers' transplantation programs.

Results. Among all patients, 57% had early-onset IA (i.e., occurred during the first 3 months after transplantation). Risk factor analysis in this group identified as significantly associated risk factors a more complicated postoperative period, repeated bacterial infections or cytomegalovirus disease, and renal failure or the need for dialysis. Among patients with late-onset infections (i.e., occurred >3 months after transplantation), who comprised 43% of cases, the patients at risk were older, were in an overimmunosuppressed state because of chronic transplant rejection or allograft dysfunction, and had posttransplantation renal failure.

Conclusions. Risk factors in patients with early-onset cases and patients with late-onset cases of posttransplantation invasive aspergillosis are not the same, a fact that could have implications for the preventive approaches used for this infection.

Invasive aspergillosis (IA) is still a life-threatening complication in patients who undergo solid-organ transplantation (SOT). Although the incidence among these patients is slightly lower than that among other risk groups, such as allogenic hematopoietic stem cell transplant recipients [1], the incidence of IA can be similar among certain SOT recipients, such as lung transplant recipients [2]. Despite the advances in early diagnosis and the development of new antifungal agents, IA-related mortality is still >70% for SOT recipients [3, 4]. Identification of SOT recipients at high risk for IA is crucial to select those who will benefit from preemptive therapy. Nevertheless, current information is insufficient for this purpose, since the available studies involve a very small series or are directed toward all invasive fungal infections [5–14]. In earlier studies, most cases of IA were reported to occur within the first 100 days after transplantation [5, 8, 13]. However, a number of more recent articles describe later development of the disease [6, 12, 15]. According to Singh et al. [15], 55% of these infections occur >3 months after transplantation. This has led to the differentiation of IA into early-onset infection, occurring <90 days after transplantation, and late-onset infection, occurring after that period [6]. Delayed occurrence of IA among transplant recipients is a relevant observation. A bimodal pattern of timing for the development of IA suggests that different risk factors may be present for each group of patients. Demonstration of this hypothesis could provide useful information for optimizing IA prevention strategies for SOT recipients. The aim of this study was to describe the epidemiology, clinical features, and risk

Received 10 September 2004; accepted 19 February 2005; electronically published 26 May 2005.

Presented in part: 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, September 2003 (abstract M-999).

Reprints or correspondence: Dr. Joan Gavaldà, Infectious Diseases Div., Hospital Vall d'Hebron, P. Vall d'Hebron 119-129, 08035 Barcelona, Spain (jgavalda@vhebron.net).

Clinical Infectious Diseases 2005; 41:52–9

1058-4838/2005/4101-0009$15.00

Table 1. Variables analyzed in the present study of invasive aspergillosis (IA) among solid-organ transplant recipients.

Variable, by class
Patient characteristic
Age
Sex
Diabetes
Long-term obstructive pulmonary disease
Previous transplant
Pretransplantation
Type and severity of underlying disease
According to Child [18] criteria
According to NYHA [19] criteria
Renal failure (serum creatinine level >2.5 mg/dL for >7 days)
Hemodialysis
Leukopenia (<3000 leukocytes/mm^3)
ICU stay
With mechanical ventilation
Without mechanical ventilation
CMV matching
Corticosteroid therapy
Antibiotic or antifungal therapy for >7 days
Aspergillus respiratory colonization in the past 6 months
Urgent clinical status at time of transplantation
Intraoperative
Length of surgery
Cold ischemia time
Total no. of blood product units given
Postoperative
Length of ICU stay
Mechanical ventilation requirement
Vascular amine requirement
Additional operation (new surgical procedure)
Urgent retransplantation (within 7 days after the first transplanation)
Additional ICU stay (excluding patients with retransplantation or IA)
With mechanical ventilation
Without mechanical ventilation
Renal failure
Hemodialysis
Neoplastic disease (time to development and type)
Bacterial infections
CMV prophylaxis
CMV infection or disease[a]
Fungal prophylaxis
Fungal infection other than IA
Immune-related factors[b]
IA-associated
Type of IA
Time to diagnosis
ICU stay
Need for mechanical ventilation
Multiorgan failure
Anemia and transfusion requirement
Thrombocytopenia
Treatment
Outcome

NOTE. CMV, cytomegalovirus; ICU, intensive care unit.

