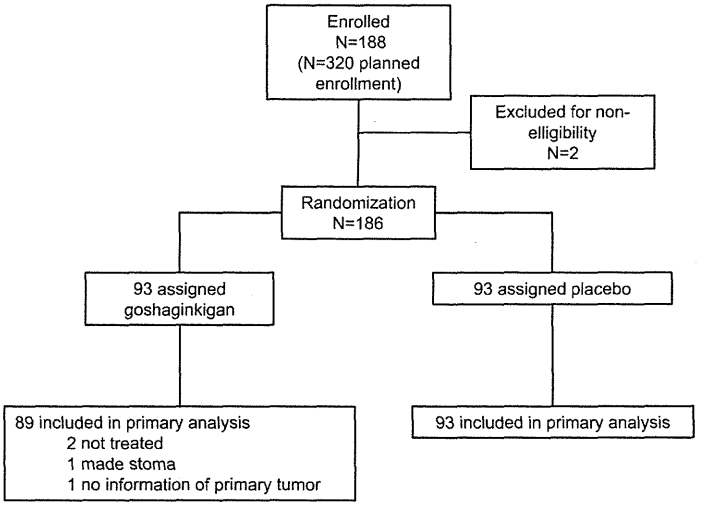


**Fig. 1** CONSORT flow diagram for this study. The initial plan was to enroll 310 patients in this clinical trial. This flow diagram pertains to the 188 patients who were enrolled before the trial was discontinued. Two patients in the *Goshajinkigan* (GJG) group were excluded from the primary analysis because one patient had a stoma and the other patient had a primary tumor that could not be assessed

## Results

After including 155 patients (one-half the number of the planned registration), a preplanned interim analysis was undertaken for 142 patients who had undergone more than one course of adjuvant chemotherapy by May 7, 2012. After careful examination of the interim data, the independent data monitoring committee (IDMC) recommended that the entry of new patients into the study should be halted. During the temporary halting of the trial, the IDMC inspected the placebo formulation and the storage conditions in some hospitals. No switching of the placebo and GJG was found to have taken place in any hospital, and on August 2, 2012 the IDMC finally recommended that the study should be discontinued.

At the time of study closure, 189 patients were enrolled and 186 patients had been randomly assigned from 39 hospitals. *Goshajinkigan* had been prescribed to 91 patients and the placebo had been prescribed to 93 patients (Fig. 1). Two patients from the GJG group were excluded: one patient had a stoma and the other patient had a primary tumor that could not be assessed. There was no difference between the groups in patient background (Table 1). The most common comorbidities were hypertension and diabetes, but there was no difference between the groups in the incidence of either disorder.

The incidence of grade 1 peripheral neuropathy based on the NCI CTCAE classification was 43.8 % in the GJG group and 62.4 % in the placebo group. By contrast, the incidence of grade 2 or greater peripheral neuropathy was 50.6 % in the GJG group and 31.2 % in the placebo group (Table 2). Figure 2 shows the TTN curve, based on the incidence of grade 2 peripheral neuropathy, which was the primary endpoint of the study. Surprisingly, in the interim analysis, the TTN was significantly less in the GJG group. The TTN was measured according to the criteria of NCI CTCAE version 3.0 (Fig. 2) and DEB-NTC (see Fig. S1 in the Electronic supplementary material, ESM). It was significantly less in the GJG group in both assessments. The difference was more significant in the updated analysis performed 8 months after the first data cutoff point (Figs. S1C and S1D in the ESM). A Cox proportional hazards model analysis indicated that the hazard ratio (HR) was 1.908 ($p = 0.007$) in the final analysis performed in accordance with NCI CTCAE criteria. Figure S2 in the ESM shows the TTN for each grade by course. *Goshajinkigan* did not reduce the TTN, even for grade 1. The forest plot indicated that the risk of peripheral neuropathy was greater among patients in the GJG group in most parameters (Fig. 3). Adverse events other than neurotoxicity were also evaluated, and no between-group differences in hematologic and nonhematologic events were noted (Table 3).

A secondary endpoint of this trial was a comparison of the FOLFOX6 dose intensity and of the rates of discontinuation as a result of neuropathy in the two arms. The total dose of L-OHP was 793.47 mg/m$^2$ in the GJG group and 749.69 mg/m$^2$ in the placebo group. The average cycle of chemotherapy was 9.0 in the GJG group and 8.3 in the placebo group. The mean relative dose intensity planned protocol of oxaliplatin up until the onset of grade 2 or greater

**Table 1** Background data for the patients

| Variables | *Goshajinkigan* 89 | Placebo 93 | $p$* |
|---|---|---|---|
| Sex | | | |
| Male | 48 (53.9) | 51 (54.8) | 1.000 |
| Female | 41 (46.1) | 42 (45.2) | |
| Age (years, mean ± SD) | 62.4 ± 10.6 | 60.4 ± 11.5 | 0.215 |
| Stage | | | |
| p-Stage IIIA | 61 (68.5) | 67 (72.0) | 0.629 |
| p-Stage IIIB | 28 (31.5) | 26 (28.0) | |
| Tumor location | | | |
| Right | 32 (36.0) | 29 (31.2) | 0.532 |
| Left | 57 (64.0) | 64 (68.8) | |
| Tumor | | | |
| T1 + T2 | 13 (14.6) | 10 (10.8) | 0.506 |
| T3 + T4 | 76 (85.4) | 83 (89.2) | |
| Lymph node metastasis | | | |
| N1 | 60 (67.4) | 66 (71.0) | 0.633 |
| N2, N3 | 29 (32.6) | 27 (29.0) | |
| Cr | | | |
| <60 mL/min | 12 (13.5) | 9 (9.7) | 0.490 |
| ≥60 mL/min | 77 (86.5) | 84 (90.3) | |
| Comorbidities | | | |
| None | 58 (65.2) | 71 (76.3) | 0.105 |
| Positive | 31 (34.8) | 22 (23.7) | |
| Performance status (ECOG) | | | |
| 0 | 85 (95.5) | 92 (98.9) | 0.204 |
| 1 | 4 (4.5) | 1 (1.1) | |

*ECOG* Eastern Cooperative Oncology Group; *SD* standard deviation

*All variables were compared using Fisher's exact test

**Table 2** Proportions of the patients who experienced peripheral neuropathy, listed according to grade (NCI CTCAE; version 3.0)

| Grade | *Goshajinkigan* | | Placebo | |
|---|---|---|---|---|
| | *N* | (%) | *N* | (%) |
| 0 | 5 | 5.6 | 6 | 6.5 |
| 1 | 39 | 43.8 | 58 | 62.4 |
| 2 | 30 | 33.7 | 19 | 20.4 |
| 3 | 15 | 16.9 | 10 | 10.8 |
| Grade 1 and greater | 84 | 94.4 | 87 | 93.5 |
| Grade 2 and greater | 45 | 50.6 | 29 | 31.2 |
| Grade 3 and greater | 15 | 16.9 | 10 | 10.8 |
| Total | 89 | 100 | 93 | 100 |

*NCI CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events

peripheral neuropathy was 94.7 % in the GJG group and 94.9 % in the placebo group. The mean dose intensity by course was 70.9 mg/m$^2$/course in the GJG group and 67.1 mg/m$^2$/course in the placebo group ($p = 0.033$). As a result, the relative dose intensity of oxaliplatin in the GJG group was 83.1 %, which was higher than that of the placebo group (Table 4). Thus, the dose intensity and treatment cycle were higher in the GJG group. Whether the dose intensity influenced the effect of the chemotherapy was not determined. It was specified in the protocol that the assessment of prognosis would be investigated five years later.

