HCC.[27,28] Further, AFP and DCP are directly associated with HCC progression through the induction of cancer cell proliferation and angiogenesis, respectively.[29,30] Thus, our results are in good accordance with previous basic investigations and suggest that hepatic inflammation as well as elevated AFP and DCP levels independently accelerate the progression of NBNC-HCC.

Diagnostic year of HCC was also directly associated with the Milan criteria in this study. Although the reason for this association is unclear, a progress in serum tumor markers is a possible explanation. Because sensitivities of AFP and DCP were improved during this study period (1995–2006),[31–33] one would think that serum AFP and DCP levels are confounding factors for an association between diagnostic year of HCC and the Milan criteria.

Recently, lifestyle-related factors including alcohol intake and diabetes mellitus have been noted as risk factors for the development of NBNC-HCC.[2,10–12,34–38] Previous *in vitro* studies showed that ethanol and glucose stimulate the proliferation and migration of HCC,[39,40] indicating the direct association of alcohol intake and diabetes mellitus with NBNC-HCC progression. However, in this study, these factors were not directly associated with the Milan criteria. Although the reason for this discrepancy remains unclear, alcohol intake and diabetes mellitus were associated with the Milan criteria through diagnosis of liver cirrhosis in this study. Both ethanol consumption and diabetes mellitus can activate fibroblasts,[41,42] which are crucial components of the tumor microenvironment promoting the growth and invasion of cancer cells.[43,44] Thus, alcohol intake and diabetes mellitus may be associated with the clinical progression of NBNC-HCC through the tumor microenvironment.

Then, we created a decision tree algorithm to identify the clinical feature profiling associated with the staging of NBNC-HCC; the reproducibility of this model was confirmed by the independent validation datasets. Serum AFP level was selected for the initial classification, and serum DCP level was selected for the third division, creating groups 3 and 4. Although it is still unclear why the serum AFP level was associated with the Milan criteria to a greater extent than the serum DCP level, an association of the serum AFP level with the pathological features of HCC is a possible explanation. The AFP level is related to the number of HCC, whereas the DCP level is more specific to vascular invasion.[45–47] In this study, the staging of HCC was evaluated by using the Milan criteria, which include number and size of HCC but not vascular invasion,[26] explaining why serum AFP level was selected for the initial classification.

Diagnosis of liver cirrhosis was selected for the second division in the decision tree algorithm. Although liver cirrhosis is a well-known major risk factor for the development of HCC,[5,10,12,25,34,42] our result indicates that liver cirrhosis may suppress the progression of NBNC-HCC. We do not have any data accounting for the association between diagnosis of liver cirrhosis and suppression of the NBNC-HCC progression, the following is, however, a possible explanation for this contradiction. HCC surveillance may be performed more often in patients with liver cirrhosis than in those without liver cirrhosis,[12,25] so HCC could be identified at an early stage in patients with liver cirrhosis.

A limitation of this study is that a relationship between progression of NBNC-HCC and non-alcoholic steatohepatitis (NASH) was not evaluated. The reason is that NASH-related HCC is often diagnosed as cryptogenic cirrhosis-related HCC because of reduction of hepatic triglycerides according to the progression of NASH, so-called "burned-out NASH".[48] However, NASH is deeply involved in the development of HCC and a major reason for the increase in number of NBNC-HCC patients.[8,49,50] Recently, visceral fat accumulation is also reported to be an independent risk factor for HCC recurrence after curative treatment.[51] Thus, further study will be focused on a relationship between the progression of NBNC-HCC and NASH.

In conclusion, data mining disclosed complex associations of risk factors and clinical feature profiling associated with the staging of NBNC-HCC.

## REFERENCES

1 El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1752–63.
2 Kawaguchi T, Taniguchi E, Itou M, Sumie S, Yamagishi SI, Sata M. The pathogenesis, complications and therapeutic strategy for hepatitis C virus-associated insulin resistance in the era of anti-viral treatment. *Rev Recent Clin Trials* 2010; **5**: 147–57.
3 Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. *Hepatology* 2010; **51**: 1820–32.
4 Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. *Intervirology* 2010; **53**: 39–43.
5 Nordenstedt H, White DL, El-Serag HB. The changing pattern of epidemiology in hepatocellular carcinoma. *Dig Liver Dis* 2010; **42** (Suppl 3): S206–14.
6 Nouso K, Kobayashi Y, Nakamura S *et al.* Evolution of prognostic factors in hepatocellular carcinoma in Japan. *Aliment Pharmacol Ther* 2010; **31**: 407–14.

7 Tanaka H, Imai Y, Hiramatsu N *et al.* Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. *Ann Intern Med* 2008; **148**: 820–6.
8 Taura N, Fukushima N, Yatsuhashi H *et al.* The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area. *Med Sci Monit* 2010; **17**: PH7–11.
9 Abe H, Yoshizawa K, Kitahara T, Aizawa R, Matsuoka M, Aizawa Y. Etiology of non-B non-C hepatocellular carcinoma in the eastern district of Tokyo. *J Gastroenterol* 2008; **43**: 967–74.
10 Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35–50.
11 Hassan MM, Hwang LY, Hatten CJ *et al.* Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206–13.
12 Kiyosawa K, Umemura T, Ichijo T *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127**: S17–26.
13 Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. *Int J Med Inform* 2008; **77**: 81–97.
14 Kalisch M, Fellinghauer BA, Grill E *et al.* Understanding human functioning using graphical models. *BMC Med Res Methodol* 2010; **10**: 14–23.
15 Edwards D. *Introduction to Graphical Modelling*. New York: Springer-Verlag, 2000.
16 Pielou EC. *The Interpretation of Ecological Data. A Primer on Classification and Ordination*, 1st edn. New York: John Wiley&Sons, Inc., 1984.
17 Legendre P, Legendre L. *Numerical Ecology*, 2nd edn. Amsterdam: Elsevier Science, 1998.
18 Tsai CL, Camargo CA Jr. Methodological considerations, such as directed acyclic graphs, for studying "acute on chronic" disease epidemiology: chronic obstructive pulmonary disease example. *J Clin Epidemiol* 2009; **62**: 982–90.
19 Kurosaki M, Matsunaga K, Hirayama I *et al.* A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. *Hepatol Res* 2010; **40**: 251–60.
20 Kurosaki M, Sakamoto N, Iwasaki M *et al.* Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis. *J Gastroenterol* 2010 (in press).
21 Pauker SG, Kassirer JP. The threshold approach to clinical decision making. *N Engl J Med* 1980; **302**: 1109–17.
22 Lee A, Joynt GM, Ho AM, Keitz S, McGinn T, Wyer PC. Tips for teachers of evidence-based medicine: making sense of decision analysis using a decision tree. *J Gen Intern Med* 2009; **24**: 642–8.
23 Guo J, Wang W, Liao P *et al.* Identification of serum biomarkers for pancreatic adenocarcinoma by proteomic analysis. *Cancer Sci* 2009; **100**: 2292–301.
24 Warwick J, Vardaki E, Fattizzi N *et al.* Defining the surgical management of suspected early-stage ovarian cancer by estimating patient numbers through alternative management strategies. *BJOG* 2009; **116**: 1225–41.
25 Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007; **72** (Suppl 1): 2–15.
26 Mazzaferro V, Regalia E, Doci R *et al.* Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693–9.
27 Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57–70.
28 Sanz-Cameno P, Trapero-Marugan M, Chaparro M, Jones EA, Moreno-Otero R. Angiogenesis: from chronic liver inflammation to hepatocellular carcinoma. *J Oncol* 2010; article no.: 272170.
29 Inagaki Y, Tang W, Xu H *et al.* Des-gamma-carboxyprothrombin: clinical effectiveness and biochemical importance. *Biosci Trends* 2008; **2**: 53–60.
30 Wang XW, Xie H. Alpha-fetoprotein enhances the proliferation of human hepatoma cells in vitro. *Life Sci* 1999; **64**: 17–23.
31 Weitz IC, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. *Hepatology* 1993; **18**: 990–7.
32 Fujiyama S, Tanaka M, Maeda S, Ashihara H, Hirata R, Tomita K. Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology* 2002; **62** (Suppl 1): 57–63.
33 Marrero JA, Lok AS. Newer markers for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S113–19.
34 El-Serag HB. Epidemiology of hepatocellular carcinoma in USA. *Hepatol Res* 2007; **37** (Suppl 2): S88–94.
35 El-Serag HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460–8.
36 Kawaguchi T, Sata M. Importance of hepatitis C virus-associated insulin resistance: therapeutic strategies for insulin sensitization. *World J Gastroenterol* 2010; **16**: 1943–52.
37 Kawaguchi T, Taniguchi E, Morita Y *et al.* Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int* 2010; **30**: 479–86.
38 Tazawa J, Maeda M, Nakagawa M *et al.* Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; **47**: 710–15.
39 Brandon-Warner E, Sugg JA, Schrum LW, McKillop IH. Silibinin inhibits ethanol metabolism and ethanol-dependent cell proliferation in an in vitro model of hepatocellular carcinoma. *Cancer Lett* 2010; **291**: 120–9.
40 Chang YJ, Chiu CC, Wu CH *et al.* Glucose-regulated protein 78 (GRP78) silencing enhances cell migration but does not influence cell proliferation in hepatocellular carcinoma. *Ann Surg Oncol* 2010; **17**: 1703–9.