[a] Disease was defined as a consistent clinical picture associated with direct tissue culture or histological evidence of invasive CMV disease or CMV syndrome. Infection was defined as detectable CMV by antigen assay and shell vial culture of blood or by PCR, regardless of clinical manifestations [20].

[b] Included crossmatching for HLA compatibility; leukopenia; acute and chronic graft rejection; number of methylprednisolone boluses (≥500 mg each) administered and cumulative dose at 1, 3, 6, 9, 12, and 18 months; cyclosporine and FK506 maximum blood levels at 1, 3, 6, 9, 12, and 18 months; and concomitant use of azathioprine, mycophenolate, or anti-CD3 monoclonal antibody.

factors for the development of IA and IA-related mortality in a large, representative population of SOT recipients, with a possible distinction between early and late episodes taken into account.

METHODS

Study patients. This retrospective study included all patients who underwent SOT in the hospitals included in the Spanish Network for Research on Infection in Transplantation (Red de Estudio de la Infección en el Transplante [RESITRA]) from the start of the transplantation program in each institution to December 2001. To obtain accurate incidence figures, only complete years were analyzed. Eleven centers from all over Spain took part in the study.

Patients were identified by a review of records of microbiological and pathological studies (i.e., from autopsies and biopsies) and discharge diagnosis to identify all IA episodes that occurred after transplantation. A case patient was defined as any SOT recipient with a proven or probable diagnosis of IA [16]. For the purposes of this study, ulcerative tracheobronchitis in lung transplant recipients was not included in the diagnosis of IA. All cases were reviewed by 2 infectious disease physicians who rejected the reported cases that did not fulfill the diagnostic criteria.

The comparison population (controls) included the 2 patients who underwent the same type of transplantation immediately before and after each identified case patient did but did not develop aspergillosis infection and had a minimum of 18 months of follow-up. The day of diagnosis was defined as the day the first isolate of *Aspergillus* or the first histopathological specimen positive for *Aspergillus* was obtained. For patients for whom the diagnosis of IA was confirmed only after death, the date of death was used as the date of diagnosis. Cases of IA were classified according to 4 clinical types: nodular IA,

pneumonic IA, and disseminated IA (i.e., with involvement of ⩾2 noncontiguous organs) either with or without CNS involvement. Response to treatment was classified as either a success or a failure. Complete response was defined as the resolution of all clinical signs and symptoms and of >90% of the lesions due to IA that were visible on X-ray examination. Partial response was defined as clinical improvement and >50% improvement seen in radiographic findings. Stable response was defined as the absence of changes from baseline or a <50% improvement seen in radiographic findings. Failure of therapy was defined as clinical or radiological worsening of disease. Complete and partial responses were classified as successful outcomes, whereas stable and indeterminate responses and therapy failures were regarded as unsuccessful outcomes [17].

The variables analyzed as possible risk factors for developing IA were divided into the following 4 groups: patient characteristics; and pretransplantation, intraoperative, and postoperative variables. For the analysis of factors associated with mortality, we also compiled data on variables associated with the IA episode. The preoperative period was the 1 month immediately before transplantation. The variables analyzed in this study are shown in table 1.

Data analysis. Cases were divided into 2 groups, early IA and late IA, according to whether onset occurred before or after month 3 after transplantation. For the early IA analysis, we included data for all variables except the data for postoperative variables from later than month 3 after transplantation. The late IA analysis included data for all variables except the pretransplantation factors, which were excluded because of the belief that the recipient's pretransplantation status would have little bearing on an event occurring several months after the procedure and might introduce confounding factors in the statistical analysis.

Data for continuous variables were expressed as the mean ± SD and 95% CI, for those with a normal distribution, and as the median and interquartile range, for those with a skewed distribution. Data for discrete variables were expressed as percentages. Continuous variables were compared using Student's unpaired *t* test or the Mann-Whitney *U* test, where appropriate, and proportions were compared using the χ^2 test or Fisher's exact test. All statistical tests were 2-tailed, and the threshold of statistical significance was $P < .05$.

ORs were calculated for variables with statistically significant different values for cases and for controls. Binary logistic regression was applied individually to each variable to obtain the OR in the univariate analysis. Quantitative variables were previously converted into qualitative variables for that task.

Statistically significant variables ($P < .05$) in the univariate analysis were introduced in a multivariate model by use of forward stepwise logistic regression to identify the independent risk factors for IA and IA-related mortality. In addition, clinically relevant factors with *P* values of <.1 that were considered to be potential confounders (on the basis of experience and the literature) were forced into the multivariate model to investigate their effect.

