Management of Fungal Colonization and Infection after Living Donor Liver Transplantation.

Running title: Fungal infection after liver transplantation

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Abstract:

Background/Aims: Control of infection is important in liver transplant patients under immunosuppressive conditions. In particular, invasive fungal infection is often fatal if diagnosis and therapy are delayed. The aim of this study was to analyze the incidence of fungal colonization and infection after living donor liver transplantation (LDLT).

Methodollgy: Retrospective analysis was performed with 60 consecutive adult recipients of LDLT.

Results: Fungi were isolated from specimens of 16 (26.7%) patients after LDLT. All the fungi were *Candida species*. One patient for whom *Candida species* were isolated in ascites and blood was complicated with systemic methicillin-resistant Staphylococcus aureus and cytomegalovirus infection. In the univariate analysis, fungal carriage before surgery ($p=0.01$) was associated with fungal isolation after LDLT. In the multivariate analysis, fungal carriage was found to be an independent predictor of fungal isolation (odds ratio: 15.7, $p=0.03$). Of the 60 recipients, 16 (26.7%) showed serum levels of β-D glucan above 60pg/ml after surgery. Among these, 4 died and were all complicated with severe bacterial infection.

Conclusion: Preoperative fungal carriage was associated with fungal isolation after LDLT. If fungal infection was suspected after LDLT, along with treatment against fungi, control of complicated infections with other pathogens to be simultaneously considered.

Introduction

Living donor liver transplantation (LDLT) is widely accepted as an effective modality for various types of irreversible liver disease. Due to advances in new immunosuppressive agents and strategies, refinement of surgical techniques, and more effective postoperative care, the outcomes of LDLT have improved significantly. Recipients, however, tend to suffer from infection due to poor medical state, the technical complexity of the surgical procedure, and immunosuppressive conditions. Microorganisms, which are not a problem after usual surgery, can be pathogens and threaten patient lives. Among them, invasive fungal infection occurs in 7–42% of deceased donor liver transplantation (DDLT) recipients, and *Candida* and *Aspergillus species* are the majority pathogens (1). After DDLT, the mortality rates of invasive fungal infection by *Candida* and *Aspergillus species* are 70% and 92 – 100% respectively. Although there have been several reports of fungal infection in DDLT patients, few studies of LDLT patients have been reported (2, 3). However, to overcome the problem of fungal infection after LDLT, the details regarding these types of infection should be investigated and we review herein the present state of fungal infection in 60 consecutive adult LDLT cases.

Methodology

Study population

From August 1997 to June 2007, 70 consecutive cases of LDLT were carried out at our hospital in 67 patients by a single team. Of these patients, 9 who were younger than 15 years old, and 1 patient, whose medical record could not be located were excluded.

Immunosuppression after LDLT

Basically, patients were administered calcineurin inhibitor (tacrolimus or cyclosporine A) and corticosteroids as primary immunosuppressive therapy. Oral predonine discontinued in 3 months to 1 year. Patients with poor renal function received basiliximab, interleukin-2 receptor monoclonal antibodies, as induction to delay the use of calcineurin inhibitor. If acute cellular rejection was suspected, percutaneous liver biopsy was carried out to make a diagnosis, and bolus injection of methylprednisolone was started. In addition to the above-mentioned therapy, ABO-incompatible LDLT recipients underwent more intensive immunosuppressive therapy. They were given rituximab, a monoclonal chimeric human-murine anti-CD20 antibody, to deplete the B cells 1 week before surgery, and took apheresis and/or double-filtration plasmapheresis to reduce anti-A or anti-B antibody titers perioperatively. To control the local intravascular coagulation in graft, prostaglandin E1 for 3 weeks and methylprednisolone for 2 weeks were infused through the catheter that is put into the hepatic artery or portal vein.