41 Flanders KC. Smad3 as a mediator of the fibrotic response. *Int J Exp Pathol* 2004; **85**: 47–64.
42 Gyamfi MA, Wan YJ. Pathogenesis of alcoholic liver disease: the role of nuclear receptors. *Exp Biol Med (Maywood)* 2010; **235**: 547–60.
43 Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. *Exp Cell Res* 2010; **316**: 1324–31.
44 Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Front Biosci* 2010; **15**: 166–79.
45 Hamamura K, Shiratori Y, Shiina S *et al.* Unique clinical characteristics of patients with hepatocellular carcinoma who present with high plasma des-gamma-carboxy prothrombin and low serum alpha-fetoprotein. *Cancer* 2000; **88**: 1557–64.
46 Miyaaki H, Nakashima O, Kurogi M, Eguchi K, Kojiro M. Lens culinaris agglutinin-reactive alpha-fetoprotein and protein induced by vitamin K absence II are potential indicators of a poor prognosis: a histopathological study of surgically resected hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 962–8.
47 Toyoda H, Kumada T, Kiriyama S *et al.* Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2006; **4**: 111–17.
48 Ong J, Younossi ZM, Reddy V *et al.* Cryptogenic cirrhosis and posttransplantation nonalcoholic fatty liver disease. *Liver Transpl* 2001; **7**: 797–801.
49 Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972–8.
50 Hashimoto E, Yatsuji S, Kaneda H *et al.* The characteristics and natural history of Japanese patients with nonalcoholic fatty liver disease. *Hepatol Res* 2005; **33**: 72–6.
51 Ohki T, Tateishi R, Shiina S *et al.* Visceral fat accumulation is an independent risk factor for hepatocellular carcinoma recurrence after curative treatment in patients with suspected NASH. *Gut* 2009; **58**: 839–44.

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***Original Article***

# Efficacy of splenectomy in preventing anemia in patients with recurrent hepatitis C following liver transplantation is not dependent on inosine triphosphate pyrophosphatase genotype

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*Aim:* A genetic polymorphism of inosine triphosphate pyrophosphatase (ITPA) has been associated with pegylated-interferon/ribavirin (PEG-IFN/RBV)-induced anemia in chronic hepatitis C patients. However, correlation of the genetic variant with anemia following liver transplantation has not been determined.

*Methods:* Sixty-three hepatitis C virus (HCV)-positive patients who underwent liver transplantation and PEG-IFN/RBV therapy were enrolled. The rs1127354 was determined for each individual.

*Results:* There was no relationship with anemia or RBV dosage in patients carrying the CC allele (CC group, $n = 43$) and those carrying the CA allele (CA group, $n = 20$). The incidence of hemoglobin (Hb) decline >3 g/dL (CC: 4.7%, CA: 0%) was relatively low, whereas the incidence of Hb levels <10 g/dL (CC: 18.6%, CA: 30.0%) was high. Univariate analysis revealed that splenectomy inversely correlated with Hb levels <10 g/dL at 4 weeks ($P = 0.04$). Among the 22 patients who did not undergo splenectomy, the incidence of Hb levels <10 g/dL tended to be lower in the seven patients carrying the CA allele (28.6%) than in the 15 patients with the CC allele (60.0%).

*Conclusion:* The ITPA genetic polymorphism does not correlate with post-transplant PEG-IFN/RBV-induced anemia. Splenectomy is useful in preventing anemia regardless of the ITPA genotype.

**Key words:** inosine triphosphate pyrophosphatase genetic polymorphism, liver transplantation, recurrent hepatitis C, splenectomy

## INTRODUCTION

HEPATITIS C VIRUS (HCV) and its related diseases are the leading cause of liver transplantation (LT) worldwide.[1] The incidence of HCV re-infection is increased in almost all cases after LT and the outcome of post-transplant antiviral therapy is very poor.[2] Although the combination of pegylated interferon and ribavirin (PEG-IFN/RBV) is the standard antiviral therapy for HCV, it is expensive and has some side effects such as flu-like symptoms, thrombocytopenia, and anemia. Of these problems, anemia is a serious matter, especially for Japanese patients, as erythropoietin replacement therapies are not covered by public medical insurance and are seldom performed. Furthermore, the incidence of anemia after LT is as high as 50%, even without anti-HCV therapy.[3] PEG-IFN/RBV therapy for recurrent hepatitis C after LT has been reported to cause anemia in no less than 71% of recipients.[4] To prevent these side effects, various techniques have been trialed, including: (i) simultaneous splenectomy at transplantation,[5] and (ii) PEG-IFN-α2 therapy with 200 mg RBV daily followed by an increase in dosage according to the tolerance of the individual.[6]

Recently, two single nucleotide polymorphisms (SNPs) in the inosine triphosphatase pyrophosphatase

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(ITPA) gene were reported to correlate with treatment-induced anemia in chronic hepatitis C patients. These were identified as rs1127354 and rs7270101.[7] These two SNPs are known to be responsible for ITPA deficiency[7] and inosine triphosphate (ITP) accumulation in erythrocytes, and are thought to confer protective effects in ribavirin-related hemolytic anemia. Of these two SNPs, rs7270101 is not polymorphic in Japanese people,[8] but variants at rs1127354 have been demonstrated to be significantly associated with treatment-induced anemia in Japanese hepatitis C patients.[9,10]

If this genetic polymorphism is a predictor of anemia after post-transplant PEG-IFN/RBV therapy, then that would lead to a new tailored anti-HCV treatment post-LT. In this article, we describe the relationship of the ITPA genetic polymorphism to anemia in LT patients undergoing PEG-IFN/RBV therapy, and demonstrate the usefulness of our strategies against the aforementioned side effects.

