Risk Factors for Invasive Aspergillosis in Liver Transplant Recipients

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Aspergillosis is a potential, severe, and usually early complication of liver transplantation. New promising strategies, such as detecting *Aspergillus* antigenemia, have been used for the diagnosis of aspergillosis in immunosuppressed patients, but the impact in solid organ transplantation is not well known. A case-control study in 260 adults who underwent liver transplantation from January 1994 to June 2000 was performed. A case was defined as any liver transplant recipient with a proven or probable diagnosis of invasive aspergillosis. Controls were defined as a liver transplant recipient without aspergillosis infection with a survival longer than two months after transplantation. Clinical and analytical variables, including *Aspergillus* antigenemia, were compared. A special analysis was performed in patients in whom late aspergillosis developed (after day 100 posttransplantation). Among 260 patients, invasive aspergillosis developed in 15 (5.6%). Median time from transplantation to aspergillosis in 13 patients with sufficient data for analysis was 126 days (range, 22 to 1117). Seven (54%) developed the infection after day 100 posttransplantation. Thirty-eight patients were used as controls. Antigenemia was available in nine of 13 cases and in 33 of 38 controls. By multivariate analysis, retransplantation (OR, 29.9 [95% CI, 2.1 to 425.1]), dialysis requirements after transplantation (OR, 24.5 [95% CI, 1.25 to 354]), and the presence of *Aspergillus* antigenemia in serum at any time point after transplantation (OR, 50.0 [95% CI, 3.56 to 650]) were independently associated to aspergillosis. In the subgroup of patients that developed late aspergillosis, cytomegalovirus infection (OR, 6.7 [95% CI, 1.0 to 42.5]) was the only independent factor associated. Hepatic and renal dysfunction predispose to *Aspergillus* infection in liver transplant recipients. Cytomegalovirus infection and increased immunosuppression favor invasive aspergillosis during the late posttransplantation period. *Aspergillus* antigenemia seems to be a good predictor of invasive aspergillosis. (*Liver Transpl 2002;8:1065-1070.*)

Aspergillosis is a feared complication in liver transplant recipients. Although the incidence of the disease is low, mortality in these patients is higher than 80%.[1-4] The lack of nontoxic, efficacious antifungal drugs and the limited efficacy of prophylactic measures for avoiding *Aspergillus* infections represent important challenges for the clinical management of invasive aspergillosis.

Risk factors for the development of invasive aspergillosis in transplant recipients have been assessed in several reports.[5-9] Recently, new promising strategies have been applied in the diagnosis of aspergillosis in immunosuppressed patients. One of these strategies is the detection of *Aspergillus* antigenemia.[10-13] Our group has previously shown the use of *Aspergillus* antigenemia detection as a serodiagnosis method for invasive aspergillosis in liver transplant patients.[14] *Aspergillus* antigenemia has not been previously evaluated as a risk factor in other studies, and the impact in solid organ transplantation is not well known.

On the other hand, classically, it has been stated that most cases of invasive aspergillosis develop in the early posttransplantation period, within the first 100 days,[5-7] although some reports have described the occasional occurrence of invasive aspergillosis in a later period.[8,9,15]

A retrospective case-control study was performed to describe the global risk factors for *Aspergillus* infections among liver transplant recipients and to evaluate the impact of *Aspergillus* antigenemia as a marker for patients at special risk of developing aspergillosis. A secondary analysis was focused to assess the frequency of late aspergillosis and to identify risk factors associated with the disease in this period.

Methods

Study Population: Definitions

This is a case-control study, performed at the Liver Transplantation Unit of the Hospital Ramón y Cajal, in Madrid, Spain. All liver transplant recipients from January 1994 to June 2000 were included. A case was defined as any liver transplant recipient with a proven or probable diagnosis of invasive aspergillosis. Controls were defined as a liver transplant recipient without aspergillosis infection and with a survival longer than 2 months after transplantation. To avoid a

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nosocomial exposure bias in cases, controls were chosen among patients who had undergone transplantation within one month of cases. Three control patients were included for each case.

