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ORIGINAL ARTICLE

Risk Factors for Invasive Aspergillosis in Living Donor Liver Transplant Recipients

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Invasive aspergillosis (IA) is a severe complication of liver transplantation. Risk factors for IA after deceased donor liver transplantation (DDLT) have been presented in several reports, but are not well established for living donor liver transplant recipients. Here, a retrospective case-control study was performed. Five cases with IA were investigated after living donor liver transplantation (LDLT) between January 1999 and December 2002 at Kyoto University Hospital. For comparison, living donor liver transplant recipients without IA were taken as controls. These patients had undergone LDLT 1 month before or after each IA case and had the same survival times as the latter. We evaluated the clinical and laboratory findings for both groups up until their demise. Patients with IA after LDLT had a very poor prognosis. By univariate analysis, risk factors for IA were preoperative intensive care unit stay (P = 0.02) and preoperative steroid administration (P = 0.02). Preoperative steroid administration for fulminant hepatitis possibly predisposed to the development of IA after LDLT. ***Liver Transpl 13:566-570, 2007.*** © **2007 AASLD.**

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Living donor liver transplantation (LDLT) has been a predominant procedure in Asian countries. Increased indications and growing numbers of referrals for deceased donor liver transplantation (DDLT) have led to an increasing disparity between the number of patients waiting for transplantation and the number of donor organs, resulting in high mortality among candidates on the waiting list. Since the indications for LDLT were expanded from pediatric to adult liver diseases, such as hepatitis virus infections with or without hepatocellular carcinoma, LDLT has been accepted as a therapeutic alternative even in the United States and Europe.[1] Although small-for-size in LDLT leads to septic complications and higher mortality,[2] recent studies have shown comparable results in LDLT and DDLT for hepatitis C virus-positive patients.[3] Furthermore, the incidence of surgical site infections in LDLT was also similar to that reported in DDLT.[4] However, few studies have reported other infectious complications in LDLT.

Aspergillus spp. is the second most common fungal pathogen responsible for infection of liver transplant recipients. The frequency of invasive aspergillosis (IA) among DDLT recipients ranges from 1 to 8%,[5] but the IA-related mortality rate for these patients exceeded 90%[6] and an estimated 16.9% of all deaths among DDLT recipients were due to *Aspergillus* infections.[7] Several risk factors for IA in DDLT, such as renal dysfunction, retransplantation, the presence of *Aspergillus* antigenemia,[8] fulminant hepatic failure,[9] and use of OKT3,[10] have been reported. On the other hand, risk factors for IA in LDLT recipients have not been reported so far. We therefore conducted a retrospective case-control study to assess the risk factors for IA in LDLT.

PATIENTS AND METHODS

Study Population and Definition of IA

All 430 adult patients (≥18 yr old) who underwent LDLT at Kyoto University Hospital from January 1999 to De-

Abbreviations: IA, invasive aspergillosis; LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; CMV, cytomegalovirus.

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cember 2002 were included in this retrospective case-control study. IA was defined as any LDLT recipient meeting the criteria for proven or probable IA described in a previous report from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Co-operative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group.[11] *Aspergillus* antigen was detected by the latex agglutination test for galactomannan. Controls included the 2 recipients without *Aspergillus* infection who had undergone LDLT 1 month before or after each IA patient, and whose survival time was similar. No patient underwent retransplantation.

Immunosuppression

The details of donor evaluation, donor surgery protocols, surgical techniques, and perioperative management of recipients in Kyoto University Hospital have been described elsewhere.[3] The standard immunosuppressive regimen consisted of tacrolimus and low-dose corticosteroid. The whole-blood trough level of tacrolimus was adjusted to 10–15 ng/mL during the first 2 weeks and tapered thereafter. Methylprednisolone was administered intraoperatively before graft reperfusion, tapered from 1 mg/kg/day on day 1 to 0.3 mg/kg/day until the end of the first month, followed by 0.1 mg/kg/day until the end of the third month. If liver function was stable, steroids were discontinued after this time. Moderate or severe acute rejection was initially treated by bolus methylprednisolone. When mild liver dysfunction persisted after steroid therapy, azathioprine was added to the standard regimen until liver function again stabilized.