## METHODS

### Patients

FROM APRIL 1999 to March 2009, 112 HCV-RNA-positive patients underwent LT at our institute, of which 78 patients were administered PEG-IFN/RBV therapy. Of these 78 patients, five patients who were under treatment, six patients who dropped out from treatment because of its side effect other than anemia such as depression, and three patients whose Hb levels after treatment were unavailable, were excluded from this study. Therefore, 63 patients were retrospectively analyzed. The current study was approved by the ethics committee of Kyushu University.

### Antiviral treatment

The primary doses of PEG-IFN α2b (Pegintron®; Schering-Plough Inc, Kenilworth, NJ, USA) and RBV (Rebetol®; Schering-Plough Inc) were 0.5 μg/kg per week and 200 mg daily, respectively. They were increased to 1.5 μg/kg per week and 800 mg daily in a stepwise manner according to individual tolerance as previously described.[6] Neither granulocyte colony-stimulating factor nor erythropoietin was used in any individual.

### Assessment of the therapeutic effects and anemia

A virological response (VR) was defined as a lack of HCV RNA in response to the treatment regimen regardless of whether a relapse occurred when treatment was terminated. A sustained virological response (SVR) was defined as a lack of HCV RNA at 6 months after completion of the treatment. Treatment-induced anemia was defined as a decline in hemoglobin (Hb) greater than 3 g/dL at 4 weeks, or a Hb level less than 10 g/dL at 4 weeks as previously described.[7]

### DNA extraction and ITPA genotyping

DNA was extracted from the recipient's exenterated liver tissue at transplantation, and direct sequencing was performed using a Big Dye Terminator v1.1 Cycle Sequence Kit (Applied Biosystems Inc., Tokyo, Japan) according to the manufacturer's protocol. The primers used to identify the ITPA genetic polymorphism (rs1127354) were 5′-AGA GTT ATC GAT GAG AAA-3′ (sense) and 5′-GAG AAA TCC AAC CAT CTT-3′ (antisense).

### Statistical analysis

All data was analyzed using JMP® statistical software. A $\chi^2$ test was performed for qualitative variables and a Wilcoxon test was performed for quantitative variables.

## RESULTS

### ITPA genotyping and anemia

THE ITPA MAJOR homozygote allele (rs1127354: CC) was seen in 43 recipients (68.3%) and the heterozygote allele (CA) was seen in 20 recipients (31.7%). No recipient enrolled in the current study carried the minor homozygote allele (AA). The patients' backgrounds between these two genotypes have been outlined in Table 1. None of the pre-transplant, operative, and pre-treatment factors exhibited any differences, except for pre-treatment viral titre.

Among those carrying the CC allele, only two recipients (4.7%) showed a decline in Hb greater than 3 g/dL at 4 weeks after the commencement of PEG-IFN/RBV therapy; whereas none of the recipients carrying the CA allele showed a Hb decline greater than 3 g/dL ($P = 0.311$; Fig. 1a). In contrast, eight recipients whose Hb level was less than 10 g/dL at 4 weeks carried the CC allele and six carried the CA allele ($P = 0.327$; Fig. 1b). In addition, the progression of anemia during the treatment between two groups were compared by each Hb decline at 4, 8, and 12 weeks after commencement of the therapy to reveal that there was no difference (−0.92 g/dL vs. −0.59 g/dL; $P = 0.59$, −1.33 g/dL vs. −0.74 g/dL; $P = 0.27$, −1.39 g/dL vs.

**Table 1** Comparison of the data among patients carrying CC allele and CA allele at rs1127354

| rs1127354 | CC ($n = 43$) | CA ($n = 20$) | *P*-value |
|---|---|---|---|
| *Pretransplantation factor* | | | |
| Recipient's age (years), mean ± SD | 57 ± 1 | 56 ± 2 | n.s |
| Recipient's sex (male / female), *n* | 24 / 19 | 14 / 6 | n.s |
| Recipient's BMI ($kg \cdot m^{-2}$), mean ± SD | 24.9 ± 0.62 | 24.0 ± 0.88 | n.s |
| Donor's age (y), mean ± SD | 33 ± 2 | 34 ± 2 | n.s |
| Donor's sex (male / female), *n* | 31 / 12 | 12 / 8 | n.s |
| Donor's BMI ($kg \cdot m^{-2}$), mean ± SD | 23.3 ± 0.61 | 21.3 ± 0.89 | n.s |
| Pretransplant Hb level (g/dL), mean ± SD | 10.9 ± 0.36 | 11.2 ± 0.48 | n.s |
| MELD score, mean ± SD | 10.3 ± 0.79 | 10.8 ± 1.1 | n.s |
| *Operative factor* | | | |
| Operative time (min), mean ± SD | 793 ± 31 | 839 ± 44 | n.s |
| Simultaneous splenectomy (yes/no), *n* | 28 / 15 | 13 / 7 | n.s |
| Intraoperative bleeding (mL), mean ± SD | 5752 ± 891 | 6105 ± 1260 | n.s |
| GV/SLV (%), mean ± SD | 40.5 ± 1.4 | 42.3 ± 2.0 | n.s |
| *Post-transplantation factor* | | | |
| Bile duct complication (yes / no), *n* | 40 / 3 | 16 / 4 | n.s |
| Pretreatment viral load (logIU/mL), mean ± SD | 6.2 ± 0.1 | 6.6 ± 0.2 | 0.02 |
| Pathological activity score, mean ± SD | 1.3 ± 0.12 | 1.4 ± 0.16 | n.s |
| Pathological fibrosis score, mean ± SD | 1.1 ± 0.20 | 0.88 ± 0.28 | n.s |
| Immunospressive agents (CyA / FK), *n* | 21 / 22 | 15 / 5 | n.s |
| Total dose of RBV during the first 4 weeks (mg), mean ± SD | 8882 ± 703 | 8755 ± 1034 | n.s |
| Pretreatment Hb level (g/dL), mean ± SD | 12.3 ± 0.27 | 11.9 ± 0.40 | n.s |

BMI, body mass index; CyA, cyclosporine; FK, tacrolimus; GV, graft volume; Hb, hemoglobin; MELD, model for end-stage liver disease; n.s, not significant; SLV, standard liver volume.

−1.59 g/dL; $P = 0.81$, respectively, Fig. 1c). The ITPA genetic polymorphism did not correlate with PEG-IFN/RBV-induced anemia after LT.

