

Significance of serum  $\beta$ -D-glucan levels in recipients of living donor liver transplantation.

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## Abstract

**Background/Purpose:** Early identification and treatment of fungal infections is essential for recipients of liver transplants, but the sensitivity of surveillance culture is insufficient. Measurement of the serum level of  $\beta$ -D-glucan is a rapid diagnostic strategy for invasive fungal infection. We aimed to evaluate the significance of serum  $\beta$ -D-glucan levels in transplant recipients after living donor liver transplantation (LDLT).

**Methods:** We retrospectively analyzed the clinical and laboratory data of 100 consecutive adult transplant recipients after LDLT performed between August 1997 and August 2009.

**Results:** 71 had high serum  $\beta$ -D-glucan levels ( $>20$  pg/ml) after LDLT. Nearly half (47.2%) of the episodes of increase occurred within the first 5 days after surgery. The mortality rate of the recipients with high serum  $\beta$ -D-glucan levels was similar to that of the recipients without high levels. However, in terms of the time line of increase, the recipients with high serum  $\beta$ -D-glucan levels from 15 days onward after surgery showed a significantly higher mortality rate than those with high levels before 15 days after surgery (33.3% and 4.3%, respectively;  $p < 0.001$ ).

**Conclusions:** High serum levels of  $\beta$ -D-glucan at late time points after LDLT indicate established fungal infection and higher mortality.

## Introduction

Liver transplantation is widely accepted as an effective modality for irreversible liver diseases. However, because of their poor medical state, involving liver failure, surgical invasion, and immunosuppressive management, recipients tend to suffer from infection. Invasive fungal infection has been noted in 7%–42% of the deceased recipients of liver transplants, and the associated mortality rates are 70%–100% (1). Although early identification and treatment is essential, the sensitivity of surveillance culture is insufficient (2). Further, fungal identification is time consuming, as it can take days to weeks. Measurement of the serum levels of  $\beta$ -D-glucan, a fungal cell-wall component, has emerged as a rapid adjunct diagnostic strategy for invasive fungal infection, especially in Japan (4, 5). However, as reported previously, measurement of serum  $\beta$ -D-glucan can yield a false-positive result immediately after liver transplantation (3). In this retrospective study, we aimed to evaluate the significance of the serum  $\beta$ -D-glucan levels of transplant recipients after living donor liver transplantation (LDLT).

## Methods

We reviewed the clinical and laboratory data of 100 consecutive adult transplant recipients after LDLT performed between August 1997 and August 2009 at our hospital.

### Immunosuppression after LDLT

Basically, the patients were administered a calcineurin inhibitor (tacrolimus or cyclosporine A) and corticosteroids as primary immunosuppressive therapy. Methylprednisolone was injected intravenously during surgery at a dose of 20 mg/kg and at a dose of 2 to one mg/kg/day tapered for 1 to 6 postoperative days followed by oral predonine at 0.3 mg/kg/day (7 to 28 days), 0.2 mg/kg/day (after 28days), and discontinued in 3 months to one year. If acute cellular rejection was proven, bolus injections of methylprednisolone were started. In addition to the above-mentioned therapy, the ABO-incompatible LDLT recipients underwent more intensive immunosuppressive therapy, in that they were given rituximab, a monoclonal chimeric human-murine anti-CD20 antibody, to deplete the B cells one week before surgery, and took apheresis and/or double-filtration plasmapheresis to reduce the anti-A or anti-B antibody titers perioperatively. To control the local

intravascular coagulation in the graft, prostaglandin E1 for 3 weeks and methylprednisolone for 2 weeks were infused through a catheter that was put into the hepatic artery or portal vein. In addition, mycophenolate mofetil (MMF) was given as soon as possible after transplantation.

#### Surveillance of fungal infection

Before LDLT, specimens from pharyngeal swabs, nasal swabs, urine, and feces were cultured and the serum levels of  $\beta$ -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 at our hospital. Routine surveillance cultures after LDLT were obtained and the serum levels of  $\beta$ -D-glucan were determined weekly. When the recipients developed high fever, specimens of blood, pharyngeal swabs, nasal swabs, urine, and feces were cultured.