Aspergillosis was categorized as proven or probable.[16] Proven aspergillosis was considered when tissue histopathology showed septate, acute branching hyphae with or without a positive culture for *Aspergillus* spp from the same site, or, in the absence of histopathology, a positive culture from tissue obtained by an invasive procedure. Probable aspergillosis applied only to patients with pulmonary disease with chest radiographic appearance of new nodules or cavities, and two sputum cultures or one bronchoalveolar lavage, washing, or brushing culture for *Aspergillus* spp. In absence of pulmonary infiltrates, the isolation of *Aspergillus* spp in sputum but not confirmed in bronchoalveolar lavage was considered colonization. Colonized patients with *Aspergillus* spp were excluded because, based on invasive aspergillosis definitions, we were unable to classify them either as cases or as controls, and invasive aspergillosis had not been completely excluded in some of these patients. Episodes of aspergillosis that developed after day 100 were considered late cases.[15]

Cytomegalovirus disease was defined according to standard clinical criteria.[17] Cytomegalovirus disease was defined as a compatible picture associated with direct tissue culture or histologic evidence of invasive cytomegalovirus disease, or when cytomegalovirus viral syndrome was present. Cytomegalovirus infection was defined as the presence of detectable cytomegalovirus by antigenemia shell vial culture of blood, or by polymerase chain reaction regardless of clinical manifestation.

Collection of Data

Medical charts were reviewed, and for each patient included in the study, clinical and surgical variables were collected and grouped in four different periods. The preoperative period included the last month before transplantation. For patients who underwent retransplantation, the last transplant was considered the reference. Preoperative variables included: age, sex, Child-Pugh class, administration of antibiotic therapy for more than 7 days, hyperglycemia, insulin requirements, admission to the intensive care unit, surgical procedures within this period, serum creatinine, and total bilirubin levels. Data collected during the intraoperative period were urgent clinical status at the time of transplantation, fulminate hepatic failure, retransplantation, number of packed red blood cells required, and length of graft cold ischemia. The immediate postoperative period included events that occurred within the first month after transplantation. The following variables were analyzed: dialysis requirement, number of days in the intensive care unit, length of intubation, reintervention (a new surgical procedure in the first month), prolonged antibiotic therapy (more than 14 days), and cytomegalovirus infection. Finally, in the subgroup of patients with late aspergillosis (after day 100), a late posttransplantation period was defined as the time after the first month posttransplantation. We analyzed in this period the following variables: requirement of boluses of steroids, OKT3 administration, chronic rejection episodes, cytomegalovirus infection, cytomegalovirus disease, reintervention, and insulin requirement.

In addition to these variables, stored frozen serum specimens obtained during the posttransplantation period from patients included in the case-control study were used to determine *Aspergillus* antigen using a sandwich—enzyme-linked immunosorbent assay technique (Platelia; Sanofi Diagnostic Pasteur, Paris, France), following the method described by the manufacturer. For the antigenemia analysis, a patient was considered valid if more than three serum samples, drawn at separate times, were available. In case patients, one of the samples had to be obtained within the month before aspergillosis diagnosis. An optical density index *Aspergillus* antigen detection with a value of 1 ng per μL or higher was considered positive. Positive samples were retested in parallel in another assay. A sample was considered true positive when the results were also positive in the retesting assay.

Transplantation Procedures

Donor management and organ retrieval were generally standardized during the period of this review. Organs were preserved using Wisconsin solution. Biliary reconstruction was accomplished with either choledocholedocostomy or Roux-en-Y choledocojejunostomy when appropriate.

Postoperative prophylaxis included ampicillin and cefotaxime during 48 hours and was prolonged to five days in patients with allograft dysfunction. During the hospitalization period, all patients received oral fluconazole, 100 mg once a day, as fungal prophylaxis. From 1994 to 1996, oral ofloxacin was also given as selective bowel decontamination.

During the first six months, all patients received cotrimoxazole as *Pneumocystis carinii* prophylaxis. Cytomegalovirus prophylaxis (intravenous ganciclovir 5 mg/kg twice daily for 14 days followed by oral acyclovir [800 mg four times daily] for three months) was given to seronegative recipients of seropositive donors. Seropositive recipients receiving OKT3 monoclonal antibodies for acute rejection treatment received intravenous ganciclovir six mg/kg once a day for 14 days. For the rest of the patients, pre-emptive cytomegalovirus prophylaxis guided by antigenemia (pp65) was used. Intravenous ganciclovir was administered for patients with cytomegalovirus antigenemia values higher than 15 positive cells per 200,000 until the value became a negative result.

Immunosuppression regimen protocols generally included cyclosporine or tacrolimus, a tapering course of corticosteroids, and azathioprine or mycophenolate. Rejection episodes were initially treated with corticosteroid boluses or with increasing doses of tacrolimus if it was the immunosuppressor used. Refractory cases were treated with a 14-day course of OKT3 monoclonal antibody.