RESULTS

Eleven Spanish hospitals participated in the study. Among 11,014 SOT recipients, 156 cases of IA (70 probable and 86 proven) were diagnosed and 312 corresponding controls were identified between 1990 and 2001. The group of patients with IA had a mean age of 52 years (range, 14–76 years) and included 113 men (72.4%). Fifty-nine cases of IA (37.8%) were diagnosed at autopsy. Eighty cases (51.3%) occurred in liver transplant recipients (including 4 recipients of liver-kidney transplants), 47 (30.1%) in heart recipients, 17 (10.9%) in lung recipients, 10 (6.4%) in kidney recipients, and 2 (1.3%) in pancreas-kidney recipients (table 1). The overall incidence of IA was 1.4%, and the incidence according to the type of allograft received was as follows: 3% (17 of 566) for lung recipients, 2.4% (47 of 1920) for heart recipients, 2% (80 of 3981) for liver recipients, 0.9% (2 of 230) for pancreas-kidney recipients, and 0.2% (10 of 4317) for kidney recipients.

Distribution of IA episodes according to the time of diagnosis after transplantation is shown in table 2. The mean time to the development of IA was 234 days (range, 2–3025 days). Pulmonary aspergillosis accounted for 92 (59%) of the cases, com-

Table 2. Distribution of cases of invasive aspergillosis (IA) according to the time of diagnosis, by type of solid-organ transplant.

Type of transplant	No. of patients	No. (%) of patients with episode of IA, by time of diagnosis				
		1–30 days	31–90 days	3–6 months	6–12 months	>1 year
Liver	80	27 (33.7)	17 (21.2)	15 (18.7)	11 (13.7)	10 (12.5)
Heart	47	12 (25.5)	20 (42.5)	5 (10.6)	5 (10.6)	5 (10.6)
Lung	17	3 (17.6)	2 (11.7)	2 (11.7)	5 (29.4)	5 (29.4)
Kidney	10	3 (30)	3 (30)	2 (20)	0	2 (20)
Pancreas-kidney	2	0	1 (50)	1 (50)	0	0
Total	156	45 (28.8)	43 (27.6)	25 (16)	21 (13.5)	22 (14.1)

Table 3. Distribution of cases of invasive aspergillosis (IA) according to the clinical form, by type of solid-organ transplant.

Type of transplant	No. of patients	No. (%) of patients with IA episode, by clinical form of IA			
		Pulmonary nodular	Pulmonary pneumonia	Disseminated with no CNS involvement	Disseminated with CNS involvement
Liver	80	8 (10)	31 (38.7)	28 (35)	13 (16.2)
Heart	47	16 (34)	14 (29.7)	9 (19.1)	8 (17)
Lung	17	5 (29.4)	7 (41.1)	3 (17.6)	2 (11.7)
Kidney	10	3 (30)	6 (60)	0	1 (10)
Pancreas-kidney	2	0	2 (100)	0	0
Total	156	32 (20.5)	60 (38.5)	40 (25.6)	24 (15.4)

prising 32 (20.5%) nodular cases and 60 (38.4%) pneumonia cases. Disseminated aspergillosis was diagnosed in 64 patients (41%), with CNS involvement in 24 patients (15.4%). Table 3 gives detailed information by type of SOT. Eighty-nine episodes of IA (57%) that occurred before month 3 after transplantation were analyzed separately as early IA, and 67 episodes (43%) that occurred after month 3 were analyzed as late IA.