## ITPA genotype and RBV dosage

The dosage of PEG-IFNα2b and RBV were adjusted for each individual so as not to cause any side effects, including anemia. If the ITPA minor allele was able to protect post-transplant patients from RBV-related hemolytic anemia, the RBV dosage could be increased in recipients carrying the CA allele. As described in Table 1, total dose of RBV administered during the first 4 weeks were similar in each group (8882 mg vs. 8875 mg, $P = 0.787$). It was possible to increase the RBV dosage in 16 recipients (40%) carrying the CC allele and eight recipients (40%) carrying the CA allele ($P = 1.00$; Fig. 2a). Twelve patients carrying the CC allele and four carrying the CA allele had their RBV dosage decreased because of anemia ($P = 0.409$; Fig. 2b).

Ochi *et al.*[9] reported that there was marginal correlation between ITPA genetic polymorphism and the outcome of PEG-IFN/RBV therapy, probably because of the dose reduction of RBV in patients showing severe anemia. In patients enrolled in the current study, the therapeutic effects between recipients carrying the CC and those carrying the CA allele were not significantly different; with a VR of 68.9% and 72.7% ($P = 0.746$; Fig. 3a), respectively. The SVR for these two groups was 38.9% and 42.7% ($P = 0.768$, Fig. 3b), respectively.

## Efficacy of splenectomy

We performed simultaneous splenectomy at transplantation for HCV-related liver diseases to prevent PEG-IFN/RBV therapy-induced blood cytopenia. Univariate analysis showed that splenectomy was significantly related to a Hb level less than 10 g/dL after 4 weeks (Table 2). Therefore, to prove the efficacy of splenectomy against treatment-induced anemia, the incidence of anemia and RBV dose reduction were compared between 41 recipients who had undergone spontaneous splenectomy (Spx group) and 22 recipients who had not undergone splenectomy (Non-Spx group). Although the incidence of Hb decline greater than 3 g/dL was not significantly different between the two groups (2.4 *vs.* 4.5%, $P = 0.649$; Fig. 4a), the Spx group showed a

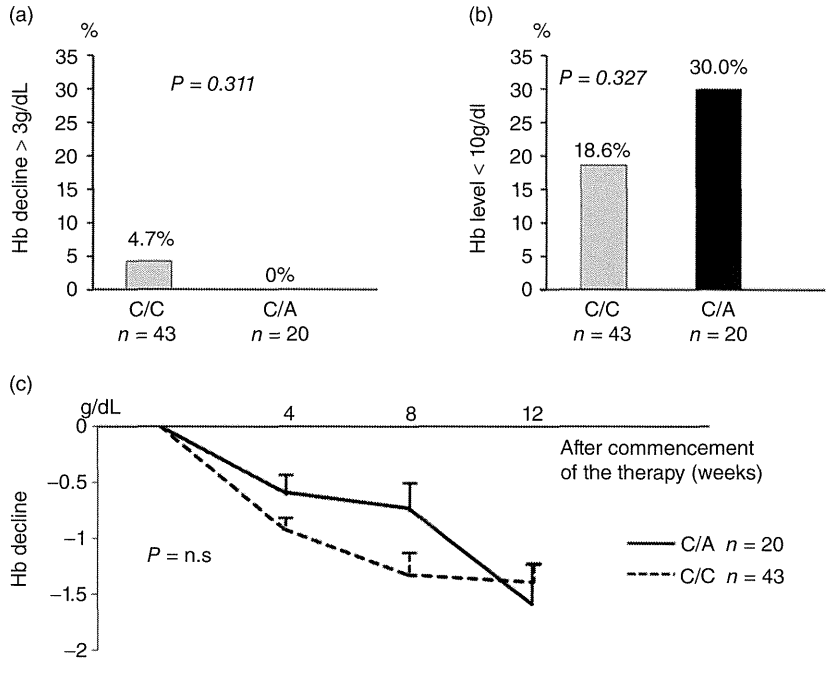


**Figure 1** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and pegylated-interferon/ribavirin (PEG-IFN/RBV)-related anemia after liver transplantation (LT). (a) Hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks after the commencement of therapy was found in 4.7% of CC allele carriers and in none of the CA allele carriers. (b) hemoglobin (Hb) levels less than 10 g/dL at 4 weeks were found in 18.6% of CC allele carriers and in 30.0% of CA allele carriers. (c) Hb decline at 4, 8, and 12 weeks after commencement of the therapy were compared. There was no statistical difference in the progression of anemia during the treatment between two groups. (—): C/A $n = 20$; (----): C/C $n = 43$.

significantly lower incidence of Hb levels lower than 10 g/dL compared with the Non-Spx group (14.6 *vs.* 36.4%, $P < 0.05$; Fig. 4b). Additionally, the RBV dosage tended to be increased more often in the Spx group than in the Non-Spx group (46.3 *vs.* 22.7%, $P = 0.09$; Fig. 4c); and at the same time was not reduced because of anemia (19.5 *vs.* 36.4%, $P = 0.09$; Fig. 4d), though there was no statistical difference.

The incidence of treatment-induced anemia between those carrying the CC and CA alleles among the non-Spx group was evaluated. Of the 22 recipients in the non-Spx group, 15 carried the CC allele and seven carried the CA allele. Although there was no significant difference because of the small numbers involved, a Hb decline greater than 3 g/dL and Hb levels less than 10 g/dL at 4 weeks were found more often in recipients carrying

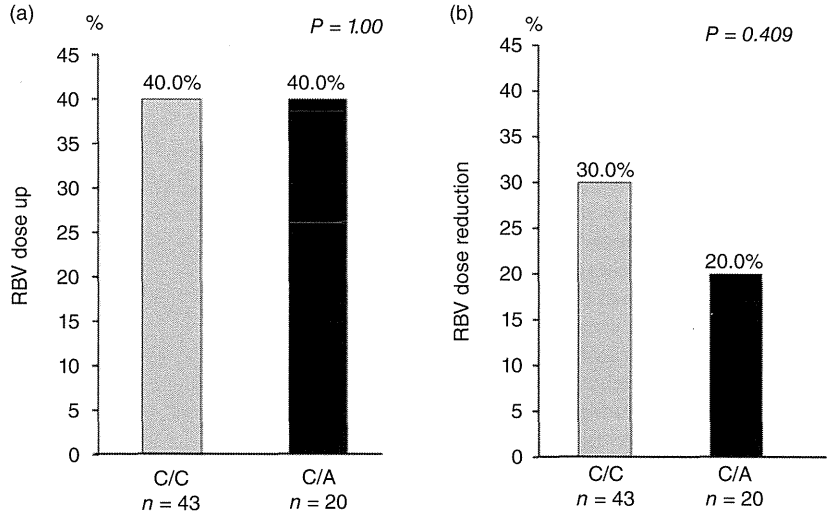


**Figure 2** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and ribavirin (RBV) dosage. (a) The dosage of RBV was increased in 40% of each genotype group. (b) RBV dose reduction due to anemia was found in 30% of those carrying the CC allele and 20% of those carrying the CA allele.