#### Preventive therapy and treatment

Amphotericin B syrup was first administered through nasogastric tubes and then orally to all the recipients during the first 4 weeks after LDLT, for prophylaxis against fungal infection in the gastrointestinal tract. When fungal infection or colonization was suspected or proved, fluconazole (100–200

mg/day) or micafungin sodium (after 2004, 50–150 mg/day) was administered intravenously.

### Statistical analyses

The data are presented as the mean  $\pm$  standard deviation (SD). The Student's t-test was used for comparisons of continuous variables and the chi-square ( $\chi^2$ ) test was used for categorical variables. A p-value of  $<0.05$  was considered statistically significant. Statistical analysis was performed with StatMate III for Macintosh (ATMS Co., Ltd, Tokyo, Japan).

## Results

### Characteristics of the study population

Of the 100 patients, 63 were male, and the age range was 16–68 years (mean = 51.6 years). The underlying liver diseases were as follows: hepatitis C virus-related liver cirrhosis (LC) with or without hepatocellular carcinoma (HCC; 27 cases); hepatitis B virus-related LC with or without HCC (25 cases); hepatitis C+B virus-related LC with or without HCC (three cases); fulminant hepatic failure (seven cases); primary biliary cirrhosis (10 cases); alcohol-induced LC (nine cases); graft failure (four cases); cryptogenic LC with or without HCC (four cases); primary sclerosing cholangitis (three cases); biliary atresia (two cases); nonalcoholic steatohepatitis (two cases); Budd-Chiari syndrome, idiopathic portal hypertension, Caroli disease, and autoimmune hepatitis (one case each).

### Characteristics of the recipients who had high levels of $\beta$ -D-glucan

Seventy-one recipients had 89 episodes of increased serum levels of  $\beta$ -D-glucan, greater than 20 pg/ml, which is the manufacturer's recommended cutoff value. The time line of increase in the  $\beta$ -D-glucan levels is shown in Figure 1. Forty-two episodes (47.2%) of increase occurred within the first 5

days after LDLT. Among the 89 episodes of high serum  $\beta$ -D-glucan levels, only 21 (23.6%) were associated with positive fungal cultures. The recipients with high levels of  $\beta$ -D-glucan had positive fungal cultures more frequently than those without high levels of  $\beta$ -D-glucan (29.6% and 10.3%, respectively;  $p < 0.05$ ; Figure 2). Fungi were isolated from the pharynx (nine cases), feces (nine cases), ascites (three cases), urine (two cases), endotracheal tubes (two cases), bile (two cases), nasal cavity (one case), central venous catheter (one case), sputum (one case), surgical wound (one case), and blood (one case). All the fungi that were isolated were *Candida* (*C.*) *species*, namely, *C. albicans* (20 cases, 62.5%), *C. parapsilosis* (five cases, 15.6%), *C. glabrata* (four cases, 12.5%), *C. krusei* (one case, 3.3%), *C. tropicalis* (one case, 3.3%), and *C. guilliermondii* (one case, 3.3%).

#### Association between serum $\beta$ -D-glucan levels and prognosis after LDLT

During hospitalization, the mortality rate of the 71 recipients who had high serum levels of  $\beta$ -D-glucan ( $>20$  pg/ml) was similar to that of the 29 recipients who did not have high levels (14.1% and 6.9%, respectively;  $p = 0.32$ ; Figure 3a). However, when the time line of increase was considered, the recipients with high serum levels from 15 days onward after LDLT showed a significantly higher mortality rate than those with high levels before 15 days



after LDLT (33.3% and 4.3%, respectively;  $p < 0.001$ ) and those without high levels (6.9%,  $p < 0.05$ ; Figure 3b). When the 25 recipients who showed high serum levels of  $\beta$ -D-glucan from 15 days onward after surgery were analyzed, the deceased recipients showed higher levels of  $\beta$ -D-glucan than the living ones (159.6 pg/ml and 56.3 pg/ml, respectively;  $p < 0.05$ ; Figure 4). The rate of positive fungal cultures between the groups, however, was not different statistically (data not shown). Six of 10 deceased recipients with high serum levels of  $\beta$ -D-glucan died at least partly because of proven (by culture) infection including sepsis or bacteremia.