Surveillance in fungal infection

Before LDLT, specimens from pharyngeal swabs, nasal swabs, urine, and feces were cultured. Serum levels of β-D glucan were measured by a kinetic chromogenic method with a commercially available kit (Fungitec G-Test MK, Seikagaku Corporation, Tokyo, Japan), in the clinical laboratory in our hospital. Routine surveillance cultures after LDLT were obtained from pharyngeal swabs, nasal swabs, endotracheal tubes, urine, feces, abdominal drains (ascites), and bile from biliary external stent and serum levels of β-D glucan were determined

once a week until the patients were discharged.

Preventive therapy and treatment

Amphotericin B syrup is generally given through nasal-gastric tubes, and then orally to all the recipients for prophylaxis against fungal infection in gastro-intestinal tract for the first 4 weeks after LDLT. In our protocol, however, other perioperative prophylaxis against fungal infection was not usually given to the recipients. When fungal infection or colonization was suspected, such as with patients having positive specimens with fungus or high serum levels of β-D glucan regardless of their symptom, or a high fever without any other identified reasons, fluconazole (FLCZ) (100 – 200mg/day) or micafungin sodium (MCFG) (after 2004, 50–150mg/day) was given intravenously.

Risk factor analysis

The following variables were assessed in relation to fungal isolation after LDLT. The data assessed included age, sex, diabetes mellitus, fulminant hepatic failure, MELD (the model for end-stage liver disease) score, fungal carrier state before surgery, actual graft volume/standard liver volume ratio, hemodialysis/apheresis before LDLT, emergency LDLT, duration of surgery, amount of blood loss, splenectomy, ABO-incompatible, hapaticojejunostomy, basiliximab injection, hemodialysis/apheresis after LDLT, cytomegalovirus serostatus, bacterial infection, reoperation, and mycophenolate mofetil (MMF) administration.

Statistical analysis

Univariate analysis was used to clarify the relationships between each variable and fungal infection. The chi-square test or, for small numbers, Fisher's exact test was used for

comparison of categorical data. Continuous variables were compared using the Student's *t*-test. A p value <0.05 was considered statistically significant. For multivariate analysis, the factors identified to be associated with p value <0.20 were entered into a stepwise logistic regression analysis to make out independent risk factors for infections.

Results

Characteristics of the study population

Out of 60 patients, 38 (63.3%) were male, and the age range was 16 to 68 years (mean: 50.3 years). Underlying liver diseases were as follows: hepatitis C virus-related liver cirrhosis (LC) with or without hepatocellular carcinoma (HCC) (19 patients, 31.7%), hepatitis B virus-related LC with or without HCC (13 patients, 21.7%), fulminant hepatic failure (7 patients, 11.7%), primary biliary cirrhosis (5 patients, 8.3%), alcohol-induced LC (3 patients, 5.0%), graft failure (3 patients, 5.0%), congenital biliary atresia (2 patients, 3.3%), and non-alcoholic steatohepatitis, Budd-Chiari syndrome, cryptogenic LC with HCC, primary sclerosing cholangitis, idiopathic portal hypertension, Caroli disease, and autoimmune hepatitis (1 patient each).

Fungal isolation

After LDLT, fungi were isolated by culture in 16 (26.7%) patients before they were discharged (Table 1). The mean time for the first isolation of fungus was 16.9 days (range, 0-88 days), and 8 (50.0%) patients were isolated within 7 days of surgery. The sites where fungi were isolated were pharynxes in 7 patients, feces in 6, urine in 3, ascites in 2, endotracheal

tubes in 2, nasal cavity in 1, bile in 1, gastric juice in 1, central venous catheter in 1, and blood in 1. All the fungi that were isolated were *Candida* (*C.*) *species*, namely, *C. albicans* in 6 (37.5%) patients, *C. glabrata* in 3 (18.8%), *C. parapsilos* in 3 (18.8%), *C. krusei* in 1 (6.3%), *C. tropicalis* in 1 (6.3%), and *C. guilliermondii* in 1 (6.3%). Of all the patients for whom fungi were isolated in cultures, 1 male patient died 48 days after surgery. He showed *Candida species* in ascites, urine, and blood, following systemic methicillin-resistant Staphylococcus aureus and cytomegalovirus infection.