Overall mortality was 76.3% (119 of 156 cases), with no significant differences between the SOT groups: pancreas-kidney recipients, 100% (2 of 2); liver recipients, 83.7% (67 of 80); lung recipients, 70.6% (12 of 17); kidney recipients, 70% (7 of 10); and heart recipients, 65.9% (31 of 47). Nor were there significant differences in mortality relative to the time of diagnosis; the mortality rate was 80% (36 of 45 cases) when IA was diagnosed on or before day 30 after onset; 69.7% (30 of 43), from day 31 to day 90; 70.8% (17 of 24), from months 3 to 6; 85.7% (18 of 21), from months 6 to 12; and 81.8% (18 of 22), after month 12. Mortality varied slightly, depending on the form of IA. For disseminated IA with CNS involvement, the mortality rate was 95.8% (23 of 24 cases); for pneumonia, 81.6% (49 of 60); for disseminated IA without CNS involvement, 80% (32 of 40), and for pulmonary nodular IA, 46.8% (15 of 32). The mortality rate did not change over time and showed a range from 64.7% in 1994 to 100% in 2000. Thirty-nine case patients died without receiving antifungal therapy. The other case patients were treated as follows: 68 patients (43.6%) received a lipid formulation of amphotericin B (46 died), 38 (24.4%) received conventional amphotericin B (28 died), and 7 (4.5%) received itraconazole (3 died) as first-line therapy. The multivariate analysis of mortality risk factors (table 4) showed that severely ill patients (i.e., those needing mechanical ventilation [OR, 25.5; 95% CI, 6.4–101.9] or transfusion therapy [OR, 4.03; 95% CI, 1.01–16]) and patients with IA with CNS involvment (OR, 12.3; 95% CI, 1.1–136.6) were at a higher risk of death.

Table 5 analyzes the overall risk factors for the development of early IA after SOT. IA was independently associated with variables that categorized patients as more seriously ill in the

Table 4. Risk factors for mortality due to invasive aspergillosis (IA) in solid-organ transplant (SOT) recipients.

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Liver transplant	2.3 (1.1–5.1)	.027	…	…
Renal failure after SOT	3.2 (1.4–7.3)	.004	…	…
Hemodialysis after SOT	3.6 (1.8–10.8)	.024	…	…
Infectious episodes after SOT	2.9 (1.4–6.2)	.006	…	…
Methylprednisolone dose >2 g during the first 30 days after SOT	3.5 (1.5–7.7)	.002	…	…
Disseminated IA with CNS involvement	8.6 (1.1–65.7)	.038	12.3 (1.1–136.6)	.041
Anemia during IA episode	25.1 (9.7–65)	<.0001	…	…
Thrombocytopenia during IA episode	31.6 (11–91)	<.0001	…	…
Multiorgan failure during IA episode	16.9 (16.6–43)	<.000	…	…
Mechanical ventilation during IA episode	48 (16–143.5)	<.0001	25.5 (6.4–101.9)	<.0001
Transfusion therapy during IA episode	15.1 (6.1–37.4)	<.0001	4.03 (1.01–16)	.047
Treatment with LAmB	0.4 (0.2–0.9)	.031	0.3 (0.1–1.1)	.066

NOTE. LAmB, lipid formulation of amphotericin B.

Table 5. Risk factors for early invasive aspergillosis in solid-organ transplant (SOT) recipients.

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*
Urgent transplantation	2.4 (1.2–4.8)	.01	…	…
ICU stay in the month before SOT	1.9 (0.9–3.8)	.057	…	…
CMV mismatch	2.8 (1.2–6.7)	.018	2.6 (0.9–7.2)	.055
Steroid therapy in the month before SOT	2.7 (1.1–6.7)	.028	…	…
Antimicrobial therapy in the month before SOT	2.3 (1.1–4.8)	.026	…	…
Use of vascular amines for >24 h	2.4 (1.5–4)	<.0001	2.2 (1.2–4.1)	.012
ICU stay of >5 days	2.5 (1.4–4.3)	.002	…	…
Mechanical ventilation for >2 days	2.5 (1.5–4)	<.0001	…	…
Urgent retransplantation	7 (2.5–19.5)	<.0001	…	…
Additional operation	3.2 (1.8–5.6)	<.0001	…	…
Additional ICU stay	5.6 (12.7–11.5)	<.000	2.9 (1.2–7)	.021
Posttransplantation renal failure	7.7 (4.5–13.2)	<.0001	4.9 (2.4–9.8)	<.0001
Posttransplantation hemodialysis	13 (6.1–27.4)	<.0001	3.2 (1.3–8.1)	.014
>1 Episode of bacterial infection	5.2 (2.6–10.5)	<.0001	3.2 (1.4–7.4)	.006
OKT3 use	1.6 (1–2.7)	.047	1.7 (0.9–3.2)	.071
CMV disease	2.1 (1.1–3.8)	.016	2.3 (1.1–4.9)	.029

NOTE. CMV, cytomegalovirus; ICU, intensive care unit; OKT3, anti-CD3 monoclonal antibody.

postoperative period; these included the use of vascular amines for >24 h after surgery (OR, 2.2; 95% CI, 1.2–4.1), intensive care unit (ICU) readmission (OR, 2.9; 95% CI, 1.2–7), development of renal failure (OR, 4.9; 95% CI, 2.4–9.8), and the requirement of hemofiltration or hemodialysis (OR, 3.2; 95% CI, 1.3–8.1). Bacterial infections (OR, 3.2; 95% CI, 1.4–7.4) and cytomegalovirus (CMV) disease (OR, 2.3; 95% CI, 1.1–4.9) were also more frequent among the early IA cases.