## Discussion

The usefulness of the serum  $\beta$ -D-glucan level for diagnosing invasive fungal infections in critically ill patients has been reported (5). In our study, the transplant recipients frequently showed high serum levels of  $\beta$ -D-glucan during the early period after LDLT: nearly half of the episodes of increase occurred within 5 days of surgery. Further, the recipients with high serum  $\beta$ -D-glucan levels at the late time points showed a significantly higher mortality rate. From these results, high  $\beta$ -D-glucan levels at late time points after LDLT are likely to indicate established, invasive fungal infection in recipients.

Measurement of serum  $\beta$ -D-glucan can yield a false-positive result because of the influence of cellulose membranes, which are used to process blood products (6). Usami et al. analyzed blood products consisting of albumins, immunoglobulins, and blood-coagulation factors from various brands: 75%, 40%, and 63% of the samples, respectively, were positive for  $\beta$ -D-glucan in vitro (6). Intraoperatively or immediately after operation, the blood of many recipients can be affected by these products; for instance, albumin is often administered to recipients to compensate for drained ascites until the graft liver regenerates to a sufficient size and the portal vein pressure decreases after LDLT. Use of cotton gauze during surgery is also associated with increase in the serum levels

of  $\beta$ -D-glucan (7). Further, the phagocytic function of Kupffer cells, which eliminate  $\beta$ -D-glucan (8), is impaired immediately after liver transplantation (9). Consequently, recipients could frequently show a false-positive result at early time points after surgery.

In our study, the recipients with high serum levels of  $\beta$ -D-glucan showed positive fungal cultures more frequently than those without high levels. However, the rate of positive fungal cultures in all the recipients with the high levels was below 30%. Obayashi et al. reported that the sensitivity of blood culture for detecting fungal infection is only 8.3% in autopsy cases (10). Moreover, colonization should be considered among the cases of positive fungal detection especially at unsterile sites such as feces, endotracheal tubes, and the nasal cavity. The sensitivity and specificity of fungal culture should have been low in our series, as previously reported.

As shown in Figure 4, the  $\beta$ -D-glucan levels were significantly higher in the deceased recipients than in the living ones. Theoretically, high serum levels of  $\beta$ -D-glucan suggest greater amounts of fungi in the blood stream and reflect the severity of infection, because  $\beta$ -D-glucan is a component of the fungal cell wall.

Regarding the cutoff level of  $\beta$ -D-glucan, in the autopsy study with 41 patients by Obayashi et al. (10), the sensitivity and specificity of the assay were

85.4% and 95.2%, respectively, at a cutoff value of 60 pg/ml and the specificity with this cutoff value was much higher than that at a cutoff value of 30 pg/ml. Further studies are necessary to determine the appropriate cutoff value in the case of transplantation, which may demonstrate diverse values of serum  $\beta$ -D-glucan at different time points.

At early time points after LDLT, serum levels of  $\beta$ -D-glucan could be very confusing. Pazos et al. have reported data indicating that potential false-positive results should be considered with sudden rises and falls (12). Recipients showing high serum levels of  $\beta$ -D glucan soon after surgery with no other symptoms or findings, may not need to be given antifungal agents under a strict follow-up.

In conclusion, high serum levels of  $\beta$ -D-glucan at late time points after LDLT indicate established fungal infection and higher mortality, even if fungi are undetected by standard culture.