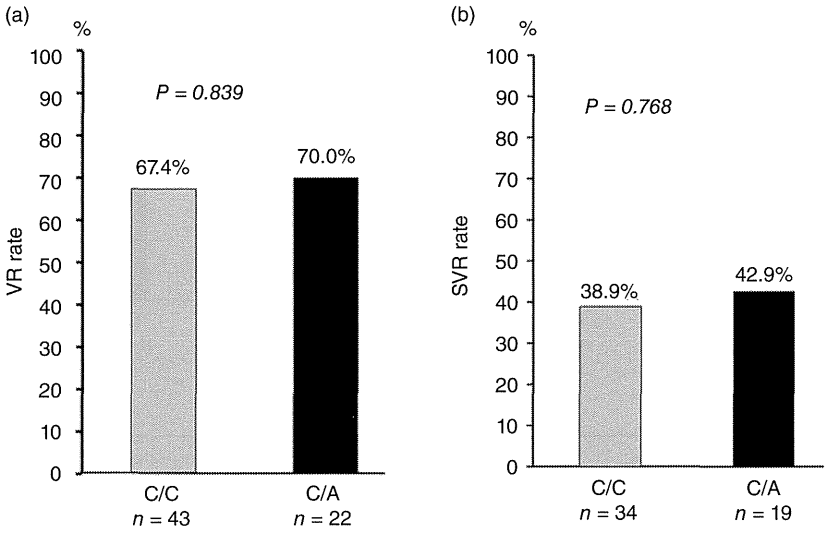


**Figure 3** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and virological response. (a) Cirological response (VR) between the two genotypes was 68.9% and 72.7%. (b) The incidence of the sustained virological response (SVR) was 38.9% and 42.9%.

the CC allele (6.6 *vs.* 0% and 60 *vs.* 28.6%, respectively; Fig. 5a,b). In addition, tolerance to RBV seemed better in recipients carrying the CA allele. The dosage of RBV was able to be increased in 15.4% of those carrying the CC allele and in 42.9% of those carrying CA (Fig. 5c). At the same time, RBV dose reduction due to anemia was found in 46.7% of those carrying the CC allele and in 28.6% of those with the CA allele (Fig. 5d).

**Table 2** Comparison of the data among patients whose Hb level < 10 g/dL and ≥10 g/dL at 4 weeks

| Hb level at 4 weeks | Hb ≥ 10 g/dL ($n$ = 49) | Hb < 10 g/dL ($n$ = 14) | $P$-value |
|---|---|---|---|
| ***Pretransplantation factor*** | | | |
| Recipient's age (year), mean ± SD | 56 ± 1 | 58 ± 2 | n.s |
| Recipient's sex (male/female), $n$ | 32 / 17 | 6 / 8 | n.s |
| Recipient's BMI (kg·m$^{-2}$), mean ± SD | 24.6 ± 0.6 | 24.5 ± 1.3 | n.s |
| Donor's age (year), mean ± SD | 33 ± 2 | 34 ± 4 | n.s |
| Donor's sex (male/female), $n$ | 33 / 16 | 10 / 4 | n.s |
| Donor's BMI (kg·m$^{-2}$), mean ± SD | 23.0 ± 0.6 | 21.5 ± 1.2 | n.s |
| Pretransplant Hb level (g/dL), mean ± SD | 11.2 ± 0.32 | 9.9 ± 0.68 | n.s |
| MELD score, mean ± SD | 10.2 ± 0.73 | 10.9 ± 1.2 | n.s |
| ***Operative factor*** | | | |
| Operative time (min), mean ± SD | 823 ± 29 | 730 ± 63 | n.s |
| Simultaneous splenectomy (yes/no), $n$ | 35 / 14 | 6 / 8 | 0.04 |
| Intraoperative bleeding (mL), mean ± SD | 5721 ± 786 | 5332 ± 1260 | n.s |
| GV / SLV (%), mean ± SD | 40.2 ± 1.0 | 44.5 ± 2.3 | n.s |
| ***Post-transplantation factor*** | | | |
| Bile duct complication (yes/no), $n$ | 40 / 3 | 16 / 4 | n.s |
| Pretreatment viral load (logIU/mL), mean ± SD | 6.2 ± 0.1 | 6.7 ± 0.2 | 0.03 |
| Pathological activity score, mean ± SD | 1.3 ± 0.11 | 1.2 ± 0.22 | n.s |
| Pathological fibrosis score, mean ± SD | 0.9 ± 0.19 | 1.2 ± 0.38 | n.s |
| Immunospressive agents (CyA / FK) | 25 / 24 | 11 / 3 | n.s |
| Total dose of RBV during the first 4 weeks (mg), mean ± SD | 9282 ± 633 | 7000 ± 1294 | n.s |
| Pretreatment Hb level (g/dL), mean ± SD | 12.7 ± 0.21 | 10.4 ± 0.40 | <0.0001 |

BMI, body mass index; CyA, cyclosporine; FK, tacrolimus; GV, graft volume; Hb, hemoglobin; MELD, model for end-stage liver disease; n.s, not significant; RBV, ribavirin; SLV, standard liver volume.

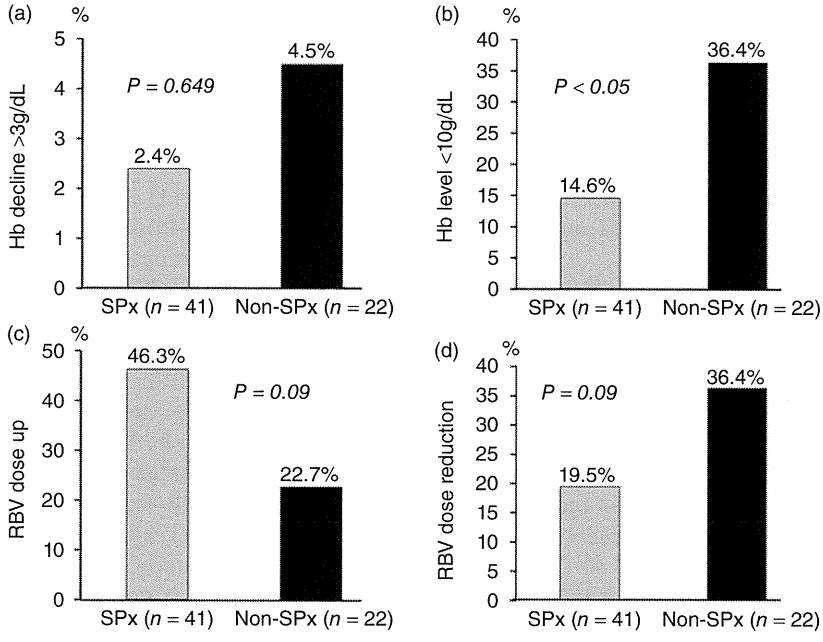


**Figure 4** The efficacy of splenectomy for anaemia and ribavirin (RBV) tolerance. (a) The incidence of a hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks was evident in 2.4% of recipients who had simultaneous splenectomy at liver transplantation (LT) (Spx group) and 4.5% of recipients were not subjected to a splenectomy (non-Spx group). (b) The incidence of Hb level less than 10 g/dL at 4 weeks was significantly lower in the Spx (14.6%) as compared with the non-Spx group (36.4%). (c) The dosage of RBV tended to increase more often in the Spx group (46.3%) than in the non-Spx group (22.7%). (d) RBV dose reduction due to anemia tended to be less frequent in the Spx group (19.5%) than in the non-Spx group (36.4%).