The analysis of data for the group with late IA (table 6) revealed some very different risk variables. Independent risk factors for late IA were age >50 years (OR, 2.5; 95% CI, 1.3–5.1), chronic transplant rejection (OR, 5; 95% CI, 1.9–13), and several variables indicating overimmunosuppression. Overimmunosuppression was defined by the following: tacrolimus blood levels of >15 ng/mL or cyclosporine blood levels >500 ng/mL at month 3 after transplantation (OR, 2.5; 95% CI, 1.2–5), tacrolimus and cyclosporine use in the same patient (OR, 3.2; 95% CI, 1.1–9.4), and occurrence of a immunosuppression-related neoplasm (3 cases of non-Hodgkin lymphoma and 1 case of Kaposi sarcoma in the IA group, compared with only

Table 6. Risk factors for late invasive aspergillosis in solid-organ transplant (SOT) recipients.

	Univariate analysis		Multivariate analysis	
Variables	OR (95% CI)	*P*	OR (95% CI)	*P*
SOT at age >50 years	1.9 (1.1–3.3)	.027	2.5 (1.3–5.1)	.009
Additional intervention	2.1 (1.1–3.9)	.021	…	…
Additional ICU stay	3 (1.2–7.1)	.015	…	…
Renal failure after SOT	3.9 (2.2–7)	<.0001	3.9 (1.9–7.8)	<.0001
Hemodialysis after SOT	5.2 (2.1–12.5)	<.0001	…	…
>6 g Cumulative dose of steroids at month 3	2.5 (1.3–4.6)	.005	…	…
>2 Boluses of steroids	2.3 (1.3–4)	.003	…	…
Blood levels of Tac >15 ng/mL or CyA >500 ng/mL at month 3	2.9 (1.7–5.2)	<.0001	2.5 (1.2–5)	.011
Use of Tac and CyA for the same patient	5.7 (2.4–13.7)	<.0001	3.2 (1.1–9.4)	.032
>1 Episode of bacterial infection	8.2 (4–16.6)	<.0001	7.5 (3.2–17.4)	<.0001
>1 Episode of CMV disease	2.2 (1.2–4.3)	.015	…	…
Significant leukopenia (<3000 leukocytes/mm^3)	3.3 (1.9–5.8)	<.0001	1.9 (0.9–3.7)	.056
Immunosuppression-related neoplasm	19 (2.1–173.3)	.009	69.3 (6.4–753)	<.0001
Chronic graft rejection	4.4 (2–9.5)	<.0001	5 (1.9–13)	.001

NOTE. CMV, cytomegalovirus; CyA, cyclosporine; ICU, intensive care unit; Tac, tacrolimus.

1 case of non-Hodgkin lymphoma in the control group). This last variable was the most important independent risk factor, with an OR of 69.3 (95% CI, 6.4–753). Finally, the late IA group had 2 independent risk factors in common with the early IA group: renal failure (OR, 3.9; 95% CI, 1.9–7.8) and >1 episode of bacterial infection (OR, 7.5; 95% CI, 3.2–17.4).

Separate analyses were performed to identify specific risk factors for each SOT subgroup. Statistically significant factors for early IA were similar to those described for all types of SOT, except in the case of lung transplantation, for which the only independent risk factor was *Aspergillus* colonization of the recipient in the 6 months before transplantation (OR, 11.2; 95% CI, 1.4–89). Results similar to those for the overall risk factor study for late IA were also seen in the SOT group analyses; however, hepatitis C virus (HCV) infection (OR, 5.9; 95% CI, 2.1–17) was an independent risk factor for liver transplant recipients.

DISCUSSION

This study analyzes the risk factors for developing IA in a large population of SOT recipients. Previous studies designed for this purpose are scarce, and they usually included a limited number of cases and frequently considered yeast and filamentous fungal infections together to identify risk factors for invasive fungal infection [7, 8]. The strengths of the present study are the large number of patients examined and the analysis of >100 variables, which confers high sensitivity for the detection of risk factors and provides enough control variables to adjust for confounding factors.