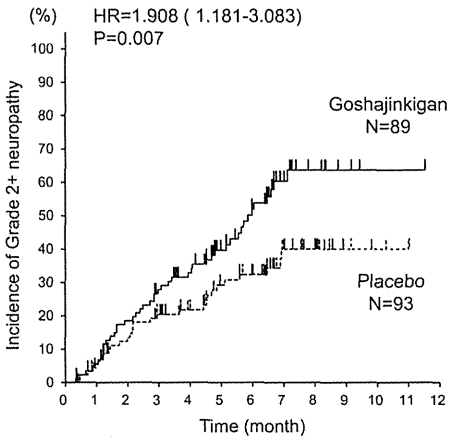


**Fig. 2** Time to grade 2 or greater sensory neuropathy (TTN), as measured according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, version 3.0). The *black* and *dotted lines* represent the *Goshajinkigan* (GJG) group and the placebo group, respectively

## Discussion

Oxaliplatin is metabolized to oxalate and dichloro(1,2-diaminocyclohexane) platinum (Pt(dach)$Cl_2$) [22]. Oxalate is a well-known chelator of $Ca^{2+}$ and $Mg^{2+}$. Oxaliplatin causes severe peripheral neuropathy, which is characterized by two types of neurological symptoms. One symptom occurs within hours of oxaliplatin infusion in 90 % of patients and includes acral paresthesia and dysesthesia triggered or enhanced by exposure to cold (i.e., acute neuropathy); this is caused by oxalate [10]. The other symptom is characterized by a loss of sensation and motor dysfunction after long-term oxaliplatin therapy (i.e., chronic neuropathy); this is possibly caused by Pt(dach)$Cl_2$ [22]. Acute neuropathy is peculiar to oxaliplatin. Some in vitro studies have shown that oxaliplatin modulates voltage-gated sodium ($Na^+$) and potassium ($K^+$) channels. In clinical studies, Ca and Mg infusions have been attempted as a

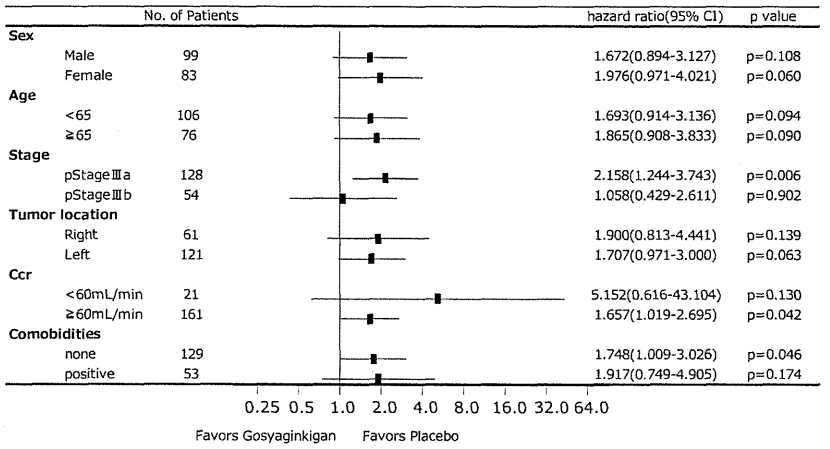


**Fig. 3** Hazard ratios (along with the 95 % confidence intervals) for the time to neuropathy

means to reduce oxaliplatin-induced neurotoxicity, because these elements are known chelators of oxalate [8, 23, 24]. However, a recent phase III clinical study did not show that these infusions were able to prevent oxaliplatin-induced neurotoxicity [25]. Therefore, neurotoxicity remains the greatest difficulty associated with the use of oxaliplatin.

*Goshajinkigan* is composed of 10 herbs, each of which contains numerous active ingredients. It is primarily used in Japan to improve symptoms such as numbness, cold sensation, and limb pain associated with diabetic neuropathy [13, 26, 27]. Two mechanisms have been suggested by which GJG may alleviate peripheral neurotoxicity. The first mechanism is its promotion of the release of dynorphin, which improves numbness/paresthesia via the opiate system. The second mechanism is its promotion of nitric oxide production, which improves circulation and the blood supply to the nerves. *Goshajinkigan* prevents oxaliplatin-induced acute peripheral neuropathy in rats [28]. A phase II study to investigate the efficacy of GJG against peripheral neurotoxicity induced by FOLFOX therapy has shown promising results [29]. Another small retrospective study reported that the incidence of peripheral neuropathy was markedly lower among GJG recipients [19]. Therefore, we proposed a phase III study to investigate the preventive effect of GJG on peripheral neurotoxicity associated with FOLFOX6 therapy. This is the first clinical double-blind phase III study to evaluate the usefulness of this herbal medicine and to ascertain if it can suppress oxaliplatin-induced peripheral neurotoxicity.

**Table 3** Hematologic and nonhematologic adverse events

| | All grades (%) | | | Grade 3/4 (%) | | |
|---|---|---|---|---|---|---|
| | *Goshajinkigan* | Placebo | *p** | *Goshajinkigan* | Placebo | *p** |
| Anorexia | 62 (68.9) | 68 (73.1) | 0.625 | 3 (3.3) | 3 (3.2) | 1.000 |
| Fatigue | 59 (65.6) | 62 (66.7) | 0.877 | 0 (0.0) | 0 (0.0) | N.E. |
| Nausea | 65 (72.2) | 71 (76.3) | 0.612 | 2 (2.2) | 2 (2.2) | 1.000 |
| Vomiting | 23 (25.6) | 31 (33.3) | 0.261 | 1 (1.1) | 0 (0.0) | 0.492 |
| Diarrhea | 32 (35.6) | 28 (30.1) | 0.529 | 3 (3.3) | 2 (2.2) | 0.679 |
| Allergic reaction | 15 (16.7) | 17 (18.3) | 0.847 | 1 (1.1) | 2 (2.2) | 1.000 |
| Chromatosis | 19 (21.1) | 17 (18.3) | 0.711 | 0 (0.0) | 0 (0.0) | N.E. |
| Anemia | 54 (60.0) | 52 (55.9) | 0.654 | 0 (0.0) | 1 (1.1) | 1.000 |
| Leucopenia | 55 (61.1) | 59 (63.4) | 0.762 | 5 (5.6) | 6 (6.5) | 1.000 |
| Neutropenia | 63 (70.0) | 70 (75.3) | 0.507 | 32 (35.6) | 39 (41.9) | 0.448 |
| Thrombocytopenia | 55 (61.1) | 47 (50.5) | 0.181 | 2 (2.2) | 2 (2.2) | 1.000 |

*NE* not evaluated

*All comparisons were performed using Fisher's exact test

**Table 4** Dose intensity of oxaliplatin until the onset of grade 2 or greater peripheral neuropathy

| Dose intensity | *Goshajinkigan* | Placebo | $p$* |
|---|---|---|---|
| | 89 | 93 | |
| Relative dose intensity planned protocol (%) | | | |
| Case number | 89 | 93 | |
| Mean ± SD | 94.72 ± 7.05 | 94.98 ± 7.16 | 0.803 |
| Median | 96.37 | 96.75 | |
| Dose intensity (mg/m$^2$/course) | | | |
| Case number | 86 | 91 | |
| Mean ± SD | 70.90 ± 10.17 | 67.14 ± 12.90 | 0.033 |
| Median | 72.78 | 67.83 | |
| Relative dose intensity (%) | | | |
| Case number | 86 | 91 | |
| Mean ± SD | 83.41 ± 11.96 | 78.99 ± 15.17 | 0.033 |
| Median | 85.62 | 79.80 | |

*SD* standard deviation

*All comparisons were performed using the Student $t$ test

To evaluate subjective adverse neurotoxic events, we assumed a primary endpoint of TTN (i.e., the interval between treatment initiation and grade 2 neuropathy, based on NCI CTCAE criteria). A discrepancy between the NCI CTCAE and DEB-NTC criteria has been reported in relation to their use in the evaluation of oxaliplatin-related neurotoxicity. Thus, it may be that the concomitant use of NCI CTCAE and DEB-NTC would be useful for ensuring better quality oxaliplatin-based chemotherapy. Because it was very important to perform an objective assessment of neuropathy, we used both NCI CTCAE and DEB-NTC to investigate peripheral neuropathy. In the interim analysis, the TTN was consequently significantly less in patients in the GJG group than in recipients in the placebo group. This tendency was detected when using both NCI CTCAE and DEB-NTC. Even if this study had continued, GJG could not have been significantly superior to placebo. Therefore, the IDMC recommended termination of the study after it had confirmed that the placebo and GJG had not been switched at the participating institutions. As with infusions of Ca and Mg, GJG cannot suppress chronic neuropathy that may be induced by Pt(dach)$Cl_2$. Calcium/magnesium infusions have also been reported as promising in retrospective and small prospective studies, but not in double-blind clinical studies [9, 30]. Some pharmacological agents may improve mild symptoms by suppressing acute neuropathy, and this type of bias may have led to the promising results of a small-scale study. However, this small advantage may have been balanced out by the placebo effect in a double-blind study. Further studies should be undertaken to determine how chronic damage induced by Pt(dach)$Cl_2$ can be suppressed by pharmacological agents.

Dose intensity was a secondary endpoint in this trial. The dose intensity of oxaliplatin and average cycle of adjuvant chemotherapy were higher in the GJG group than in the placebo group. *Goshajinkigan* may prevent acute mild neuropathy and increase the dose intensity of oxaliplatin, but it may consequently cause severe neuropathy. In 5 years, we plan to re-evaluate the overall survival and relapse-free survival. There is a possibility that GJG has greater effect on chemotherapy. It is also necessary to investigate the administration time of GJG to prevent L-OHP neurotoxicity. After the oral administration of GJG, the ingredient that provides neuroprotection is absorbed rapidly and achieves a maximum concentration ($C_{max}$) value within 60 min (unpublished data). The time when GJG was administered may be associated with the prevention of chronic neuropathy and the dose intensity of L-OHP.

In conclusion, GJG did not prevent oxaliplatin-associated peripheral neuropathy in this clinical trial. At present, herbal medicine (Kampo) should not be used to reduce acute mild neuropathy because they may induce chronic severe neuropathy.