Statistical Analysis

Risk factors for the development of infection were identified by comparison of patients with the control group. Characteristics of

Table 1. Characteristics of Patients With Invasive Aspergillosis After Liver Transplantation

Patient	Age/ Gender	Retrans-plantation	IA Diagnosis (Days Post-OLT)	Positive Antigenemia (Days Post-OLT)	Localization	Diagnosis	Outcome
1	50/M	No	95	52 (Ag: 5.87)	Disseminated	Proven	Death
2	61/M	No	245	Neg	Disseminated	Proven	Death
3	40/M	Yes	470	Neg	Pulmonary	Proven	Death
4	65/M	Yes	56	51 (Ag: 6.43)	Disseminated	Proven	Death
5	58/M	No	364	Neg	Disseminated	Proven	Death
6	53/F	No	1117	1110 (Ag: 2.9)	Disseminated	Proven	Death
7	25/M	Yes	22	NA	Disseminated	Proven	Death
8	30/M	No	308	NA	Disseminated	Proven	Death
9	66/M	No	365	365 (Ag: 6.6)	Pulmonary	Proven	Death
10	63/M	Yes	72	1 (Ag: 1.7)	Pulmonary	Proven	Death
11	51/F	No	126	NA	Pulmonary	Probable	Death
12	46/M	Yes	35	NA	Disseminated	Proven	Alive
13	62/F	Yes	33	Neg	Pulmonary	Probable	Alive

NOTE. Performed using sandwich ELISA assay (Plateli, Snofi Diagnostic Pasteur) and the detection level is measured in ng/ml. Abbreviations: OLT, orthotopic liver transplantation; NA: not avalaible; POS: positive result; NEG: negative result; IA: invasive aspergillosis; Ag: Aspergillus antigenemia.

cases and controls were compared using Student's *t*-test for continuous data (separate estimates of variance were used when variances differed significantly) and with Mantel-Haenszel extended Chi-squared test for categorical data. Fisher's exact test was used when the expected number of cases per cell was below five. Odds ratio and 95% confidence intervals have been given with Chi-squared and *P* values to illustrate the amount of risk associated with some of the effects. Multiple logistic regression analysis was used to determine the independent risk factors associated with early or late aspergillosis.

All statistical associations were assessed with two-tailed test. A P value $\leq .05$ was considered to indicate statistical significance. Statistical analysis of the data was performed with Epi-Info software package, version 6 (CDC; Atlanta, GA) and SPSS 9.0 for Windows.

Results

From January 1994 through June 2000, 260 adults underwent orthotopic liver transplantation at our hospital. A total of 15 patients (5.6%) developed invasive aspergillosis. Enough data for analysis were not available in two patients. Finally, 13 cases and 38 controls were included in the study.

Characteristics of the 13 cases are shown in Table 1. Eleven (85%) patients had aspergillosis histologically proven. *Aspergillus* infection was limited to the lungs in five cases, and it was disseminated in the other eight cases. Six patients had undergone retransplantation. The median time from transplantation to the diagnosis of invasive aspergillosis was 126 days (range, 22 to 1117 days). Seven patients developed the disease after day 100. Aspergillosis-related mortality was high (11 of 13 patients, 85%).

Table 2 analyzes the risk factors for the development of aspergillosis. In univariate analysis, aspergillosis was associated with previous intensive care unit stay (OR, 7.2 [95% CI, 1.34 to 41.83]), high serum bilirubin levels at the moment of transplantation ($P = .03$), retransplantation (OR, 7.3 [95% CI, 1.3 to 43.6]), dialysis requirement after transplantation (OR, 8.0 [95% CI, 1.0 to 77.1]), a prolonged stay in the intensive care unit ($P = .03$) and cytomegalovirus disease (OR, 8.0 [95% CI, 1.0 to 77.0]). In addition, the presence of *Aspergillus* antigenemia in serum in some determination after transplantation was significantly associated with the development of aspergillosis (OR, 19.4 [95% CI, 2.1 to 228.4]).

The multivariate analysis (Table 3) showed an independent association of development of aspergillosis with retransplantation (OR, 29.9 [95% CI, 2.1 to 425.1]), the dialysis requirements after transplantation (OR, 24.5 [95% CI, 1.25 to 354.1]), and *Aspergillus* antigenemia (OR, 50.0 [95% CI, 3.56 to 650]).