In a recent study, Singh et al. [15] reported that 55% of *Aspergillus* infections are diagnosed >3 months after transplantation, a rate similar to the 43% of cases of late-onset IA diagnosed in the present study. Considering the hypothesis that risk factors might be different for early-onset and late-onset IA cases, we performed a separate analysis for each of these populations.

Two of the most important independent risk factors for early-onset IA in SOT recipients were posttransplantation renal failure and the need for hemodialysis. The association between these factors and invasive fungal infection has been widely reported, particularly in liver transplantation [7, 9, 10], although the basis for this relationship has not been completely explained [21–24]. These clinical conditions probably indicate the profile of a critical patient and were supported by other variables with similar meaning, such as the persistent need for administration of vascular amines after surgery and for readmission to the ICU, which were also associated with the development of early IA. One important risk factor that has not been reported previously is the occurrence of >1 episode of bacterial infection after transplantation. Lastly, CMV disease also seemed to be associated with early IA, because of the immunologic effect of cytokine deregulation [12].

In contrast to the patients at high risk for early IA, who were critically ill or had a poor postoperative course, transplant recipients who developed late IA were older, overimmunosuppressed patients with chronic impaired graft function. The relationship between age and increased risk of developing IA has not been reported before. Also for the first time, presence of an immunosuppression-related neoplasm was found to be an important risk factor for late-onset IA. The use of steroid treatment to manage these tumors might favor the development of IA, but the occurence of neoplasms itself could also imply a high state of immunosuppression. Other risk factors retained in the final model, such as high blood levels of tacrolimus or cyclosporine, or the use of both agents for the same patient, also point to an overimmunosuppressed state. Chronic rejection probably indicates deteriorated organ function and, once again, high immunosuppression. As for early IA, hemodialysis and bacterial infections also determined a higher risk for late IA. Finally, CMV disease has been described as a risk factor for late IA in liver transplant recipients in 2 previous studies [6, 9], although the number of cases studied was small (6 and 7 patients, respectively). In the present study, CMV disease was identified as a risk factor for late IA in the univariate analysis (OR, 2.22; 95% CI, 1.2–4.3), but its statistical significance decreased to 1% in the multivariate study. This was probably a result of the important effects of the variables associated with overimmunosuppression, which could have masked the significance of CMV disease.

Specific risk analyses were also performed according to the type of organ transplanted. Liver transplant recipients comprised the majority of cases in this series (80 patients) and were the group with the highest incidence of disseminated disease (51.2%). This has been explained by the fact that hepatic reticuloendothelial phagocytes are the major line of defense against dissemination of *Aspergillus* organisms; thus, liver transplant recipients with abnormal allograft function are thought to have increased susceptibility to disseminated aspergillosis [25]. In previous studies, liver transplant recipients with IA had evidence of significant hepatic and/or renal dysfunction. In particular, the requirement for dialysis has been shown to portend a 15- to 25-fold greater risk for invasive fungal infections [9, 10], and approximately one-fourth of infection cases were seen after retransplantation [6]; thus, the risk is 30-fold higher for liver transplant recipients than for other patients [9]. In the present study, renal failure and, in particular, hemodialysis were associated with a special risk for both early and late IA in liver transplant recipients. A high cumulative dose of steroids was also associated with increased risk (OR, 5.9; 95% CI, 1.11–31), a fact that seems reasonable on the basis of the higher risk of fungal infection inherent to use of these agents [26, 27];

nevertheless, this risk factor has not been reported in previous studies, including studies of liver transplant recipients [7]. Another surprising risk factor was HCV infection as the reason for transplantation. Some studies have shown that the evolution of the graft is slightly poorer for HCV-associated transplants than for non–HCV-associated transplants, partially because of the high rate of recurrence of HCV infection in the graft [28–30]. Regarding HCV's role as an immunomodulator, documented clinical experience has shown a higher number of infectious complications in transplant recipients with HCV infection [31]. Again, immunologic studies of these patients showed a significant decrease in circulating CD4-cell populations and a lower response to mitogenic stimulation [32].