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Figure 1

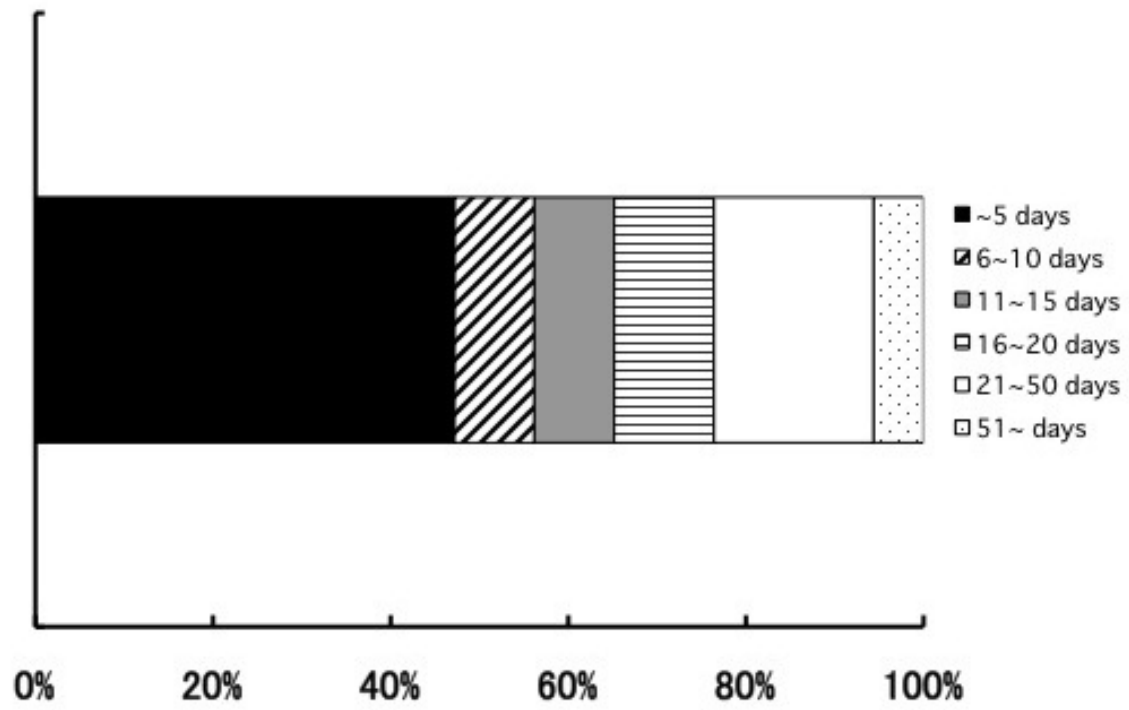


Figure 2