Risk factor analysis

The characteristics of fungus-isolated and non-isolated patients are compared in Table 2. In the variables, fungal carriage before surgery ($p=0.01$) was found to be associated with fungal isolation after LDLT in the univariate analysis. In the multivariate analysis, 6 risk factors with $p<0.2$ were tested, and only fungal carriage before surgery was found to be an independent predictor of fungal isolation (odds ratio: 15.7, $p=0.03$).

β-D glucan

The characteristics of all the 16 patients whose serum levels of β-D glucan above 60pg/ml after surgery are shown in Table 3. On the basis of previous reports (4), 60pg/ml was chosen as the cutoff. Fungi were isolated in cultures from the specimens in only 4 out of 16 patients. Twelve patients were given antifungal agents (FLCZ to 6, MCFG to 6) when the serum levels of β-D glucan were above high values regardless of the isolation of fungi. Four deceased patients showed high serum levels of β-D glucan at relatively late POD (23, 25, 12, and 39, respectively) and they were all complicated with bacterial infections.

Discussion

In a clinical setting, distinguishing between fungal colonization and infection is often difficult because non-sterile sites such as the pharynx or nasal cavity frequently harbor fungi without evidence of inflammation or invasion (5). Generally, in order not to delay therapy, we begin administration of antifungal agents if fungus is isolated in cultures from specimens normally considered sterile, as immunosuppressive conditions easily lead to fungal colonization overgrowth and infection. Historically, although *C. albicans* has accounted for more than 80% of isolated *Candida* species in liver transplant recipients, the incidence of FLCZ-resistant *non-albicans Candida* increased after the late 1990s partially due to widespread use of FLCZ (6). In our assessed period, the emergent rate of *C. albicans* was only 37.5% and we chose MCFG for treatment after 2004.

We assessed some known factors that are associated with an increased risk of developing fungal infection (Table 2). In the present study, multivariate analysis indicated that only fungal carriage is associated with fungal isolation after surgery. One reason for the dissociation from previous reports probably originated from our sample extraction; namely, we assessed fungus-identified patients as including both colonization and infection.

Early identification and treatment should be quite essential but sensitivity of surveillance culture is not sufficient (8). Obayashi et al. have reported that the sensitivity of blood culture for the detection of fungal infection is only 8.3% (4). In addition, identification of fungi can take days to weeks (9). Measurement of β-D Glucan, which is derived from fungal cell walls, has emerged as a rapid adjunct diagnostic strategy for invasive fungal infection, especially

in Japan. Recently, this test was included as one of the microbiological criteria for probable invasive fungal disease in the Consensus Revised Definitions Draft VI produced by the joint committee of the European Organization for Research and Treatment of Cancer and the United States Mycology Study Group (10). Forty-three (71.7%) patients showed serum levels of β-D glucan above 20pg/ml, which is the manufacturer's recommended cutoff. Autopsy study showed the sensitivity and specificity of the assay to be 85.4% and 95.2%, respectively, at a cutoff value of 60pg/ml, with the same commercial kit as ours (4). Based on this report, the patients who showed serum levels of β-D glucan at greater than 60pg/ml were chosen in this study, and we then evaluated their characteristics (Table 3). Serum β-D glucan can show a false-positive by the influence of blood product (11), dialysis membrane (12), and use of cotton gauze during surgery (13), and intra- or immediately after operation, many recipients can be affected by these products. Additionally, one of the major sites of eliminating β-D glucan is Kupffer cells in the liver (14), whose phagocytic function is impaired immediately after liver transplantation (15). Less β-D glucan may be eliminated from the blood stream, and it is likely to show a false-positive at early time points after surgery. Recipients showing positive serum levels of β-D glucan soon after surgery with no other symptoms or findings, may not need to be given antifungal agents under a strict follow-up. If recipients are suspected of having fungal infection with high serum levels of β-D glucan or any other findings, however, not only control of fungal infection but also bacterial infection is quite important.