Lastly, the highest incidence of IA occurred in the subgroup of lung transplant recipients, even though ulcerative tracheobronchitis was not included in the study. However, the incidence of IA in lung recipients has decreased considerably since the first lung transplant recipients were enrolled in this study, in great part because of the introduction of effective prophylaxis with nebulized amphotericin B [33]. Although this subgroup was smaller than the other subgroups in our study, and the sensitivity for detecting possible risk factors is lower, some conclusions can be extracted. Prior airway colonization with *Aspergillus* was the only independent risk factor for early IA. Previous studies have reported a higher incidence of invasive infection among lung transplant recipients with previous colonization than among those without it [11, 34–37], and the present results support those data. Interestingly, in recipients of most types of SOT, the majority of IA cases were diagnosed within 90 days, whereas, in lung transplant recipients, most cases were diagnosed after 90 days (table 2); this fact may be related to withdrawal of preventive measures (i.e., nebulized amphotericin B therapy).

Like other retrospective studies, the present report has some limitations. The most important is the difficulty in establishing a precise cause-effect relationship between the detected risk factors and the studied event. Nevertheless, the results of this case-control study could be highly useful in clinical practice for identifying SOT recipients at risk for developing early or late IA. Directed prophylaxis strategies could be implemented with higher efficiency, and patients at the highest risk could be considered for preemptive therapy. Some local experiences that have had good results with this approach have been reported [10, 33, 38], and the findings of the present multicenter study could reinforce the evaluation of these strategies on a broader basis. On the basis of recently presented results [39], we currently use nebulized liposomal amphotericin B prophylaxis for all lung transplant recipients. It is likely that this strategy would also be useful in the other risk populations identified in the present study.

In conclusion, IA is still a major threat for SOT recipients because of its high associated mortality. Among transplant recipients who have a postoperative course complicated by IA, 2 groups can be differentiated according to the time of onset of IA, and each group has specific risk factors. Early IA is more likely to occur in patients who have a more complicated postoperative course, in those who experience repeated bacterial infections or CMV disease, and in those who develop renal failure or who need hemodialysis. Late IA develops more commonly in older patients with an overimmunosuppressed state because of chronic graft rejection or allograft dysfunction and in patients with posttransplantion renal failure. Guided by the risk factors reported in this study, clinicians should identify the groups at highest risk, to evaluate directed prophylactic strategies and to select patients that might benefit from preemptive treatment.

Acknowledgments

We thank Celine Cavallo for assistance with our written English.

Financial support. This work was supported in part by Fondo Investigaciones Sanitarias de la Seguridad Social (FISS grant G03/075 RESITRA).

Potential conflicts of interest. All authors: no conflicts.

References

1. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogenic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood **2003**; 102: 827–33.
2. Minari A, Husni R, Avery RK, et al. The incidence of invasive aspergillosis among solid organ transplant recipients and implications for prophylaxis in lung transplants. Transpl Infect Dis **2002**; 4:195–200.
3. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. Clin Infect Dis **2001**; 32:358–66.
4. Denning DW. Therapeutic outcome in invasive aspergillosis. Clin Infect Dis **1996**; 23:608–15.
5. Kusne S, Torre-Cisneros J, Mañez R, et al. Factors associated with invasive lung aspergillosis and the significance of positive *Aspergillus* culture after liver transplantation. J Infect Dis **1992**; 166:1379–83.
6. Singh N, Arnow PM, Bonham A, et al. Invasive aspergillosis in liver transplant recipients in the 1990s. Transplantation **1997**; 64:716–20.
7. 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**; 14:375–82.
8. Collins LA, Samore MH, Roberts MS, et al. Risk factors for invasive fungal infections complicating orthotopic liver transplantation. J Infect Dis **1994**; 170:644–52.
9. Fortun J, Martin-Davila P, Moreno S, et al. Risk factors for invasive aspergillosis in liver transplant recipients. Liver Transpl **2002**; 8: 1065–70.
10. 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**; 71:910–3.
11. Cahill BC, Hibbs JR, Savik K, et al. *Aspergillus* airway colonization and invasive disease after lung transplantation. Chest **1997**; 112:1160–4.
12. Husni RN, Gordon SM, Longworth DL, et al. Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients. Clin Infect Dis **1998**; 26:753–5.
13. Patel R, Portela D, Badley AD, et al. Risk factors of invasive *Candida*