## DISCUSSION

HCV-RELATED LIVER DISEASES are the main reason for liver transplantation worldwide.[1] The post-transplant prognosis for HCV is worse than with other diseases because of the recurrence of hepatitis C[11]. Although PEG-IFN/RBV is the only standardized anti-HCV therapy after LT, the outcome is poor with less than 30% of cases exhibiting a SVR. This is likely because of immunosuppressive agents used and severe side

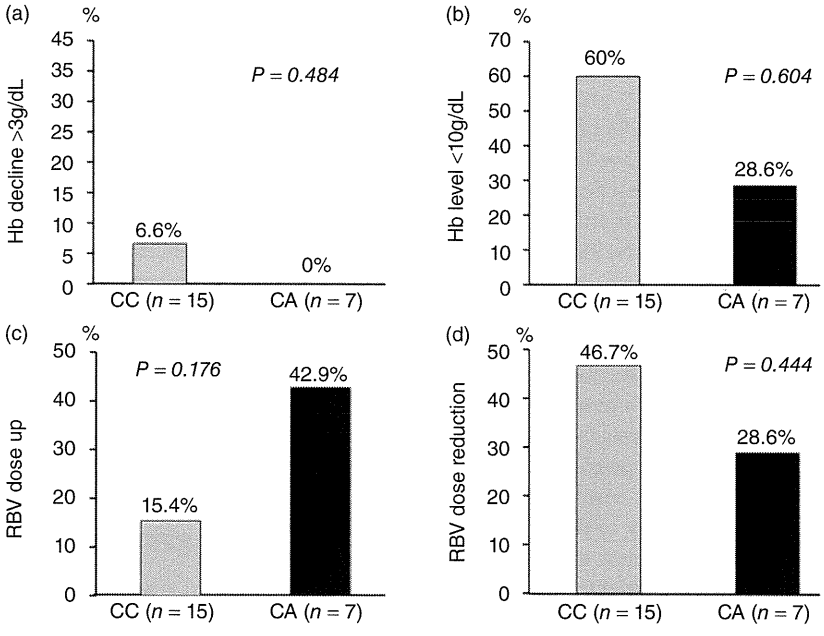


**Figure 5** Inosine triphosphate pyrophosphatase (ITPA) genotypes and anemia among non-Spx group. (a) The incidence of hemoglobin (Hb) decline at 4 weeks was higher in CC allele carriers compared with CA allele carriers (6.6 *vs.* 0%). (b) The incidence of Hb levels less than 10 g/dL at 4 weeks was higher in the CC allele carriers (60 *vs.* 28.6%). (c) The dosage of RBV could be increased more often in CA allele carriers than in CC allele carriers (42.9 *vs.* 15.4%). (d) RBV dose reduction due to anemia was found more often in CC allele carriers than in CA allele carriers (46.7 *vs.* 28.6%).

effects, including anemia.[2] Treatment-induced anemia is an important issue in Japan where erythropoietin-replacement therapy is seldom performed. The ITPA genetic polymorphism was recently reported to be associated with PEG-IFN/RBV-induced anemia in chronic hepatitis C patients.[7,9,10] However, the correlation between this genetic polymorphism and post-transplant PEG-IFN/RBV induced anemia has never been examined until now.

We have made many attempts to prevent side effects, such as minimal dose of PEG-IFN/RBV at therapy commencement followed by dose adjustment in a stepwise manner.[6] In the current study, we hypothesized that the CA allele at rs1127354 correlated to post-transplant PEG-IFN/RBV therapy-induced anemia, and that those who carried the CA allele could tolerate a full dose of PEG-IFN/RBV without reduction.

Among the 63 recipients enrolled in this study, the CA allele was found in 20 (31.7%) patients, a frequency corresponding to previous reports regarding Japanese people.[8] Contrary to the hypothesis, the ITPA genetic polymorphism did not correlate with treatment-induced anemia after LT as shown in Figures 1 and 2. The incidence of Hb decline greater than 3 g/dL was relatively low, whereas the numbers of patients with Hb levels less than 10 g/dL were high at 4 weeks of post-transplant PEG-IFN/RBV therapy compared with those in previous reports for chronic hepatitis C patients.[7,10] In the current study, Hb decline was found in 4.7% of individuals in the CC group and none in the CA group, whereas this was 47.6–48.7% and 0.8–4.5%, respectively, in chronic hepatitis C patients.[7,10] In contrast, a Hb level below 10 g/dL was found in 18.6% and 30.0% in the CC and CA groups, respectively, and in 9.3–15.9% and 0.0–0.8% of chronic hepatitis C patients.[7,10] These findings may reflect that post-transplant patients are originally subject to severe anemia with or without PEG-IFN/RBV treatment and that our stepwise manner protocol in PEG-IFN/RBV therapy prevents the progression of anemia.

Ochi *et al.*[9] demonstrated that the ITPA genetic polymorphism correlated not only with anemia but with treatment efficacy. In the present study, however, the VR was not different between the two genotypes. It can be assumed that this was because of similar RBV tolerance, although another possibility is that the difference for each pretreatment viral load (6.2 *vs.* 6.6 logIU/mL) affected the efficacy of the ITPA minor genotype. It was recently reported that treatment-related anemia would possibly be associated with a greater occurrence of VR[12]. The correlation of the ITPA genetic polymorphism or anemia with the efficacy of PEG-IFN/RBV therapy requires further investigation.

Another strategy against the side effects of post-transplant PEG-IFN/RBV therapy at our institute is simultaneous splenectomy at LT[5]. Splenectomy is known to be effective and safe in combination with PEG-IFN/RBV therapy for thrombocytopenic patients with HCV-related cirrhosis,[13–15] but its efficacy in alleviating anemia is yet to be demonstrated. In the guidelines for the treatment of chronic hepatitis and cirrhosis due to HCV in Japan,[16] a splenectomy is recommended for patients with a platelet count less than 50 000/mm$^3$. Kishi *et al.*[17] described that a splenectomy was effective for treating leukocytopaenia, thrombocytopenia, but not for anemia in post-transplant recurrent HCV patients. In fact, there was no difference in pretreatment Hb levels between the Spx and non-Spx groups (12.0 *vs.* 12.4 g/dL, $P = 0.39$; data not shown) in the present study. However, the incidence of Hb levels less than 10 g/dL after treatment was significantly lower in the Spx group as compared with the non-Spx group, which shows the efficacy of a splenectomy for treatment-induced anemia after LT. At the same time, the ITPA genetic polymorphism tended to be associated with treatment-induced anemia and RBV tolerance in the non-Spx group only, similar to chronic hepatitis C patients. Conversely, it could be said that a splenectomy prevents PEG-IFN/RBV-related anemia regardless of the ITPA genetic polymorphism. However, neither splenectomy nor other factors that were suggested to be significantly associated with anemia by univariate analysis was shown to be associated with Hb level <10g/dl at 4 weeks after commencement of the therapy by multiple logistic regression (data not shown). The proof of the efficacy of splenectomy in preventing PEG-IFN/RBV induced anemia needs further investigation.