Antimicrobial Prophylaxis

Perioperative prophylaxis consisted of flomoxef, an oxacefem antibiotic, for 72 hours. Trimethoprim and sulfamethoxazole once daily was administered as prophylaxis against *Pneumocystis jirovecii* infection. Selective bowel decontamination was performed for 3 days before transplantation using oral kanamycin. Miconazole was administered for 7 days in all patients, followed by fluconazole as antifungal prophylaxis in severe cases after transplantation. In the IA-free controls, 2 patients were treated with fluconazole followed by miconazole. *Aspergillus* antigen detection was performed based on clinical criteria only. Cytomegalovirus (CMV) antigen detection was performed at regular intervals or based on clinical criteria only. Samples that had at least 1 CMV pp65 antigen-positive cell/50,000 polymorphonuclear cells were defined as positive for CMV antigenemia. In the presence of such CMV infection, regardless of clinical manifestations, preemptive ganciclovir treatment was started.

Risk Factors for IA

The following variables were assessed as risk factors for IA: 1) preoperative variables, including age, gender, fulminant hepatitis, Child-Turcotte-Pugh classification, blood-type compatibility, duration of the intensive care unit, duration of hospital stay, requirement for hemodialysis or hemofiltration, serum creatinine level, requirement for surgical intervention, requirement for mechanical ventilation, urgent requirement for surgery, administration of insulin, administration of corticosteroid at a cumulative dose of >0.5 gm in the 1 week before LDLT, administration of antibiotics, administration of antifungals; 2) intraoperative variables, including units of blood product transfused, operation time, graft cold ischemia time; and 3) postoperative variables, including survival time, duration of intensive care unit stay, duration of mechanical ventilation, hemodialysis, or hemofiltration, maximum serum creatinine level, requirement for surgical intervention, requirement for bolus administration of corticosteroid, and CMV infection, presence of bacteremia, and presence of fungemia.

Statistical Analysis

Risk factors for the development of IA were identified by comparison of patients with the control group. Characteristics of cases and controls were compared using Student's *t*-test for continuous data and the Mann-Whitney U-test for categorical data. Odds ratios and 95% confidence intervals are given with Fisher's exact test and *P*-values to illustrate the degree of risk associated with some of the factors considered. A *P*-value <0.05 was considered to indicate statistical significance. Statistical analysis of the data was performed with Statview 5.0 (SAS Institute Inc., Cary, NC) for Macintosh.

RESULTS

Characteristics of the Study Population

One proven case of IA and 4 probable cases developed during the study period. The incidence of IA was thus 1.2% (5/430). The characteristics of the 5 cases are shown in Table 1. Three patients (60.0%) were male, and mean age was 47.5 yr (range: 42 to 53 yr). The predominant underlying disease was fulminant hepatitis (60.0%, 3 patients). All had pulmonary involvement alone. The proven IA case was diagnosed at autopsy. *Aspergillus* was cultured from sputum in 3 cases and from bronchial lavage in 2. Chest computed tomography was performed in 3 cases at diagnosis and halo sign was observed in one. Case 1 was discharged and infected with *Aspergillus* after readmittance.

Time of Occurrence and Clinical Course

Three cases of IA occurred within 30 days after surgery and 4 within 90 days. Mean time from operation to diagnosis was 93.0 ± 135.7 days (Table 1). Antifungal prophylaxis was applied in all cases. Amphotericin B deoxycholate was administered to all IA cases at a dose of 0.2 to 2.0 mg/kg/day after diagnosis. However, all case patients died, 3 within 10 days and all within 30

TABLE 1. Clinical Course of Invasive Aspergillosis Patients

Case	Age/gender	Underlying disease	Diagnosis	Days after LDLT	Days after diagnosis	Prophylaxis	Therapy
1	48/M	Liver cirrhosis	Proven	333	3	MCZ	AMB
2	48/M	Subacute FH	Probable	29	13	MCZ	AMB, ICZ
3	45/M	FH	Probable	67	8	MCZ, FCZ	AMB, ICZ
4	53/F	PBC	Probable	22	7	MCZ, FCZ	AMB, ICZ
5	42/F	Subacute FH	Probable	14	26	MCZ, FCZ	AMB, 5-FC

Abbreviations: M, male; F, female; FH, fulminant hepatitis; PBC, primary biliary cirrhosis; LDLT, living donor liver transplantation; MCZ, miconazole; AMB, amphotericin B; FCZ, fluconazole; ICZ, itraconazole; 5-FC, flucytosine.

days. Mean time from diagnosis to death was 11.4 ± 8.9 days (Table 1).