Deceased donors are generally in the ICU, on mechanical ventilation, and receiving antibiotics and/or corticosteroids, and these clinical circumstances may allow fungi to colonize in

their oropharyngeal and respiratory tract. In contrast, donors are basically healthy in LDLT and there should be fewer chances to have fungal colonization. In addition, most LDLTs are carried out electively and it is possible to make a full investigation of infections. Recently, Kawagishi, et al. have reported that 8.3% of recipients of LDLT suffer from definitive or probable invasive fungal infection in a single institute (3). In order to compare characteristics of fungal infection between LDLT and DDLT, further investigations are needed in the future.

In conclusion, all fungi that were isolated after LDLT in our institute were *Candida species*. Preoperative fungal carriage was associated with fungal isolation after LDLT. If fungal infection is suspected, antifungal therapy should be carried out concomitantly with antibacterial management.

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Table 1. The Characteristics of the patients with fungal isolation after operation

Age/Sex	Underlying Liver Disease	Fungal Carriage Before Operation	β-Dglucan (POD)	Site of Fungal isolation (POD)	Treatment	Outcome
36/M	PBC	-	199 (1)	Ascites (3)	FLCZ	alive
45/M	PBC	Oral cavity	23 (11)	Feces (10)	-	alive
58/M	HCV-LC	-	32 (7)	Pharynx (1)	-	alive
55/F	HBV-LC	-	36 (4)	Feces (17)	-	alive
57/M	HCV-LC /HCC	-	42 (4), 28 (31)	Pharynx (32~)	-	alive
48/M	HBV-LC /HCC	-	37 (4)	Pharynx (3)	-	alive
60/F	HCV-LC /HCC	Pharynx	42 (17), 263 (24), 86 (45)	Endotracheal tube (88)	MCFG	alive
51/M	Alcohol-induced cirrhosis	-	109 (4)	Pharynx (7)	-	alive
60/M	HBV-LC /HCC	-	31 (4), 35 (26)	bile (1)	MCFG	alive
53/M	HBV-LC	Endotracheal tube	normal	Endotracheal tube, Pharynx, Feces, Gastric juice (2)	MCFG	alive
57/M	HBV-LC/HCC	-	34 (4), 58 (16)	Feces (4), CV catheter (8)	MCFG	alive
57/M	HCV-LC /HCC	-	35 (2), 195 (9), 48 (21), 106 (39)	Ascites (36), Urine (39), Blood (42)	MCFG	Died on 48 POD (sepsis, MOF)
62/F	HCV-LC	Pharynx	normal	Urine (4)	MCFG	alive
45/F	AIH	-	38 (12)	Urine (19), Feces (26)	MCFG	alive
33/F	HBV-LC /HCC	-	27 (3)	Pharynx, Feces (24)	-	alive
66/M	PSC	Pharynx, Feces	44 (13), 28 (17)	Pharynx (6), Nasal cavity (12)	MCFG	alive

Abbreviations: PBC, Primary biliary cirrhosis; HCV, hepatitis C virus; LC, liver cirrhosis; HBV, hepatis B virus; HCC, hepatocellular carcinoma; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; POD, postoperative days; FLCZ, fluconazole; MCFG, micafungin sodium; MOF, multiple organ failure.

Table 2 Comparison of patients with and without fungal isolation by univariate analysis

	Fungus isolated (n=16)	Fungus non-isolated (n=44)	p value
Age (y)	52.7	4.4	0.41
Sex (male)	11(68.8%)	27 (61.4%)	0.60
Diabetes mellitus	6 (37.5%)	17 (37.8%)	0.94
Fuluminant hepatic failure	0 (0.0%)	7 (15.9%)	0.10
MELD score	19.6	21.3	0.52
Fungal carriage	5 (31.3%)	2 (4.5%)	0.01
aGV/SLV(%)	47.3	51.8	0.08
Hemodialysis/apheresis before LDLT	3 (18.8%)	14 (31.8%)	0.26
Emergency LDLT	1 (6.3%)	7 (15.9%)	0.33
Duration of surgery (minutes)	981	1052	0.32
Amount of blood loss (ml)	12289	16989	0.41
Splenectomy	1 (6.3%)	8 (18.2)	0.25
ABO-incompatible	2 (12.5%)	5 (11.4%)	0.90
Hepaticojejunostomy	3 (18.8%)	9 (20.5%)	0.88
Basilixibab injection	1 (6.3%)	10 (22.7%)	0.14
Steroid bolus injection	6 (37.5%)	13 (29.5)	0.56
Hemodialysis/apheresis after LDLT	2 (12.5%)	10 (22.7%)	0.38
CMV antigenemia	5 (31.3%)	20 (45.5%)	0.25
Bacterial infection	3 (18.8%)	17 (37.8%)	0.15
Re-operation	8 (50.0%)	13 (19.5%)	0.32
Mycophenolate mofetil intake	4 (25.0%)	18 (40.9%)	0.26