In the analysis performed in the subgroup of patients that developed aspergillosis after day 100 posttransplantation, cytomegalovirus infection (OR, 9.38 [95% CI, 1.21 to 89.57]) and the development of chronic rejection (OR, 8.7 [95% CI, 0.95 to 91.6]) were both associated with late aspergillosis. Antigenemia was not included in this analysis because the assay was not performed in two of seven patients. In this subgroup, the

Table 2. Univariate Analysis of Risk Factors for Aspergillosis Development

Variable	Controls (%)	Cases (%)	OR (95% CI)	*P*
Preoperative variables				
Male	13/38 (34)	7/13 (54)	2.2 (0.53–9.76)	.35
Age (mean ± SD)	53 ± 14	51 ± 11	—	1.0
Child C	15/38 (39)	8/13 (61)	2.5 (0.57–10.89)	.29
Antibiotics (>7 days)	8/38 (21)	6/13 (46)	3.2 (0.79–12.6)	.14
Insulin administration	5/38 (13)	5/13 (38)	4.1 (0.78–38.4)	.09
ICU stay	3/38 (8)	5/13 (38)	7.2 (1.34–41.83)	.019
Surgical procedure	2/38 (5)	2/13 (33)	3.3 (0.28–38.4)	.26
Serum creatinine level (mean ± SD)	1.1 ± 0.8	1.6 ± 1.2	—	.09
Serum bilirubin level (mean ± SD)	8.5 ± 11.5	15.7 ± 25.2	—	.03
Intraoperative period				
Urgent indication for transplantation	3/38 (8)	4/13 (31)	5.2 (0.77–37.4)	.06
Fulminant hepatopathy	3/38 (8)	0	—	1.0
Retransplantation	4/38 (10)	6/13 (46)	7.3 (1.3–43.6)	.01
Packed red cell (mean ± SD)	8.4 ± 8.0	8.9 ± 8.1	—	.8
Graft cold ischemia length (h ± SD)	6.6 ± 2.1	6.3 ± 2.2	—	.61
Immediately postoperative period				
Dialysis requirements	2/38 (5)	4/13 (31)	8.0 (1.0–77.1)	.03
Days of ICU stay (mean ± SD)	9 ± 12	24.6 ± 23	—	.03
Days of intubation (mean ± SD)	4 ± 9	8 ± 17	—	.06
Surgical reintervention	8/38 (21)	4/13 (31)	1.67 (0.33–8.35)	.47
Prolonged antibiotic therapy (> 14 d)	11/38 (29)	7/13 (54)	2.86 (0.66–12.8)	.18
Cytomegalovirus infection	5/38 (13)	5/13 (38)	4.1 (0.78–22.8)	.09
Cytomegalovirus disease	2/38 (5)	4/13 (31)	8.0 (1–77.0)	.03
Aspergillus antigenemia in serum (galactomanan)*	2/33 (6)	5/9 (56)	19.4 (2.1–228.4)	.002

*Aspergillus antigenemia was performed in nine cases and 33 controls

only independent factor associated with aspergillosis in multivariate analysis was cytomegalovirus infection (OR, 95% CI, 6.7 [1.0-42.5]) (Table 4).

Discussion

We have documented a relatively high incidence of invasive aspergillosis in liver transplant recipients during the study period. *Aspergillus* spp. is the second most common fungal pathogen producing infection in liver transplant recipients. Invasive aspergillosis has been reported in 1.5% to 6.5% of liver transplantation patients,[18-20] although other investigators have reported higher frequencies.[6] Recently, a nationwide study carried out by the Spanish Study Group for Infections in the Transplant Patient documented the development of invasive aspergillosis in 2.8% of 1719 liver transplant recipients, with a case fatality rate of 86%.[21]

Table 3. Multivariate Analysis of Risk Factors for Aspergillosis Development

Variable	OR (95% CI)	*P*
Retransplantation	29.9 (2.10–425)	.02
Dialysis requirements	24.5 (1.25–354)	.03
Aspergillus antigenemia (galactomanan)	50.0 (3.56–650)	.003

Most cases of invasive aspergillosis in liver transplant recipients occur in an early period.[5-7] The frequency at which aspergillosis develops in the first 100 days after transplantation has ranged from 75% to 90% in most reports. Late occurrence of aspergillosis has been described only occasionally.[9,15] Surprisingly, we have found that more than half of the cases of aspergillosis in our hospital developed later in the posttransplantation period. Multiple reasons may be considered to explain the difference between the results of other series and ours. In comparison with other reports, the program of liver transplantation in our hospital began in 1994, thus allowing the incorporation of most recent innovations, including a better selection of candidates for transplantation as well as improvement in surgical procedures, prophylaxis measures, and immunosuppression. All of these factors may have led to a more prolonged survival, facilitating the development of aspergillosis in a later period.