Risk Factors

Risk factors for IA, including 17 preoperative variables, 3 intraoperative variables, and 10 postoperative variables, were examined by univariate analysis. Five IA cases and 10 controls were evaluated in this case-control study. All control patients also died, with the same mean survival time as the IA cases ($P = 0.81$). The pretransplantation donor (D)/recipient (R) CMV serostatus was 5 D+/R+ in IA cases, 9 D+/R+ and 1D+/R− in controls, and unknown in 5 donors. Thus, the CMV match did not differ ($P = 0.99$). Factors associated with the development of IA were duration of intensive care unit stay ($P = 0.02$) and administration of corticosteroid ($P = 0.02$) before LDLT (Table 2).

DISCUSSION

This is the first case-control study of IA in LDLT recipients. The incidence of IA in this study (1.2%) was comparable with the overall incidence in DDLT recipients (2%).[5] In DDLT, 50% to 75% of IA cases occur early after transplantation (less than 90 days).[12] The median time to onset after transplantation was 16 days in 1 study[13] and 31 days in another.[9] However, Singh et al.[12] reported that the median time to onset in the earlier cohort from 1990 to 1995 was 17 days, whereas in the later cohort from 1998 to 2002 it was 99 days. Significant allograft dysfunction is a known risk factor for IA. One reason for this change from early- to late-onset IA (more than 90 days after operation) may be the increasing proportion of hepatitis C virus infection as the underlying liver disease in the later cohort[12] because hepatic dysfunction after liver transplantation for hepatitis C virus infection often occurs later posttransplantation. The median time to onset after transplantation in the present study was 93.0 days (range 14-333 days). Except for case 1, who had hepatitis C virus infection and developed late-onset IA, the remaining 4 patients developed early-onset IA at an average of 33.0 days. The early-onset IA patients were predisposed to allograft failure because 3 patients had fulminant hepatitis, 5 had poor hepatic function (Child-Turcotte-Pugh "C") before transplantation, and 2 required surgery urgently.

All the IA patients in this study died, as is the case in DDLT, with mortality as high as 85 to 92%.[6-8] Singh et al.[12] reported a lower mortality in patients receiving transplants (60%) in the later cohort between 1998 and 2002 because of an overall lesser severity of underlying diseases such as hepatitis C virus infection.[8,12] Although our patients were transplanted over the same period as Singh et al's[12] later cohort, the underlying disease in the present study was more severe and was more similar to their earlier cohort from between 1990 and 1995, which showed a higher mortality rate (92%).

Most of the reported risk factors for the development of IA in DDLT recipients are postoperative, such as renal dysfunction and CMV or bacterial diseases.[8,14] In the present study, 3 cases before LDLT and all cases after LDLT underwent hemodialysis or hemofiltration and, as a result, cases stayed longer in the intensive care unit. Furthermore, 4 cases had CMV infection after LDLT. Thus, the reason why these factors were not significant in the present study might be that most controls with poor outcomes also had these factors. Postoperative administration of corticosteroid increased the risk for late-onset IA, but not for early-onset IA.[14] In contrast, preoperative administration of corticosteroid increased the risk in our patients. The difference between the previous reports and this study may be attributed to the increasing use of corticosteroid pulse therapy for fulminant hepatitis and hepatic failure before transplantation in Japan.[15] Fulminant hepatitis before transplantation has itself been reported to be a risk factor. In the present study, fulminant hepatitis tended to be a risk for IA but could not be assigned statistical significance. Corticosteroid was administered to all fulminant hepatitis cases but was not administered to only 1 control before LDLT. Thus, corticosteroid therapy for fulminant hepatitis possibly increases the risk for IA. Because the incidence of IA was small and the risk factors were preoperative, factors applying to living donor graft, such as small-for-size, are not likely to be associated with the development of IA. However, there are certain limitations to our study, such as the low numbers of cases analyzed, the retrospective nature of the data, and as a result the lack of generalizability of our results.