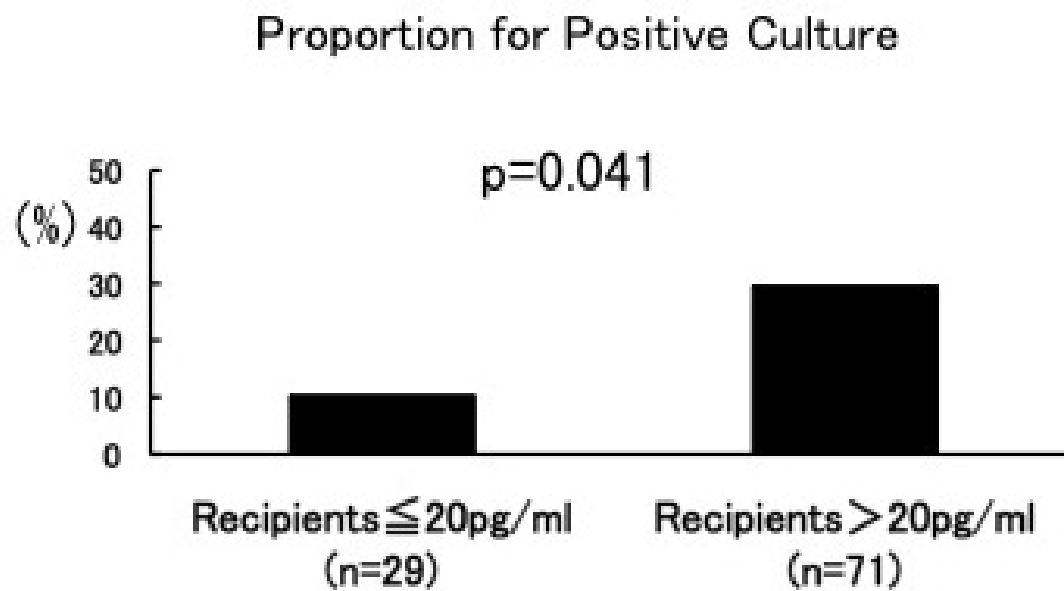




Figure 3

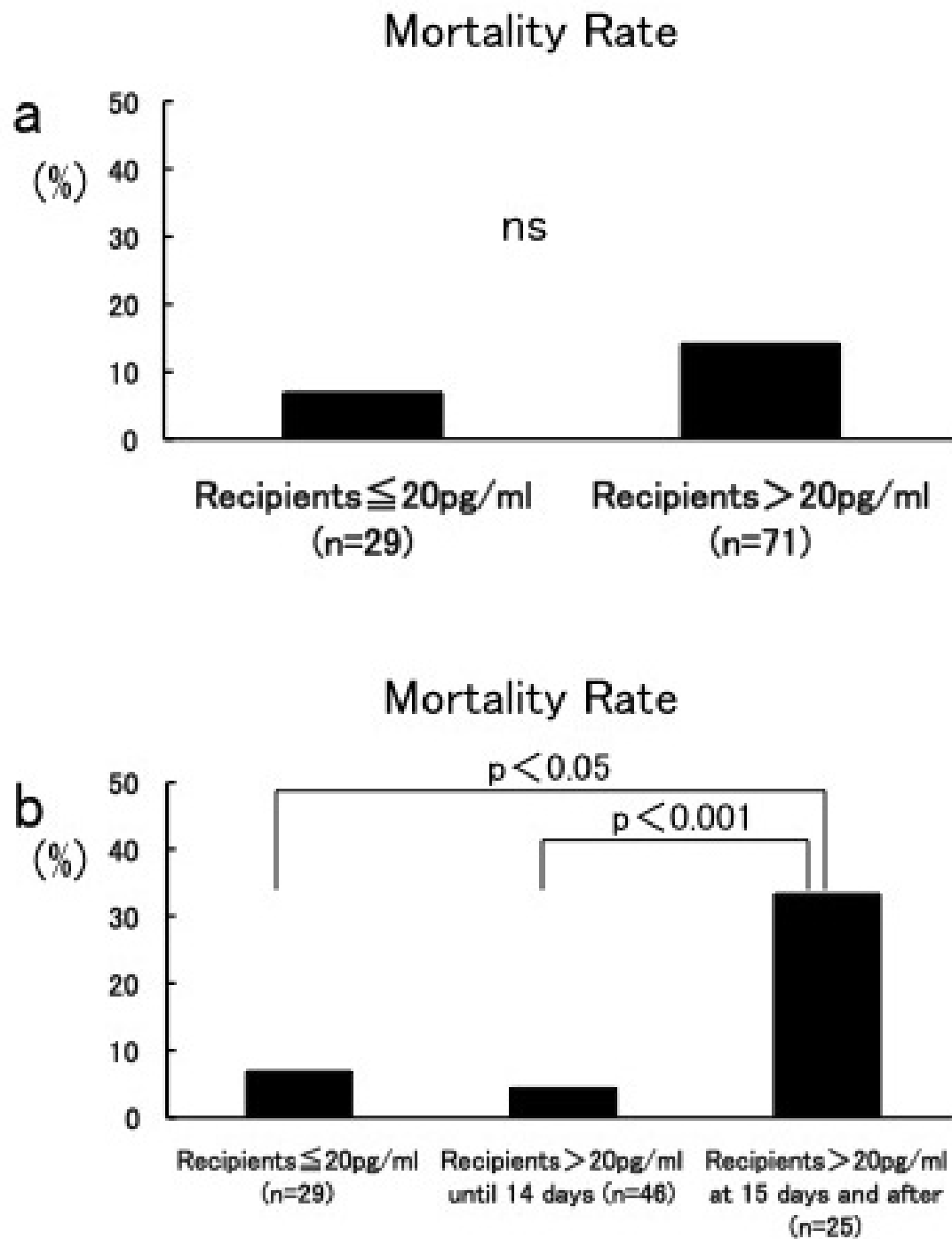


Figure 4