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Hepatology Research

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*Original Article*

# Risk factors for hepatitis B virus recurrence after living donor liver transplantation: A 17-year experience at a single center

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*Aim:* The incidence of hepatitis B virus (HBV) recurrence after liver transplantation (LT) has been reduced by prophylaxis with hepatitis B immunoglobulin (HBIG) and nucleoside analogs, but the factors associated with HBV recurrence are unclear. The aim of this study was to determine the risk factors associated with HBV recurrence after living donor LT (LDLT).

*Methods:* A retrospective review was performed for 45 patients (28 male and 17 female; median age, 54 years) who underwent LDLT for HBV-related liver disease and were followed up for at least 6 months between October 1996 and June 2013. The virological data, tumor burden, antiviral therapy and immunosuppressive therapy were evaluated and compared between the HBV recurrence ad non-recurrence groups.

*Results:* Seven of the 45 patients (15.6%) developed post-LT HBV recurrence. The median interval between LDLT and HBV recurrence was 23.7 months (range, 0.8–35.9). Three of the seven patients (42.9%) developed recurrence after cessation of HBIG, and three (42.9%) were cases with hepatocellular carcinoma (HCC) recurrence after LDLT. The remaining case underwent transplantation from a donor with positive hepatitis B surface antigen. Based on the univariate and multivariate analyses, HBIG cessation (hazard ratio [HR], 20.17; 95% confidence interval [95% CI], 2.091–194.593; $P = 0.009$) and HCC recurrence (HR, 30.835; 95% CI, 3.132–303.593; $P = 0.003$) were independent risk factors for HBV recurrence after LDLT.

*Conclusion:* In LDLT patients, cessation of HBIG and HCC recurrence were risk factors associated with HBV recurrence, so careful monitoring for serological HBV markers is needed in patients with these factors.

**Key words:** hepatitis B immunoglobulin, hepatitis B virus recurrence, hepatocellular carcinoma, living donor liver transplantation

## INTRODUCTION

HEPATITIS B IS a leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide. Before the advent of an effective means for preventing the virtually universal re-infection of the graft, the outcome of liver transplantation (LT) for hepatitis B virus (HBV)-related liver diseases was dismal, and this often led to HBV recurrence rates greater than 80% and mortality rates of 50% at 2 years.[1] Prophylaxis with hepatitis B immunoglobulin (HBIG) and nucleoside analogs, such as lamivudine, has markedly decreased the recurrence rate of HBV through their synergistic effects.[2] However, approximately 10% of transplanted patients still develop HBV recurrence.[3,4] In previous studies, the factors associated with HBV recurrence were reported to be a high pre-LT HBV DNA level,[5,6] hepatitis B e-antigen (HBeAg) positivity,[7] non-fulminant hepatitis B,[8] immunosuppression from steroids and systemic chemotherapy,[9] and pre-LT HCC and post-LT HCC recurrence.[10–12]

In this study, we noted that a group of patients still developed HBV recurrence after living donor LT (LDLT). We analyzed a retrospective series of 45 patients who underwent LDLT for HBV-related liver disease and were followed for at least 6 months, and we evaluated their virological and biochemical data, tumor burden, antiviral therapy and immunosuppressive therapy, as well as the eventual development of HCC recurrence. The aim of this

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study was to determine the risk factors associated with HBV recurrence after LDLT.

## METHODS

### Immunoprophylaxis

ALL PATIENTS WERE treated with a combination HBIG (Hebsbulin-IH; Japan Blood Products Organization, Tokyo, Japan) and at least one nucleoside agent (lamivudine, adefovir, entecavir or a combination thereof) for HBV prophylaxis after transplantation.

Nucleoside analog therapy was initiated when the patients were referred to the hospital and indicated for LDLT, if they had not yet been treated with this agent. For fulminant hepatitis B patients, lamivudine or entecavir was initiated when the etiology was verified to be HBV. HBIG at 10 000 U was administrated i.v. during the anhepatic phase during the operation, followed by 5000 U/day for 1 week after the LDLT. Thereafter, 3000–5000 U of HBIG was administrated every 2–3 months. The targeted level of hepatitis B surface antibody (HBsAb) was more than 500 IU/L for the cases before March 2004, and was more than 200 IU/L for the first year and more than 100 IU/L thereafter for the cases after April 2004.[13]

### Immunosuppression

A calcineurin inhibitor, such as cyclosporin or tacrolimus, with or without mycophenolate mofetil, was used as the immunosuppressive therapy after LDLT. The immunosuppressive dosing was adjusted according to the therapeutic drug levels and renal function. A gram of methylprednisolone was given after reperfusion, and the dose was tapered from 200 mg to 20 mg daily in a week, then switched to oral prednisolone, and finally tapered off in 6 months.

### Serological monitoring

The recurrence of the HBV was defined as the appearance of the hepatitis B surface antigen (HBsAg) in the serum after LDLT.[8,10] Standard biochemical tests of liver function were performed at each follow-up visit. The serum HBsAg, HBsAb and HBV DNA were tested monthly. From 1996 until March 2004, the HBV DNA levels were quantified with a transcription-mediated amplification assay (Mitsubishi Chemical Medience, Tokyo, Japan), which has a detection range of 3.7–8.7 log genome equivalents (LGE)/mL. Thereafter, all HBV DNA levels were tested with a polymerase chain reaction (PCR) assay (SRL, Tokyo, Japan), which has a detection range of 2.6–7.6 log copies/mL. The YMDD mutant was detected using a PCR enzyme-linked minisequence assay (SRL).

### Surveillance for HCC recurrence

After LDLT, patients with known HCC were followed regularly in our outpatient hepatology clinic. Surveillance with computed tomography was performed every 3–4 months. If there were concerns about HCC recurrence, whole-body computed tomography or magnetic resonance imaging was ordered at the discretion of the patient's physician.

### Statistical analysis

Continuous variables were compared by the Mann–Whitney *U*-test. Categorical variables were compared by the $\chi^2$-test and Fisher's exact tests. A Cox regression analysis was used to determine the predictors of the time to HBV recurrence. The variables reaching statistical significance by the univariate analysis were then included in the multivariate analysis. The cumulative incidence of patient survival and HBV recurrence after LDLT were calculated using the Kaplan–Meier method, and the difference was evaluated by the log-rank test. A value of $P < 0.05$ was considered significant. Statistical analyses were performed using the SPSS version 17.0 software package (SPSS, Chicago, IL, USA).

## RESULTS

### Demographics

A RETROSPECTIVE REVIEW of the medical record database was performed for 45 patients (28 male and 17 female; median age, 54 years) who underwent LDLT for HBV-related liver disease and were followed up at least 6 months between October 1996 and June 2013 at Kyushu University. Table 1 summarizes the patients' data at the time of LDLT. The median follow-up time after LDLT was 66 months (range, 9–174). Hepatitis C virus co-infection was present in three patients (6.7%). HCC was present in 28 patients (62.2%). Twenty-five patients with HCC (89.3%) were diagnosed by preoperative computed tomography or magnetic resonance imaging, whereas three patients (10.7%) were diagnosed incidentally by a pathological examination of the explant. Six patients (21.4%) had evidence of vascular invasion. HCC beyond the Milan criteria was present in eight patients (28.8%) by preoperative imaging, and in 15 patients (53.8%) by explant pathology, respectively.

Pre-LT HCC therapy was administrated to 17 of the 28 patients with HCC (60.7%). Among them, two patients (7.1%) received pre-LT systemic chemotherapy, which consisted of a combination of an antimetabolite (5-fluorouracil), platinum-based agent (cisplatin) and anthracycline (epirubicin). Fourteen patients (50%) received a combination of local ablative therapy in the

**Table 1** Characteristics of the 45 patients with hepatitis B virus-related liver disease

| | HBV-related transplantation ($n=45$) |
|---|---|
| Age (years) | 54 (31–67) |
| Sex | |
| Male | 28 (62.2%) |
| Female | 17 (37.8%) |
| HCV co-infection | 3 (6.7%) |
| Primary disease | |
| Acute liver failure | 12 (26.7%) |
| Liver cirrhosis | 33 (73.3%) |
| HBsAg positivity | 41 (91.1%) |
| HBeAg positivity | 10 (22.2%) |
| HBV DNA (log copies/mL) | |
| Unknown | 5 (11.1%) |
| <2.6 | 20 (44.4%) |
| 2.6–5 | 8 (17.8%) |
| >5 | 12 (26.7%) |
| HCC | 28 (62.2%) |
| By preoperative imaging | 25 (89.3%) |
| Incidental on explant | 3 (10.7%) |
| Vascular invasion | 6 (21.4%) |
| Beyond Milan criteria by: | |
| preoperative imaging | 8 (28.8%) |
| explant pathology | 15 (53.8%) |
| Pre-LT systemic chemotherapy | 2 (7.1%) |
| Post-LT systemic chemotherapy | 3 (10.7%) |
| Pre-LT antiviral therapy | |
| None | 4 (8.9%) |
| LAM | 22 (48.9%) |
| LAM + ADV | 6 (13.3%) |
| ETV | 13 (28.9%) |
| Corticosteroid therapy >6 months | 12 (26.7%) |
| Median follow-up period (months) | 66 (9–174) |

Qualitative variables are expressed as the numbers of patients, with percentages in parentheses, and quantitative variables are expressed by the medians, with ranges in parentheses.
ADV, adefovir; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LAM, lamivudine; LDLT, living donor liver transplantation.

form of arterial chemoembolization, radiofrequency ablation and/or an ethanol injection prior to LDLT. One patient (3.6%) underwent surgical resection of the tumor prior to LDLT.