In conclusion, this is the first report regarding the relationship of the ITPA genetic polymorphism and anemia caused by post-transplant PEG-IFN/RBV therapy in recurrent HCV. The ITPA genetic polymorphism does not correlate with treatment-induced anemia after LT.

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## REFERENCES

1 Brown RS. Hepatitis C and liver transplantation. *Nature* 2005; **436** (7053): 973–8.

2 Angelico M, Petolati A, Leionetti R *et al.* A randomized study on Peg-interferon alfa-2a with or without ribavirin in liver transplants recipients with recurrent hepatitis C. *J Hepatol* 2007; **46** (6): 1009–17.
3 Guitard J, Ribes D, Kamar N *et al.* Predictive factors for anaemia six and twelve months after orthotopic liver transplantation. *Transplantation* 2006 (15); **81** (11): 1525–31.
4 Castells L, Vargas V, Allende H *et al.* Combined treatment with pegylated interferon (alpha-2b) and ribavirin in the acute phase of hepatitis C virus recurrence after liver transplantation. *J Hepatol* 2005; **43** (1): 53–9.
5 Ikegami T, Toshima T, Takeishi K *et al.* Bloodless splenectomy during liver transplantation for terminal liver diseases with portal hypertension. *J Am Coll Surg* 2009; **208** (2): e1–4.
6 Ikegami T, Taketomi A, Soejima Y *et al.* The benefits of interferon treatment in patients without sustained viral response after living donor liver transplantation for hepatitis C. *Transplant Proc* 2009; **41**: 4246–52.
7 Fellay J, Thompson AJ, Ge D *et al.* ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; **464** (7287): 405–8.
8 Maeda T, Sumi S, Ueta A *et al.* Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. *Mol Genet Metab* 2005; **85** (4): 271–9.
9 Ochi H, Maekawa T, Abe H *et al.* ITPA polymorphism affects ribavirin-induced anaemia and outcomes of therapy – a genome-wide study of Japanese HCV virus patients. *Gastroenterology* 2010; **139** (4): 1190–7.
10 Sakamoto N, Tanaka Y, Nakagawa M *et al.* ITPA gene variant protects against anaemia induced by pegylated interferon-α and ribavirin therapy for Japanese patients with chronic hepatitis C. *Hepatol Res* 2010; **40** (11): 1063–71.
11 Moonka DK, Kim D, Kapke A, Brown KA, Yoshida A. The influence of induction therapy on graft and patient survival in patients with and without hepatitis C after liver transplantation. *Am J Transplant* 2010; **10**: 590–601.
12 Sulkowski MS, Shiffman ML, Afdhal NH *et al.* Hepatitis C virus treatment-related anaemia is associated with higher sustained virologic response rate. *Gastroenterology* 2010; **139** (5): 1602–11.
13 Ikezawa K, Naito M, Yumiba T *et al.* Splenectomy and antiviral treatment for thrombocytopenic patients with chronic hepatitis C virus infection. *J Viral Hepat* 2010; **17** (7): 488–92.
14 Morihara D, Kobayashi M, Ikeda K *et al.* Effectiveness of combination therapy of splenectomy and long-term interferon in patients with hepatitis C virus-related cirrhosis and thrombocytopenia. *Hepatol Res* 2009; **39** (5): 439–47.
15 Kawanaka H, Akahoshi T, Kinjo N *et al.* Impact of antithrombin III concentrates on portal vein thrombosis after splenectomy in patients with liver cirrhosis and hypersplenism. *Ann Surg* 2010; **251** (1): 76–83.
16 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40** (1): 8–13.
17 Kishi Y, Sugawara Y, Akamatsu N *et al.* Splenectomy and preemptive interferon therapy for hepatitis C patients after living-donor liver transplantation. *Clin Transplant* 2005; **19** (6): 769–72.

Hepatology Research

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***Original Article***

# Impact of tumor size, number of tumors and neutrophil-to-lymphocyte ratio in liver transplantation for recurrent hepatocellular carcinoma

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*Aim:* Hepatocellular carcinoma (HCC) is primarily treated with hepatic resection and/or locoregional therapy. When HCC recurs and further treatment is no longer possible owing to poor liver function, liver transplantation (LT) or living-donor LT (LDLT) is considered. The aim of this study was to clarify risk factors for tumor recurrence after LDLT in patients with recurrent HCC.

*Methods:* The study comprised 104 patients who had undergone LDLT because of end-stage liver disease with recurrent HCC. The recurrence-free survival rates after the LDLT were calculated. Risk factors for tumor recurrence were identified.

*Results:* The 1-, 3- and 5-year recurrence-free survival rates were 89.6%, 80.3% and 78.4%, respectively. By univariate analysis, the factors affecting recurrence-free survival were the sum of the largest tumor size and number of tumors of 8 or more ($P < 0.0001$), des-γ-carboxy prothrombin of more than 300 mAU/mL ($P = 0.0001$), and a neutrophil-to-lymphocyte ratio (NLR) of 4 or more ($P = 0.0002$), α-fetoprotein of more than 400 ng/mL ($P = 0.0001$) and bilobar tumor distribution ($P = 0.046$). A multivariate analysis identified independent risk factors for post-LDLT tumor recurrence including the sum of tumor size and number of tumors of 8 or more ($P = 0.0004$) and an NLR of 4 or more ($P = 0.01$). The 1- and 3- year recurrence-free survival rates in the recipients who had both risk factors were 30.0% and 15.0%, respectively.

*Conclusion:* LDLT should not be performed for patients who have both independent risk factors after any treatments for HCC.

**Key words:** hepatocellular carcinoma, living-donor liver transplantation, neutrophil-to-lymphocyte ratio, number of tumors, tumor size

## INTRODUCTION

A SHORTAGE OF cadaveric organs for transplantation continues to impair our ability to provide liver transplantation (LT) despite progress in surgical techniques and immunosuppression.[1,2] Currently, there is no consensus on how to manage patients with hepatocellular carcinoma (HCC) while awaiting LT. Guidelines published in the UK state that locoregional therapy, such as transarterial chemoembolization (TACE), radiofrequency ablation (RFA), ethanol injection therapy and microwave coagulation therapy (MCT), should be considered for all listed patients with HCC.[3] In Asian countries, religious, cultural and political ideologies have created significant obstacles to the transplantation of organs from cadavers. As a result, HCC is primarily treated with hepatic resection and/or locoregional therapy.[4,5] However, when HCC recurs and further treatment is no longer possible owing to poor liver function, LT is considered.[4] Organ shortages have forced patients with recurrent HCC to endure long waiting periods that are associated with tumor development. Thus, living-donor LT (LDLT) is a potential choice for treating recurrent HCC patients after the use of other

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*Conflict of interest:* none.
*Author contribution:* Tomoharu Yoshizumi designed the study; Tomoharu Yoshizumi, Ken Shirabe, Toru Ikegami, Yuji Soejima, Shohei Yoshiya Yohei Mano, Jun Muto and Tetsuo Ikeda performed the study; Tomoharu Yoshizumi, Takashi Motomura and Toru Ikegami collected the data; Tomoharu Yoshizumi, Ken Shirabe and Yoshihiko Maehara analyzed the data; and Tomoharu Yoshizumi wrote the paper.
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treatments.[4] Since the 1994 report demonstrating successful LDLT, living donors have been increasingly used because of the disparity between demand and supply, even in Western countries.[2,6] Moreover, a blood relationship between the donor and the recipient in LDLT may give the recipient a chance to receive a transplant even during the suboptimal conditions of HCC.[7-9]

Thus, it is important to focus on factors that affect tumor recurrence after LDLT in patients with recurrent HCC.