NOTE: Data are numbers (%) of patients unless indicated otherwise.
Abbreviations: MELD, the model for end-stage liver disease; aGV, actual graft volume; SLV, standard liver volume; LDLT, living donor liver transplantation; CMV, cytomegalovirus.

Table 3. The Characteristics of the patients with high levels of serum β-D glucan

Age/Sex	Underlying Liver Disease	β-D glucan (POD)	Fungal Detection (POD)	Re-operation (POD)	Bacterial infection (POD)	Pathogens of bacterial infection	Anti-fungal thearpy	Outcome
36/M	PBC	199 (1)	Ascites (3)	-	-	na	FLCZ	Alive
28/F	FHF	63 (16)	-	-	-	na	-	Alive
33/F	FHF	116 (3)	-	-	-	na	FLCZ	Alive
20/M	FHF	152 (2)	-	-	Wound infection	na	-	Alive
57/M	HCV-LC /HCC	165 (23)	-	Portal stenosis (12)	Pleural effusion, Ascites, Bactere mia (20)	MRSA	FLCZ	Dead on 30 POD (sepsis, MOF)
53/F	FHF	109 (3)	-	-	CV catheter, Bactere mia (10)	Ps. aeruginos a	FLCZ	Alive
41/M	PBC	64 (3), 81 (25)	-	Decrease in portal flow (6)	Ascited (20), Bactere mia (25)	GPC	-	Dead on 31 POD (MOF)
61/M	HCV-LC	126 (12)	-	HAT (8), Abdominal Bleeding (13)	Ascites (3), Bactere mia (10)	GNR	FLCZ	Dead on 16 POD (MOF)
56/M	HBV-LC /HCC	190 (18)	-	-	-	na	FLCZ	Alive (re-trans plant after 8 months)
22/M	Graft failure	292 (38)	-	Abdominal Bleeding (3)	Pneumo niae 14)	na	MCFG	Alive
56/M	Graft failure	96 (15)	-	Leakage of hepatico-jej unostomy	-	na	MCFG	Alive
60/F	HCV-LC /HCC	263 (24),	Endotrac heal tube	HAT (2), Portal	Ascites, Bile,	MRSA, GNR,	MCFG	Alive

		86 (45)	(88)	thrombosis (5)	Pneumoniae, Bacteremia (25~65)	GPC		
51/M	Alcohol-induced cirrhosis	109 (4)	Pharynx (17)	-	-	na	-	Alive
54/F	HCV-LC /HCC	104 (4)	-	Exploratory laparotomy (19)	-	na	MCFG	Alive
57/M	HCV-LC /HCC	195 (9), 106 (39)	Ascites (36), Urine (39), Blood (42)	Abdominal abscess (13, 29)	Ascites, Bacteremia (11~45)	MRSA	MCFG	Dead on 48 POD (sepsis, MOF)
59/F	HCV-LC	166 (3)	-	-	-	na	MCFG	Alive

Abbreviations: PBC, Primary biliary cirrhosis; FHF, fulminant hepatic failure; HCV, hepatitis C virus; LC, liver cirrhosis; HBV, hepatis B virus; HCC, hepatocellular carcinoma; POD, postoperative days; HAT, hepatic arterial thrombosi