Of the 28 patients with HCC, three patients (10.7%) received post-LT systemic chemotherapy. Among them, one patient received post-LT chemotherapy for the treatment of combined hepatocellular and cholangiocellular carcinoma. The post-LT chemotherapy regimens included a combination of antimetabolites (5-fluorouracil, gemcitabine), a platinum-based agent (cisplatin), anthracycline (epirubicin) and multikinase inhibitor (sorafenib). Prior to LDLT, 41 patients (91.1%) were treated with antiviral therapy consisting of lamivudine, adefovir, entecavir or a combination thereof.

## Overall HBV recurrence

Seven of the 45 patients (15.6%) developed post-LT HBV recurrence. The median interval between LDLT and the development of HBV recurrence was 23.7 months (range, 0.8–35.9). The overall actuarial rates of HBsAg recurrence after LDLT at 1, 3 and 5 years were 6.7%, 17.9% and 17.9%, respectively (Fig. 1). Table 2 shows the results of the univariate analysis of risk factors associated with HBV recurrence after LDLT. The factors significantly associated with HBV recurrence were cessation of HBIG ($P=0.039$) and HCC recurrence ($P=0.021$). According to the multivariate analysis, the same factors were found to be independently associated with a higher risk of HBV recurrence after LDLT: cessation of HBIG (hazard ratio [HR], 20.17; 95% confidence interval [95% CI], 2.091–194.593; $P=0.009$) and HCC recurrence (HR, 30.835; 95% CI, 3.132–303.593; $P=0.003$) (Table 3).

## HBIG cessation

Three of the seven patients (42.9%) with HBV recurrence were cases in whom HBIG was suspended during combined prophylaxis after LDLT (Table 4, cases 1–3). In case 1, the HBIG was suspended while the patient received HBV

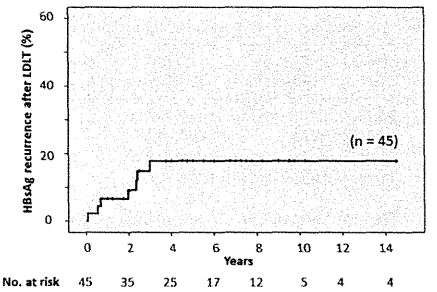


**Figure 1** Cumulative rates of hepatitis B surface antigen (HBsAg) recurrence in the 45 patients. LDLT, living donor liver transplantation.

**Table 2** Results of the univariate analysis of factors associated with HBV recurrence after LDLT

| | HBsAg recurrence ($n = 7$) | HBsAg non-recurrence ($n = 38$) | *P* |
|---|---|---|---|
| Age (years) | 53 (46–64) | 53.5 (31–67) | 0.778 |
| Male sex | 4 (57.1%) | 24 (63.2%) | 0.538 |
| Acute liver failure | 1 (14.3%) | 11 (43.2%) | 0.387 |
| Pre-LT HBeAg positivity | 1 (14.3%) | 9 (23.7%) | 0.506 |
| HBV DNA level at LT >5 log copies/mL ($n = 40$) | 2 (33.3%; $n = 6$) | 10 (29.4%; $n = 34$) | 0.595 |
| HBIG cessation | 3 (42.9%) | 3 (7.9%) | 0.039 |
| Pre-LT HCC | 4 (57.1%) | 24 (63.2%) | 0.468 |
| Beyond Milan criteria by | | | |
| preoperative imaging | 2 (28.6%) | 6 (15.8%) | 0.363 |
| explant pathology | 4 (57.1%) | 11 (28.9%) | 0.154 |
| HCC recurrence | 3 (42.9%) | 2 (5.3%) | 0.021 |
| Post-LT systemic chemotherapy | 2 (28.6%) | 1 (2.6%) | 0.059 |
| Duration of corticosteroids (months) | 3.7 (1.4–8.4) | 2.9 (0.2–23.9) | 0.309 |
| Post-LT antiviral therapy | 4/2/1 | 19/5/14 | 0.402 |
| LAM/LAM + ADV/ETV | (57.1%/28.6%/14.3%) | (50%/13.2%/36.8%) | |

Qualitative variables are expressed as the numbers of patients, with percentages in parentheses, and quantitative variables are expressed by the medians, with ranges in parentheses.
ADV, adefovir; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LAM, lamivudine; LDLT, living donor liver transplantation.

**Table 3** Results of the multivariate analysis of factors associated with HBV recurrence after LDLT

| | Hazard ratio | 95% CI | *P* |
|---|---|---|---|
| HCC recurrence | 30.835 | 3.132–303.593 | 0.003 |
| HBIG cessation | 20.170 | 2.091–194.593 | 0.009 |
| Age (>55) | – | – | 0.732 |
| Sex, male | – | – | 0.529 |
| Post-LT systemic chemotherapy | – | – | 0.863 |

CI, confidence interval; HBIG, hepatitis B immunoglobulin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LDLT, living donor liver transplantation.

vaccination. However, HBsAg and HBV DNA reemerged 2 months after the cessation of HBIG, and the vaccination had failed. Adefovir was subsequently added to his treatment regimen, and the HBV DNA immediately became undetectable. In cases 2 and 3, HBIG was suspended for financial reasons, and HBsAg reemerged 4 and 6 months after the suspension of HBIG, respectively. After HBIG was reintroduced, the HBsAg disappeared and HBsAb reappeared immediately. HBIG was also suspended in three of the 38 patients (7.9%) who did not have HBV recurrence, and these were the cases in whom HBV vaccination was successfully performed. The indication of HBV vaccination was patients who have a normal or near

**Table 4** Antiviral therapy administrated and the outcomes of patients with HBV recurrence after LDLT

| | Age (years), sex | Primary disease | HBV prophylaxis | Cessation of HBIG | Time to HBV recurrence (months) | Time to HCC recurrence (months) | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | 53, M | HCC with LC | HBIG + LAM | + | 24 | – | Alive |
| 2 | 47, M | ACLF | HBIG + LAM | + | 28 | – | Alive |
| 3 | 54, M | LC | HBIG + LAM | + | 6 | – | Alive |
| 4 | 46, M | HCC with LC | HBIG + LAM, ADV | – | 8 | 5 | Died |
| 5 | 59, F | HCC with LC | HBIG + LAM, ADV | – | 36 | 15 | Died |
| 6 | 47, F | HCC with LC | HBIG + ETV | – | 29 | 30 | Alive |
| 7 | 64, F | LC | HBIG + LAM | – | 1 | – | Alive |

ACLF, acute-on-chronic liver failure; ADV, adefovir; ETV, entecavir; HBcAb, hepatitis B core antibody; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LAM, lamivudine; LC, liver cirrhosis; LDLT, living donor liver transplantation.

normal liver function tests with a low level of immunosuppression, and the follow-up period after LDLT was at least a year. HBIG was suspended 1–4 weeks before starting HBV vaccination.[14]

As shown in Figure 2, the cumulative HBsAg recurrence rates were significantly higher in patients with HBIG cessation than in those receiving combined prophylaxis ($P = 0.013$).

## Pretransplant HCC and HCC recurrence

Hepatocellular carcinoma was present in 28 patients (62.2%), and the cumulative HBsAg recurrence rates after LDLT were not significantly higher in patients with HCC than in those without HCC ($P = 0.711$) (Fig. 3a). Between the patients with HCC beyond and within the Milan criteria based on the preoperative imaging, there was no statistically significant difference in the cumulative HBsAg recurrence rates ($P = 0.370$) (Fig. 3b). Meanwhile, in patients with HCC beyond and within the Milan criteria diagnosed by the explant pathology, the cumulative HBsAg recurrence rates were higher (with marginal significance) in the patients with HCC beyond the Milan criteria than in those with HCC within the Milan criteria ($P = 0.068$) (Fig. 3C).