and non-*Candida* fungal infections after liver transplantation. Transplantation **1996**; 62:926–34.
14. 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 patients. Am J Med **1997**; 103:106–13.
15. Singh N, Avery RK, Munoz 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.
16. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.
17. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med **2002**; 347:408–15.
18. Pugh RN, Murray-Lyon MI, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg **1973**; 60:646–9.
19. The Criteria Commitee of the New York Heart Association. Nomenclature and criteria for diagnosis of disease of the heart and great vessels. 9th ed. Boston: Little, Brown & Co, **1994**:253–6.
20. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis **2002**; 34: 1094–7.
21. Cenci E, Mencacci A, Del Sero G, et al. Interleukin-4 causes susceptibility to invasive pulmonary aspergillosis through supression of protective type I responses. J Infect Dis **1999**; 180:1957–68.
22. Cenci E, Mencacci A, Casagrande A, Mosci P, Bistoni F, Romani L. Impaired antifungal effector activity but not inflammatory cell recruitment in interleukin-6–deficient mice with invasive pulmonary aspergillosis. J Infect Dis **2001**; 184:610–7.
23. Sester U, Sester M, Hauk M, Kaul H, Kohler H, Girndt M. T-cell activation follows Th1 rather than Th2 pattern in haemodialysis patients. Nephrol Dial Transplant **2000**; 15:1217–23.
24. Kaul H, Girndt M, Sester U, Sester M, Kohler H. Initiation of hemodialysis treatment leads to improvement of T-cell activation in patients with end-stage renal disease. Am J Kidney Dis **2000**; 35:611–6.
25. Paterson DL, Singh N. Invasive aspergillosis in transplant recipients. Medicine **1999**; 78:123–38.
26. Nawada R, Amitani R, Tanaka E, et al. Murine model of invasive pulmonary aspergillosis following an earlier stage, noninvasive *Aspergillus* infection. J Clin Microbiol **1996**; 34:1433–9.
27. Berenguer J, Allende MC, Lee JW, et al. Pathogenesis of pulmonary aspergillosis: granulocytopenia versus cyclosporine and methylprednisolone-induced immunosuppression. Am J Respir Crit Care Med **1995**; 152:1079–86.
28. Macedo G, Costa Maia J, Gomes A, et al. Hepatitis C virus infection and liver transplantation: recurrence of hepatitis C virus infection after liver transplantation. Transplant Proc **2000**; 32:2667.
29. Ziolkowski J, Niewczas M, Senatorski G, et al. Liver transplantation in hepatitis C virus–related cirrhosis. Transplant Proc **2003**; 35:2275–7.
30. Loinaz C, Lumbreras C, Moreno E, et al. Liver transplantation and hepatitis C virus: results in a Spanish center since 1989. Hepatogastroenterology **2001**; 48:1435–42.
31. Singh N, Gayowski T, Wagener MM, Marino IR. Increased infections in liver transplant recipients with recurrent hepatitis C virus hepatitis. Transplantation **1996**; 61:402–6.
32. Corell A, Morales JM, Mandrono A, et al. Immunosuppression induced by hepatitis C virus infection reduces acute renal transplant rejection. Lancet **1995**; 346:1497–8.
33. 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**; 20:1274–81.
34. 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**; 2:22–8.
35. Nunley DR, Ohori P, Grgurich WF, et al. Pulmonary aspergillosis in cystic fibrosis lung transplant recipients. Chest **1998**; 114:1321–9.
36. Helmi M, Love RB, Welter D, Cornwell RD, Meyer KC. *Aspergillus* infection in lung transplant recipients with cystic fibrosis: risk factors and outcomes comparison to other types of transplant recipients. Chest **2003**; 123:800–8.
37. Ruffini E, Baldi S, Rapellino M, et al. Fungal infections in lung transplantation: incidence, risk factors and prognostic significance. Sarcoidosis Vasc Diffuse Lung Dis **2001**; 18:181–90.
38. Fortun J, Martin-Davila P, Moreno S, et al. Prevention of invasive fungal infections in liver transplant recipients: the role of prophylaxis with lipid formulations of amphotericin B in high-risk patients. J Antimicrob Chemother **2003**; 52:813–9.
39. Monforte V, Gavalda J, Roman A, et al. Pharmacokinetics and efficacy of nebulized Ambisome (n-Amb) in lung transplantation (Lt) [abstract M-1042]. In: Program and abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy (Washington DC). Washington, DC: American Society for Microbiology, **2004**:419.