TABLE 2. Univariate Analysis of Risk Factors for Invasive Aspergillosis

	Case	Control	OR (95% CI)	*P*-value
Preoperative				
Male	3/5	5/10	1.5 (0.17-13.2)	0.99
Age	47.5 ± 4.6	44.8 ± 11.7	—	0.90
Fulminant hepatitis	3/5	2/10	6.0 (0.56-64.0)	0.25
Child-Turcotte-Pugh "C"	5/5	5/10	—	0.10
ABO blood group incompatibility	0/5	3/10	—	0.51
CMV match	5/5	9/10	—	0.99
ICU stay (days)	3.0 ± 2.7	0.5 ± 1.6	—	0.02
Hospital stay (days)	41.0 ± 42.9	50.3 ± 63.7	—	0.71
Hemodialysis or hemofiltration	3/5	3/10	3.5 (0.37-33.0)	0.33
Serum creatinine level	1.1 ± 0.7	2.4 ± 3.1	—	0.38
Surgical intervention	0/5	2/10	—	0.52
Mechanical ventilation	1/5	1/10	2.3 (0.11-45.7)	0.99
Urgent operation	2/5	2/10	2.7 (0.25-28.4)	0.56
Insulin administration	1/5	0/10	—	0.33
Antibiotics administration	3/5	2/10	6.0 (0.56-64.0)	0.25
Antifungals administration	2/5	1/10	6.0 (0.39-92.3)	0.24
Steroid administration	3/5	0/10	—	0.02
Intraoperative variables				
Total units of blood product	22.4 ± 11.1	33.2 ± 21.9	—	0.24
Operation time (minutes)	649.6 ± 124.2	741.4 ± 164.0	—	0.33
Graft cold ischemia time	75.4 ± 45.5	148.7 ± 181.9	—	0.71
Postoperative				
Survival time (days)	102.4 ± 132.4	98.6 ± 83.1	—	0.81
ICU stay (days)	18.6 ± 7.8	16.5 ± 24.3	—	0.09
Days of mechanical ventilation	9.6 ± 5.8	15.8 ± 24.0	—	0.42
Hemodialysis or hemofiltration	5/5	7/10	—	0.51
Serum creatinine level	3.9 ± 0.5	4.3 ± 2.7	—	0.75
Surgical intervention	1/5	2/10	1.0 (0.07-14.6)	0.99
Steroid bolus	4/5	8/10	1.0 (0.07-14.6)	0.99
Cytomegalovirus infection	4/5	4/10	6.0 (0.48-75.4)	0.28
Bacteremia	2/5	3/10	1.6 (0.17-14.7)	0.99
Fungemia	0/5	1/10	—	0.99

NOTE: Case and control data shown is number of patients or mean ± standard deviation.
Abbreviations: ICU, intensive care unit; OR, odds ratio; CI, confidence interval.

Antifungal prophylaxis including miconazole or fluconazole has been shown to reduce invasive fungal infection caused by many *Candida* species, but to have no impact on IA[16] and also in the present study. A recent study showed efficacy of amphotericin B lipid complex for IA prophylaxis in high-risk liver transplant recipients, such as those requiring dialysis.[17] A retrospective study reported the superiority of amphotericin B lipid complex over amphotericin B deoxycholate for treating IA.[18] Chest computed tomography[19] and galactomannan assay[20] are useful as a means of radiological and serological diagnosis for IA. However, in the present study, chest computed tomography was performed only in 3 cases and the halo sign was observed in only 1. The latex agglutination test for galactomannan was positive in 3 cases but the sensitivity of the test is inferior to enzyme-linked immunosorbent assay.[21]

In conclusion, the incidence of IA in our series of 430 LDLT recipients (1.2%) was comparable with previous reports in DDLT recipients. All IA cases had poor hepatic function before transplantation and most of them developed IA in the early posttransplantation period. Preoperative steroid administration for fulminant hepatitis possibly predisposed to the development of IA. Living donor graft factors are not likely to be associated with poor outcome. Prophylaxis and treatment with amphotericin B lipid complex and monitoring by chest computed tomography and enzyme-linked immunosorbent assay test for galactomannnan is likely to decrease the incidence and improve the outcome of IA in LDLT recipients with identified risk factors.

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