Among these 28 patients with HCC, five patients (17.9%) developed HCC recurrence after LDLT, and HBV recurrence occurred in three of these five patients (60%) (Table 4, cases 4–6). In case 4, the tumors were beyond the Milan criteria at LDLT, and HCC recurred 5 months after transplantation. Despite the use of chemotherapy and radiation, the HCC had grown and the HBsAg reappeared 8 months after transplantation during systemic chemotherapy. In case 5, there were multiple HCC tumors, and

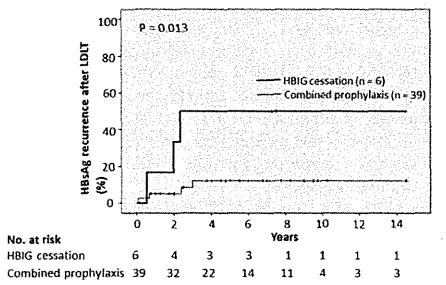


Figure 2 Cumulative rates of hepatitis B surface antigen (HBsAg) recurrence in patients with cessation of hepatitis B immunoglobulin (HBIG) and in those receiving combined prophylaxis (HBIG + antiviral agent). LDLT, living donor liver transplantation.

the disease was beyond the Milan criteria at LDLT. At 15 months after transplantation, HCC recurred as lung metastasis, and HBsAg recurrence was observed at 36 months after LDLT during systemic chemotherapy. In both cases 4 and 5, the HBV DNA increased in spite of combined therapy with entecavir, adefovir and HBIG.[15] Both patients finally died of recurrent HCC, at 12 and 43 months after transplantation, respectively.

In case 6, the HCC was within Milan criteria at LDLT, and HBsAg reemerged 29 months after LDLT, which was 1 month prior to the detection of HCC recurrence. The patient underwent several operations for the treatment of metastasis and, thereafter, the HCC has been under control. She has not received systemic chemotherapy or radiation therapy, and the HBV DNA levels have been undetectable by combined prophylaxis with entecavir and HBIG. In patients with HCC recurrence, the cumulative HBsAg recurrence rate after LDLT was significantly higher than that in patients without HCC recurrence ($P < 0.001$) (Fig. 3d).

The remaining patient with HBV recurrence (Table 4, case 7) was the only case of transplantation from a living donor with positive HBsAg.[16] This living donor was the patient's son whose blood type was identical. He had no history of liver dysfunction, and was referred to as a "healthy carrier". Because no other living donors were available and brain-dead donors are rarely available in Japan, we decided to proceed to LDLT with this donor. To date, the patient has been doing well at 12 years after transplantation and the donor has also been doing well.

## Overall survival

The overall survival after LDLT was not significantly reduced for patients with HBV recurrence, with probabilities at 1, 3 and 5 years of 100%, 91.5% and 91.5%, respectively, for patients without HBV recurrence versus 100%, 85.7% and 71.4%, respectively, for patients with HBV recurrence ($P = 0.250$) (Fig. 4a). However, if the six cases of HBIG cessation were excluded ($n = 39$), the cumulative survival rate was significantly reduced for patients with HBV recurrence, with probabilities at 1, 3 and 5 years of 100%, 90.7% and 90.7%, respectively, for patients without HBV recurrence versus 100%, 75% and 50%, respectively, for patients with HBV recurrence ($P = 0.037$) (Fig. 4b).

## DISCUSSION

IN OUR STUDY, the demographic, virological, tumor burden, antiviral therapy and immunosuppressive therapy of patients with and without HBC recurrence after LDLT were analyzed to identify the risk factors for HBV

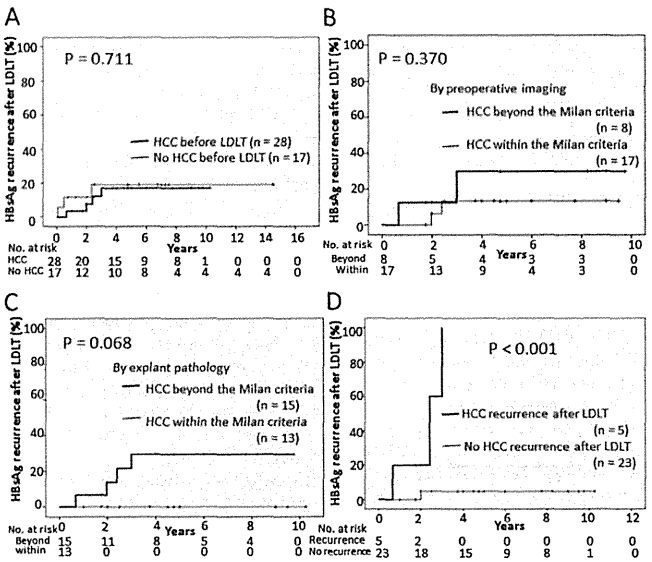


Figure 3 Cumulative rates of hepatitis B surface antigen (HBsAg) recurrence: (a) in patients with and without hepatocellular carcinoma (HCC) before living donor liver transplantation (LDLT); (b) beyond and within the Milan criteria by preoperative imaging; (c) beyond and within the Milan criteria by explant pathology; (d) with and without the recurrence of HCC. LDLT, living donor liver transplantation.

recurrence. We showed that both cessation of HBIG and HCC recurrence were independent risk factors for HBV recurrence after transplantation. Previous studies have demonstrated that pre-LT HBeAg positivity, non-fulminant hepatitis B, immunosuppression from steroids and systemic chemotherapy after LDLT are risk factors for HBV recurrence,[7–9] however, we could not find a statistically significant correlation with those factors and HBV recurrence in the present study. Although a high pre-LT HBV DNA level was also reported to be an independent risk factor for HBV recurrence,[5,6] we also found no statistically significant correlation with this factor. A limitation of our retrospective study is that not all of the HBV DNA levels were known at the time of LDLT. Therefore, the effects of antiviral therapy on the viral load were not available for all patients. However, all patients had undetectable HBsAg and HBV DNA levels after LDLT.

The mechanisms by which HBIG protects the transplanted liver against HBV reinfection are not fully understood. One hypothesis is that HBIG protects naive hepatocytes against the HBV released from extrahepatic sites by blocking a putative HBV receptor.[17,18] Previous studies reported that recurrent hepatitis B during the first 6 months post-LT is usually related to inadequate HBIG doses in patients with a high viral load pre-LT, whereas late recurrence is caused mainly by the selection of immune escape mutants.[19–21] The most common mutation involves a glycine to arginine substitution at codon 145 (G145R) of the HBV S protein. This mutation results in reduced binding to anti-HBs, and such viruses may escape neutralization by HBIG. The cessation of HBIG therapy is accompanied by a reversion to a wild-type sequence, supporting the role of HBIG in the selection of these mutations.

Due to the many drawbacks of HBIG, including its high cost, several trials have attempted to minimize the dose of HBIG in selected patients.[22,23] Recently, Fung *et al.* reported that a HBIG-free regimen of entecavir monotherapy was effective for suppressing HBV after LT.[24] Yi *et al.* also reported the efficacy of sequential entecavir monotherapy after 1-year combination therapy.[25] Compared with lamivudine, entecavir has greater antiviral potency and a

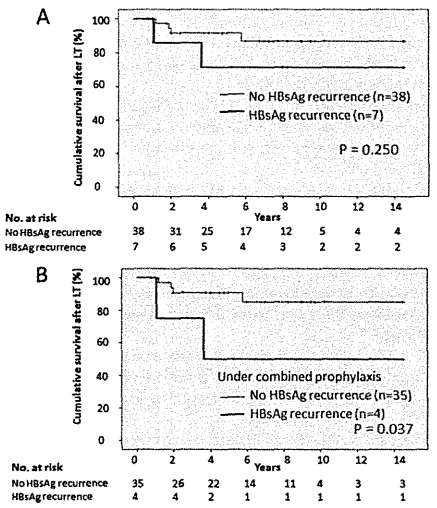


Figure 4 Cumulative survival in liver transplant recipients: (a) with and without hepatitis B surface antigen (HBsAg) recurrence; (b) with and without HBsAg recurrence under combined prophylaxis. LT, liver transplant.

higher genetic barrier to resistance, resulting in lower resistance rates in HBV-related liver disease patients. Further studies are needed to establish an optimal prophylactic regimen.

Except for a few studies that suggested a higher incidence of HBV recurrence in transplanted patients with HCC,[10–12] previous studies had not shown any association between HCC and a higher risk of HBV recurrence.[7,26,5,27,28] In 2008, Faria *et al.* reported an association between HCC recurrence and HBV re-infection.[10] In their study, the presence of HCC at transplantation and HCC recurrence after LT were independent risk factors associated with HBV recurrence. The authors demonstrated the presence of cccDNA in both HCC cells and in non-tumor cells in explanted livers, suggesting that HBV replication may also occur in tumor cells. In 2009, Saab *et al.* reported that pre-LT HCC and HCC recurrence after transplantation were associated with HBV reinfection and with decreased patient survival.[11] HCC recurrence itself is suggested to be a product of any breakthrough of the host immunity, and active cell proliferation due to malignant transformation can induce active replication of the HBV in the liver.[29] In addition, Yi *et al.* reported that chemotherapy and a high corticosteroid dose used for HCC were risk factors for HBV recurrence.[9] From that point of view, the differences in the virological kinetics in our three cases with HCC recurrence (Table 4, cases 4–6) are interesting, and may be explained by the condition of HCC and the use of systemic chemotherapy. These results require confirmation by further investigations.