Table 4. Comparison of Risk Factors in Patients Developing Aspergillosis After Day +100 of Transplantation

Variable	Controls (%)	Cases (%)	OR (95% CI)	*P*
Univariate analysis				
Late postransplant period				
Corticosteroids boluses	9/38 (24)	2/7 (28)	1.29 (0.14–10.02)	1.0
OKT3	1/38 (3)	1/7 (14)	6.1 (0–271.1)	.29
Chronic rejection	3/38 (8)	3/7 (43)	8.7 (0.95–91.6)	.04
Cytomegalovirus infection	8/38 (21)	5/7 (71)	9.38 (1.21–89.57)	.01
Cytomegalovirus disease	4/38 (10)	2/7 (28)	6.38 (0.76–58.0)	.06
Surgical reintervention	14/38 (37)	5/7 (71)	4.29 (0.59–37.98)	.11
Insulin requirements	10/38 (26)	1/7 (14)	0.47 (0.02–5.05)	.66
Multivariate analysis				
Late postransplant period				
Cytomegalovirus infection	—	—	6.7 (1.0–42.5)	.03

Risk factors for the development of invasive aspergillosis have been assessed in several reports. Most studies have analyzed risk factors for invasive aspergillosis related to the perioperative events. Recently, Singh et al[15,22] emphasized three main risk factors, which were also identified in other studies: poor allograft function or primary failure of the allograft[5,23]; renal dysfunction, particularly that requiring dialysis[5,17,24]; and increased immunosuppression, especially during cytomegalovirus coinfection and the use of OKT3 monoclonal antibody.[7,8] Other variables that reflect the allograft dysfunction, including thrombocytopenia, prolonged prothrombin values, and pretransplantation fulminate hepatic failure, have been associated with aspergillosis in other studies.[6]

In our study, univariate analysis of variables associated with aspergillosis identified most of the risk factors reported by other investigators. However, only retransplantation, the dialysis requirements after transplantation, and the presence of a positive *Aspergillus* antigenemia at any time point during the posttransplantation were independently associated with aspergillosis in the multivariate analysis.

Retransplantation and dialysis requirements are among the most reported risk factors for the development of aspergillosis in most series. However, we considerer of great importance the introduction of a new marker, not previously analyzed in other series, such as *Aspergillus* antigenemia. Antigenemia assay is promising as a trigger for pre-emptive therapy prior to overt clinical signs and symptoms of aspergillosis. Most studies assessing the value of *Aspergillus* antigenemia have been performed in high-risk hematologic patients.[10-13] A recent prospective study of hematologic patients at increased risk for aspergillosis showed sensitivity, specificity, and predictive values above 92%, and in cases of invasive aspergillosis, antigenemia was detected before clinical suspicion in more than half of the patients.[13] In transplantation, most studies assessing the efficacy of *Aspergillus* antigen detection have been performed in bone marrow recipients, and the experience in solid organ recipients is very limited.[14]

In contrast with the multiple articles assessing risk factors for early aspergillosis, the analysis of factors predisposing to late aspergillosis are scarce. Possibly, the small number of cases in the different series has precluded such an analysis. The frequent occurrence of late aspergillosis in this study has allowed us to perform an evaluation of risk factors in this group of patients. Cytomegalovirus infection and disseminated aspergillosis could both be adverse effects of previous increased immunosuppression; however, although chronic rejection was associated in univariate analysis, only cytomegalovirus infection was independently associated with aspergillosis in the multivariate analysis in this subgroup of patients. Unfortunately, antigenemia was not included in the analysis of late aspergillosis because this group of patients was small and the assay was not performed in all patients. In the only report that has previously analyzed predisposing factors to late aspergillosis, Singh et al[15] found that among the six cases that occurred after day 90 of transplantation, only cytomegalovirus infection was related with this form of aspergillosis. Previous cytomegalovirus disease or a cytomegalovirus–positive donor were shown to have an independent and striking relationship with the development of invasive fungal disease in analysis among liver transplant recipients[8] or lung transplant recipients.[9] The association between cytomegalovirus infection and aspergillosis is well explained by the immuno-

suppressive effect of this virus, which produces a dysregulation of cytokines.[9]

In conclusion, identification of high-risk patients could guide appropriate initiation of antifungal therapy or prophylactic measures in these patients. Risk factors for invasive aspergillosis in liver transplant recipients are different depending on the timing of transplantation. Hepatic and renal dysfunction predispose to *Aspergillus* infection during the early posttransplantation period. Cytomegalovirus infection and increased immunosuppression favor invasive aspergillosis during the late posttransplantation period. *Aspergillus* antigenemia seems to be a predictor of invasive aspergillosis. Monitoring high-risk groups of patients using *Aspergillus* antigenemia detection could be useful as a marker to determine when to consider starting empirical therapy with antifungal agents. These preliminary findings remain to be confirmed in larger studies.

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