Saab *et al.* reported decreased cumulative survival for patients with HBV recurrence.[11] Our present study showed no significant effect of HBV recurrence on the overall survival (Fig. 4a). However, if the analysis was limited to the patients with combined prophylaxis, the cumulative survival rates were significantly reduced in the HBV recurrence group compared with the HBV non-recurrence group (Fig. 4b). This result is consistent with previous studies,[11,30] but it should be noted that both of the two death cases in the HBsAg recurrence group (Fig. 4b) were the cases of HCC recurrence (Table 4, cases 4 and 5). The remaining two cases were another case of HCC recurrence and the case from a HBsAg positive donor (Table 4, cases 6 and 7). It is difficult to reach a definite conclusion because of small sample size, but HCC recurrence may be a strong prognostic factor for survival in a HBsAg recurrence group. Whether HBV recurrence itself is truly affecting prognosis or not should be confirmed in further studies.

Although the limitations of this study include its retrospective design and relatively small sample size, our results demonstrated the importance of combined prophylaxis, and confirmed that there is a relationship between HBV and HCC recurrence.

In conclusion, cessation of HBIG and post-LT HCC recurrence were independent risk factors for HBV recurrence in LDLT patients. Despite the improvements achieved in HBV prophylaxis following LDLT, clinicians should remain cautious concerning the risk of HBV recurrence, particularly in these groups.

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Hepatology Research

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*Original Article*

# Profile of plasma amino acids values as a predictor of sepsis in patients following living donor liver transplantation: Special reference to sarcopenia and postoperative early nutrition

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*Aim:* Sarcopenia is an independent predictor of mortality and sepsis after living donor liver transplantation (LDLT). However, the exact mechanisms by which sarcopenia affects poor prognosis or worse immunity against postoperative sepsis are unclear, particularly regarding muscular amino acid metabolism, and the authors aimed to identify the role of plasma amino acids in sarcopenia by retrospective study.

*Methods:* The area of the psoas muscle in 228 recipients of LDLT was retrospectively measured by dynamic computed tomography. Additionally, plasma amino acid levels were measured both pre- and postoperatively. The impact of plasma amino acids for postoperative sepsis and the relationship between sarcopenia and early nutrition after LDLT were analyzed.

*Results:* Among the plasma amino acids, only leucine, isoleucine and glutamine in patients with sarcopenia were significantly lower than those without sarcopenia (each, $P < 0.05$). Multivariate analysis identified the lower plasma glutamine levels as a risk factor of postoperative sepsis after LDLT (odds ratio 5.371, $P = 0.002$). In sarcopenia patients, plasma glutamine levels after LDLT were significantly decreased compared with before LDLT in patients both with and without postoperative early nutrition. However, in non-sarcopenia patients with early nutrition, plasma glutamine levels after LDLT were comparable with those before LDLT.

*Conclusion:* This is the first report to study the profile of plasma amino acid change before and after LDLT. Low preoperative glutamine values were an independent risk factor for predicting postoperative sepsis. The efficacy of postoperative early nutrition may prevent postoperative sepsis by improving glutamine levels.

**Key words:** amino acid, glutamine, liver transplantation, sarcopenia, sepsis

## INTRODUCTION

IN PATIENTS WITH end-stage liver disease requiring liver transplantation, protein–energy malnutrition is common and is closely associated with risk for morbidity and mortality after liver transplantation.[1–3] However, the loss of skeletal muscle mass, termed sarcopenia, was identified as a poor prognostic factor for patients, not only with various cancers (including pancreatic cancer, colorectal liver metastases and melanoma),[4–6] but also liver cirrhosis and liver transplantation.[7,8] Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength, with a risk of adverse outcomes such as physical disability and poor quality of life and death.[4–9] In such patients, sarcopenia reflects protein–energy malnutrition, which is characteristic of decompensated liver cirrhosis.

Our previous study demonstrated that the most frequent cause of in-hospital death after liver transplantation was infection, including sepsis.[10] Additionally, the higher mortality risk of cirrhotic patients with sarcopenia was related to a higher frequency of sepsis-related death and not to liver failure.[11] Recently, we also reported that central sarcopenia was an independent predictor of mortality and sepsis after living donor liver transplantation (LDLT).[12]

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However, the exact mechanisms by which sarcopenia, a decrease of skeletal muscle mass, affects poor prognosis or worse immunity against postoperative sepsis after liver transplantation are unclear.

Here, we describe a pilot study to examine sequential changes in levels of plasma amino acids that are metabolized or produced in skeletal muscle, before and after LDLT. This study aimed to elucidate the impact of plasma amino acids on postoperative sepsis, and to clarify the relationship between sarcopenia and early nutrition after liver transplantation.

## METHODS

### Patients

THE STUDY COHORT consisted of 228 recipients of LDLT at Kyushu University Hospital between November 2003 and December 2011 who were retrospectively investigated. Twenty-three patients with acute hepatic failure and one patient that died from operative blood loss were excluded from this study. Eighty-five patients with unavailable pre- and postoperative plasma samples were excluded.[13] Of the 143 patients, measurements of psoas muscle area by computed tomography (CT) and plasma amino acids were performed. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by our institutional review board.

### Assessment of the area of the psoas muscle

All study patients underwent preoperative CT within the month before LDLT. Measurement of the area of the psoas muscle was previously described.[12] Briefly, we measured the lengths of the major and minor axes of the psoas muscle at the caudal end of the third lumbar vertebra. The area of the psoas muscle was calculated using the following formula:

$$\text{Area} = a \times b \times \pi [12],$$

where a and b are the radii of the major and minor axes, respectively. The definition of sarcopenia was based on our previous report[14]: the cross-sectional area of the psoas muscle at the caudal end of the third lumbar vertebra of healthy donors, and an area of the psoas muscle lower than the 5th percentile for each sex was defined as sarcopenia. The cut-off levels were defined as 800 $cm^2$ for men and 380 $cm^2$ for women.

### Measurement of plasma amino acids and other prognostic factors

Plasma amino acids levels were measured on preoperative and postoperative day 7, and the following contents were measured: essential amino acids including valine, leucine, isoleucine, lysine, methionine, phenylalanine, histidine and non-essential amino acids including glutamine, arginine and tyrosine. Fisher ratio was calculated as branched-chain amino acid (BCAA) per aromatic amino acid. The predictors of sarcopenia and postoperative sepsis were evaluated only with preoperative values. The following were used as preoperative factors: recipient age, donor age, recipient sex, recipient status (hospitalized or at home), preoperative renal failure, body mass index (BMI), Child–Pugh class, Model for End-Stage Liver Disease (MELD) score and graft volume per standard liver volume ratio.

### Bacterial sepsis

Bacterial sepsis was defined as previously described.[10,15] Briefly, bacteria other than common skin contaminants were isolated from a single blood culture within 3 months after transplantation, along with clinical symptoms, including high fever, shivering, dyspnea, altered mental status, tachycardia or hypotension.[10,15] Bacterial sepsis including primary sepsis was defined as infection of undetermined origin or intravascular line infection. Secondary sepsis was defined as infection of known origin, including pneumonia, cholangitis, peritonitis, urinary tract infection and wound infection. Both phenotypes included postoperative complication of sepsis.[10,15]

### Enteral nutrition

The method of postoperative enteral nutrition was as follows.[15] Early enteral nutrition after LDLT was introduced in 2003, and the adoption of enteral nutrition was determined initially on a case-by-case basis. Since 2008, early enteral nutrition via a nasojejunal tube (new enteral feeding tube with a guide-wire, 10-F; Japan Sherwood, Tokyo, Japan) has been routinely applied for all recipients within the first 24 h after LDLT.[15] A low residual enteral liquid diet (RACOL Liquid for Enteral Use, 1 kcal/mL; Otsuka Pharmaceutical, Tokyo, Japan) was administrated starting several days after transplantation. Once a patient was able to eat 50–75% of the provided regular diet, enteral feeding was discontinued.

### Statistical analysis

All statistical analyses were performed using JMP statistical software version 7.01 (SAS Institute, Cary, NC, USA), as

previously described.[13] All values are expressed as mean ± standard deviation. Continuous variables were compared using the non-parametric Wilcoxon rank sum test for independent samples. The parametric paired Student's *t*-test was used for paired samples and categorical values were compared using the $\chi^2$-test. Multivariate analyses were performed using the logistic regression model. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of optimal cut-off values of plasma amino acids levels for the diagnosis of postoperative sepsis were calculated as described.[16] The diagnostic value of plasma amino acids levels for predicting postoperative sepsis was assessed by calculating the areas under the receiver–operator curves (ROC). The ROC is a plot of sensitivity versus 1-specificity for all possible cut-off values. The most commonly used index of accuracy is the area under the ROC, where values close to 1.0 indicate high diagnostic accuracy, and 0.5 indicates a test of no diagnostic value. All differences were considered statistically significant at $P < 0.05$.

## RESULTS

### Patient demographics

PATIENT CHARACTERISTICS WITH and without sarcopenia are summarized in Table 1. The rates were higher in the sarcopenia group ($n = 66$) for male sex ($P < 0.001$), hospitalization ($P < 0.001$), Child–Pugh class C ($P = 0.003$) and MELD score ($P < 0.001$) compared with the non-sarcopenia group ($n = 77$) (Table 1). Additionally, patients with sarcopenia had lower BMI ($P = 0.006$). Among the plasma amino acids, only three amino acids, leucine ($P = 0.001$), isoleucine ($P = 0.001$) and glutamine ($P = 0.009$) were significantly lower in patients with

**Table 1** Baseline characteristics of patients with and without sarcopenia ($n = 143$)

| Variables ($n = 143$) | Sarcopenia ($n = 66$) | No sarcopenia ($n = 77$) | *P* |
|---|---|---|---|
| Recipient age (years) | 54.8 ± 9.0 | 55.0 ± 10.4 | 0.906 |
| Donor age (years) | 35.2 ± 1.3 | 34.2 ± 1.2 | 0.532 |
| Recipient sex: male/female | 68.2/31.8 | 37.7/62.3 | 0.001 |
| (%, *n*) | (45/21) | (29/48) | |
| Recipient status: hospitalized/ home | 36.4/63.6 | 11.7/88.3 | 0.001 |
| (%, *n*) | (24/42) | (9/68) | |
| Preoperative renal failure (%, *n*) | 12.1/87.9 (8/58) | 3.9/96.1 (3/74) | 0.063 |
| Recipient BMI (kg/m$^2$) | | | |
| Child–Pugh class: | 21.2/78.8 | 24.3 ± 3.5 | 0.006 |
| A+B/C (%, *n*) | (14/52) | 44.2/55.8 | 0.003 |
| MELD score (patients) | 16.9 ± 6.1 | (34/42) | |
| GV/SLV ratio (%) | 40.1 ± 7.5 | 13.3 ± 6.9 | 0.001 |
| Skeletal muscle mass (cm$^2$) | 484.2 ± 187.0 | 39.2 ± 7.4 | 0.438 |
| Essential amino acids | | 689.6 ± 289.0 | 0.001 |
| Valine | 120.8 ± 21.1 | | |
| Leucine | 80.5 ± 18.9 | 118.1 ± 21.1 | 0.442 |
| Isoleucine | 89.4 ± 18.0 | 97.3 ± 18.1 | 0.001 |
| Lysine | 88.8 ± 15.2 | 102.4 ± 18.5 | 0.001 |
| Methionine | 18.8 ± 5.2 | 97.6 ± 17.5 | 0.110 |
| Phenylalanine | 32.0 ± 7.3 | 19.4 ± 7.4 | 0.584 |
| Histidine | 71.2 ± 32.2 | 34.1 ± 7.2 | 0.093 |
| Non-essential amino acids | | 82.3 ± 34.9 | 0.062 |
| Glutamine | 460.9 ± 65.2 | | |
| Arginine | 61.2 ± 26.4 | 495.0 ± 66.6 | 0.009 |
| Tyrosine | 140.8 ± 18.8 | 62.4 ± 25.8 | 0.787 |
| | | 144.9 ± 22.6 | 0.236 |

Mean ± standard deviation.
BMI, body mass index; MELD, Model for End-Stage Liver Disease; GV, graft volume; SLV, standard liver volume.

sarcopenia than patients without sarcopenia, and the other factors were comparable (Table 1).

### Profile of various plasma amino acids values before and after LDLT

Table 2 shows changes of plasma amino acids levels before and after LDLT in patients with and without sarcopenia. Among the essential amino acids, the levels of three amino acids, valine, leucine and isoleucine, were significantly decreased in all patients after LDLT ($n = 143$) compared with before LDLT ($P < 0.001$ each), and were also significantly decreased in patients with sarcopenia ($n = 66$; $P < 0.001$ each). Among the non-essential amino acids, only the glutamine levels in all patients after LDLT were significantly decreased compared with before LDLT ($P < 0.001$ each), which was also significantly decreased in the patients with sarcopenia ($P < 0.001$ each). Additionally, the Fisher ratio was significantly improved in all patients after LDLT than before ($P < 0.001$), which was also significantly decreased in patients with sarcopenia ($P < 0.001$).

### Diagnostic capability of plasma amino acid levels for predicting postoperative sepsis

We compared the area under ROC of plasma amino acids (valine, leucine, isoleucine and glutamine) and Fisher ratio, which were significantly decreased after LDLT compared with before LDLT for the prediction of postoperative sepsis (Supplementary Figure S1). The optimal cut-off values for amino acids levels of valine, leucine, isoleucine, BCAA (sum of valine, leucine and isoleucine) and glutamine were 110.0, 68.3, 104.3, 326.1 and 580.0, respectively, with a Fisher ratio of 2.00 (Supplementary Table S1). The area under the ROC for the diagnosis of postoperative sepsis using plasma glutamine values (0.732) was comparable with that using plasma leucine (0.707), but was significantly superior to the other surrogate markers, including valine (0.570), isoleucine (0.530), BCAA (0.558) and Fisher ratio (0.537) ($P < 0.001$ each; Supplementary Figure S1). The plasma glutamine, valine, leucine, isoleucine and BCAA levels as well as Fisher ratio cut-offs had sensitivities for predicting postoperative sepsis of 75.0%, 83.3%, 58.3%, 58.3%, 91.7% and 66.7%, respectively; specificities of 80.0%, 74.7%, 54.6%, 82.9%, 32.1% and 76.1%, respectively; PPV of 41.1%, 11.6%, 29.2%, 13.0%, 11.0% and 14.3%, respectively; and NPV of 95.2%, 96.5%, 96.0%, 93.9%, 97.7% and 94.9%, respectively (Supplementary Table S1). Thus, plasma glutamine cut-offs were more sensitive and specific for predicting postoperative sepsis.

### Impact of amino acid level profile on postoperative sepsis

The impact of the four plasma amino acids values (summarized as BCAA and glutamine) and Fisher ratio of risk factors for postoperative sepsis were examined using the individual cut-off values by univariate and multivariate analysis (Table 3). In univariate analysis, significant risk factors for postoperative sepsis were recipient age

Table 2 Changes of plasma amino acids levels before and after liver transplantation in patients with and without sarcopenia ($n = 143$)

| Levels of plasma amino acids | All patients ($n = 143$) | | Sarcopenia ($n = 66$) | |
|---|---|---|---|---|
| | Before vs after LT | *P* | Before vs after LT | *P* |
| Essential amino acids | | | | |
| Valine | 119.5 ± 21.1 vs 87.6 ± 23.8 | 0.001 | 120.8 ± 21.1 vs 92.3 ± 23.1 | 0.001 |
| Leucine | 89.5 ± 20.2 vs 73.4 ± 21.1 | 0.001 | 80.5 ± 18.9 vs 66.6 ± 19.5 | 0.001 |
| Isoleucine | 96.4 ± 19.3 vs 103.9 ± 21.3 | 0.001 | 89.4 ± 18.0 vs 102.6 ± 21.1 | 0.001 |
| Lysine | 98.9 ± 18.9 vs 108.6 ± 24.0 | N.S. | 88.8 ± 15.2 vs 104.4 ± 24.0 | N.S. |
| Methionine | 19.2 ± 6.4 vs 16.6 ± 4.0 | N.S. | 18.8 ± 5.2 vs 15.8 ± 3.7 | N.S. |
| Phenylalanine | 33.1 ± 7.3 vs 29.8 ± 4.9 | N.S. | 32.0 ± 7.3 vs 29.4 ± 5.4 | N.S. |
| Histidine | 77.2 ± 34.0 vs 81.1 ± 23.0 | N.S. | 71.2 ± 32.2 vs 75.1 ± 20.4 | N.S. |
| Non-essential amino acids | | | | |
| Glutamine | 479.3 ± 67.9 vs 437.8 ± 124.1 | 0.001 | 460.9 ± 65.2 vs 405.6 ± 116.8 | 0.001 |
| Arginine | 61.9 ± 26.0 vs 56.6 ± 18.5 | N.S. | 61.2 ± 26.4 vs 54.8 ± 18.7 | N.S. |
| Tyrosine | 143.0 ± 21.0 vs 125.7 ± 22.1 | N.S. | 140.8 ± 18.8 vs 127.6 ± 21.2 | N.S. |
| Fisher ratio (%) | 2.07 ± 0.62 vs 2.46 ± 0.29 | 0.001 | 1.86 ± 0.60 vs 2.27 ± 0.24 | 0.001 |

Fisher ratio = branched-chain amino acid/aromatic amino acid;
LT, liver transplantation; N.S., not significant.

Table 3 Univariate and multivariate analysis of the impact of plasma glutamine levels on risk factors for postoperative sepsis ($n = 143$)

| Variables ($n = 143$) | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | Odds ratio | 95% CI | *P* | Odds ratio | 95% CI | *P* |
| Recipient age (years) | 1.081 | 1.027–1.140 | 0.003 | 1.107 | 1.024–1.216 | 0.010 |
| Donor age (years) | 0.223 | 0.021–2.408 | 0.211 | 0.998 | 0.931–1.074 | 0.964 |
| Recipient sex: male/female (%, *n*) | 1.337 | 0.404–4.430 | 0.633 | 1.195 | 0.350–1.069 | 0.673 |
| Recipient status: hospitalized/home (%, *n*) | 3.850 | 1.151–12.894 | 0.033 | 1.490 | 0.607–3.711 | 0.376 |
| Preoperative renal failure | 5.125 | 1.156–22.730 | 0.019 | 1.765 | 0.518–5.540 | 0.342 |
| Recipient BMI (kg/m$^2$) | 1.033 | 0.871–1.249 | 0.716 | 1.134 | 0.697–1.111 | 0.278 |
| Child–Pugh class: A+B/C (%, *n*) | 2.706 | 0.569–12.877 | 0.171 | 1.605 | 0.486–7.990 | 0.511 |
| MELD score (patients) | 1.080 | 0.860–0.997 | 0.043 | 1.017 | 0.876–1.099 | 0.765 |
| GV/SLV ratio (%) | 0.940 | 0.873–1.013 | 0.107 | 0.931 | 0.850–1.017 | 0.110 |
| Sarcopenia | 1.934 | 1.722–8.775 | 0.013 | 1.719 | 0.673–5.033 | 0.263 |
| Plasma Fisher ratio <2.0 | 1.156 | 0.637–2.173 | 0.633 | 1.021 | 0.428–2.400 | 0.961 |
| Plasma BCAA <326.1 nmol/mL | 2.278 | 0.102–1.020 | 0.057 | 2.843 | 0.066–1.058 | 0.065 |
| Plasma glutamine levels <580 nmol/mL | 3.342 | 1.009–2.340 | 0.037 | 5.371 | 1.260–19.145 | 0.002 |

Mean ± standard deviation.
BCAA, branched-chain amino acid; BMI, body mass index; CI, confidence interval; GV, graft volume; MELD, Model for End-Stage Liver Disease; SLV, standard liver volume.

($P = 0.003$), hospitalized status ($P = 0.033$), preoperative renal failure ($P = 0.019$), MELD score ($P = 0.043$), sarcopenia ($P = 0.013$) and plasma glutamine levels of less than 580 nmol/mL ($P = 0.037$) (Table 3). Multivariate analysis identified two risk factors, recipient age (odds ratio, 1.107; 95% confidence interval [CI] 1.024–1.216; $P = 0.010$) and plasma glutamine levels of less than 580 nmol/mL (odds ratio, 5.371; 95% CI, 1.260–19.145; $P = 0.002$) (Table 3).

### Change of plasma glutamine levels in patients with postoperative sepsis

Plasma glutamine levels after LDLT in patients without postoperative sepsis ($n = 131$) were comparable with that before LDLT, whereas in patients with postoperative sepsis ($n = 12$), plasma glutamine levels after LDLT were significantly decreased than before LDLT ($P < 0.05$) (Fig. 1).

### Impact of postoperative early nutrition on plasma glutamine levels

In the sarcopenia group ($n = 66$), plasma glutamine levels after LDLT were significantly decreased compared with before LDLT in patients with and without postoperative early nutrition (each, $P < 0.05$) (Fig. 2a). However, in the non-sarcopenia group ($n = 77$), plasma glutamine levels after LDLT were significantly decreased compared with before LDLT in patients without early nutrition ($P < 0.05$), whereas values after LDLT were comparable with that before LDLT in patients with early nutrition (Fig. 2b). These results indicate the efficacy of postoperative early nutrition for the improvement of plasma glutamine values with the capability to prevent sepsis in non-sarcopenia patients but not in sarcopenia patients.

## DISCUSSION

THIS IS THE first report to clarify changes in plasma amino acid profiles before and after LDLT. The preoperative lower glutamine values were an independent risk factor for predicting postoperative sepsis. Plasma glutamine levels after LDLT with early nutrition were comparable with those before LDLT in non-sarcopenia patients, but not in sarcopenia patients, indicating the efficacy of postoperative early nutrition for the improvement of plasma glutamine values, which might prevent postoperative sepsis.

Sarcopenia, defined as the age-related loss of muscle mass and strength, was a main factor in the reduced ability to increase skeletal muscle synthesis of amino acids and proteins in response to biological responses of the immunity system.[17,18] Considering the important role of muscles in the metabolism of amino acids, muscle proteins turn over slowly and there are minimal diurnal changes

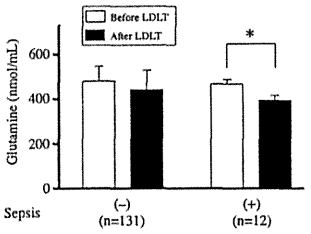


Figure 1 Change of plasma glutamine levels in patients with postoperative sepsis. Plasma glutamine levels after LDLT in patients without postoperative sepsis ($n = 131$) were comparable with that before LDLT, whereas, in patients with postoperative sepsis ($n = 12$), plasma glutamine levels after LDLT were significantly decreased than before LDLT ($P < 0.05$). *Statistically significant at $P < 0.01$. LDLT, living donor liver transplantation.

in the size of the muscle protein pool in response to feeding and fasting.[17,18] Besides, amino acids liberated from the protein pool are used for synthesis of tricarboxylic acid (TCA) cycle intermediates and glutamine. Indeed, six amino acids are mainly metabolized in resting muscle: leucine, isoleucine, valine, asparagine, aspartate and glutamate. Indeed, six amino acids are mainly metabolized in resting muscle: leucine, isoleucine, valine, asparagine, aspartate and glutamate. Only leucine and part of the isoleucine molecule can be converted to acetyl-coenzyme A and oxidized. The carbon skeleton of the other amino acids is used for synthesis of TCA cycle intermediates and glutamine. These six amino acids provide amino groups and ammonia for the synthesis of glutamine and alanine, which are subsequently released in excessive amounts by muscle tissues.[17–20] Meanwhile, glutamine, a final product synthesized by muscles from the metabolism of these various amino acids, is an important fuel and regulator of DNA and RNA synthesis in mucosal cells and immune system cells and fulfils several other important functions in human metabolism. The alanine aminotransferase reaction establishes and maintains high concentrations of TCA cycle intermediates and high TCA cycle flux in the early phase of stressed conditions such as surgery. It is proposed that maximal values in the TCA cycle are reduced because of insufficient TCA cycle anaplerosis such as muscle deficiency (quality or quantity) and that this also presents a limitation for the maximal rate of fatty acid oxidation.[17–20] In conclusion, interactions between the amino acid pool and the TCA cycle may play a central role in energy metabolism in muscle.

The effects of extracellular fluid glutamine depletion on amino acid metabolism and glutamine production in skeletal muscle of septic rodent model were demonstrated by Holecek *et al.*.[21] The sepsis model rats that underwent cecal ligation and puncture had increased glutamine release from muscle, protein breakdown and leucine oxidation, and decreased protein synthesis, allowing protection from sepsis. The sepsis rat model showed the depleted intramuscular glutamine concentration, decreased protein synthesis in muscles, and enhanced leucine oxidation and protein breakdown in skeletal muscles. The present study clinically demonstrated that plasma glutamine levels after LDLT in patients with postoperative sepsis were significantly decreased compared with before LDLT, whereas in patients without sepsis, levels after LDLT were comparable with

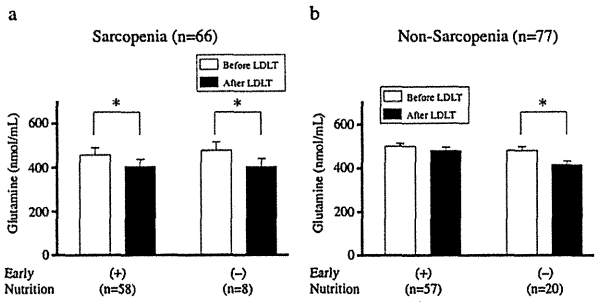


Figure 2 Impact of postoperative early nutrition on plasma glutamine levels. (a) In the sarcopenia group ($n = 66$), plasma glutamine levels after LDLT were significantly decreased compared with before LDLT in patients with and without postoperative early nutrition (each, $P < 0.05$). (b) In the non-sarcopenia group ($n = 77$), plasma glutamine levels after LDLT were significantly decreased compared with before LDLT in patients without early nutrition ($P < 0.05$), whereas values after LDLT were comparable with that before LDLT in patients with early nutrition. *Statistically significant at $P<0.01$. LDLT, living donor liver transplantation.