The neutrophil-to-lymphocyte ratio (NLR) has recently emerged as a useful prognostic factor for the recurrence of several malignancies. An NLR of 5 or more was reported to be a marker of survival in colorectal cancer patients.[10] Halazun *et al.* reported that an NLR of five or more was an independent predictor of the recurrence and poor overall survival in patients with colorectal liver metastases.[11] Recently, it was demonstrated that a preoperative NLR of 5 or more was an adverse predictor of recurrence-free survival for patients undergoing hepatic resection for HCC.[12] Furthermore, an elevated NLR significantly increased the risk of HCC recurrence after LT[13] or LDLT.[14]

Mazzaferro *et al.* recently proposed the "up-to-seven criteria", with 7 being the result of the sum of the largest tumor size (in cm) and number of tumors, to predict patient survival after LT, based on a large sample size.[15] We have reported the outcome of LDLT for otherwise unresectable and/or untreatable HCC patients[7,16] and proposed two risk factors for recurrence-free survival: a tumor size greater than 5 cm and des-γ-carboxy prothrombin (DCP) levels greater than 300 mAU/mL (Kyushu University [KU] criteria).[7] Furthermore, we previously reported a series of 68 cases of LDLT for patients who had received pretransplant treatment for HCC.[4] DCP above 300 mAU/mL was shown to be an independent risk factor for tumor recurrence after LDLT in the published work. Since this report, LDLT has become a more common treatment for such patients, thus generating a larger cohort for study.

Therefore, the aim of the present study was to clarify the risk factors of tumor recurrence after LDLT in patients with recurrent HCC.

## METHODS

### Recipients

ONE HUNDRED AND sixty-seven recipients underwent LDLT because of end-stage liver disease with HCC at Kyushu University Hospital between April 1999 and August 2012. In this study, 104 adult patients (41 female and 63 male) were enrolled who had undergone LDLT because of end-stage liver disease with recurrent HCC after treatment. The pretransplant treatments for HCC, such as RFA, TACE, MCT and/or hepatic resection, were dependent upon the recipient's liver function and tumor status. Graft types included left lobe with caudate lobe graft ($n = 63$), right lobe graft without the middle hepatic vein ($n = 37$) and posterior segment graft ($n = 4$). The etiology of liver cirrhosis included hepatitis C ($n = 75$), hepatitis B ($n = 20$), cryptogenic disease ($n = 4$), alcohol abuse ($n = 3$) and primary biliary cirrhosis ($n = 2$) (Table 1). Our selection criteria to perform LDLT for HCC patients were as follows: (i) no modality except LDLT available to cure the patients with HCC; (ii) no extrahepatic metastasis; and (iii) no major vascular infiltration.[4,7] There were no restrictions on the tumor size, number of tumors or pretransplant treatment. Since defining the KU criteria, we have not performed LDLT for HCC patients with a tumor size greater than 5 cm and DCP levels greater than 300 mAU/mL.

Pretransplant imaging was used to estimate the maximum tumor size, number of tumors and up-to-seven criteria. α-Fetoprotein (AFP), DCP and NLR were measured before the LDLT. The histological grades obtained from the explanted livers were used for tumor differentiation.

### Donor and graft selection

Donors were selected from among the candidates who hoped to be living donors.[1,8] Donors were required to be within the third degree of consanguinity with recipients or spouses, and to be between 20 and 65 years of age. For a donor who was not within the third degree of consanguinity, individual approval was obtained from the Ethics Committee of Kyushu University Hospital. Good Samaritan donations were not used.

Eligible donors proceeded to the imaging studies, including chest and abdominal X-rays and 3-mm-slice computed tomography (CT) scans for graft volumetric analysis. 3-D CT was introduced for volumetric analysis and delineation of vascular anatomy. The standard liver weight (SLW) of recipients was calculated according to the formula of Urata *et al.*[17] Graft weight (GW) was predicted by CT volumetric analysis. Decisions regarding the graft type for recipients were based on the preoperatively predicted GW to SLW (GW : SLW) ratio. The left lobe with caudate lobe graft was used when the preoperatively predicted GW : SLW ratio was more than 35%. A posterior segment graft was used when the donor's vascular variation was suitable to take the posterior segment.

**Table 1** Characteristics of recipients and donors

| Variables | *n* |
|---|---|
| Recipient | |
| Sex (male/female) | 63/41 |
| Age (years, range) | 58.0 (41–72) |
| Etiology | |
| HCV | 75 |
| HBV | 20 |
| Cryptogenic | 4 |
| Alcohol | 3 |
| PBC | 2 |
| MELD score (range) | 11.5 (4–31) |
| Diabetes mellitus (yes/no) | 31/73 |
| Splenectomy (yes/no) | 60/44 |
| CNI (TAC/CyA/None) | 44/57/3 |
| Donor | |
| Sex (male/female) | 75/29 |
| Age (years, range) | 34.3 (20–63) |
| Graft (left/right/posterior) | 63/37/4 |
| GW : SLW ratio (%, range) | 41.0 (23.6–67.6) |
| Tumor | |
| Maximum size (cm, range) | 2.4 (0–7.0) |
| *n* (range) | 17 (0–400) |
| Milan criteria (yes/no) | 52/52 |
| NLR (range) | 3.1 (0.44–20.2) |
| AFP (ng/mL, range) | 1516 (1–43 000) |
| DCP (mAU/mL, range) | 349 (3–5934) |
| Duration between first Tx and LDLT (days, median, range) | 1198 (61–4272) |
| Duration between last Tx and LDLT (days, median, range) | 349 (30–2140) |
| Times of treatment (range) | 3 (1–11) |
| Microvascular invasion (yes/no) | 39/65 |
| Pathological differentiation (well/moderate/poor) | 7/63/34 |

AFP, α-fetoprotein; CNI, calcineurin inhibitor; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus; Tx, pretransplant treatment.

## Postoperative management

The graft retrieval technique, recipient surgery and perioperative management of the recipients, including immunosuppression regimens, have been described elsewhere.[9,18] Immunosuppression was initiated using a protocol based on either tacrolimus (Prograf; Astellas Pharma, Tokyo, Japan) or cyclosporin A (Neoral; Novartis Pharma, Tokyo, Japan) with steroid and/or mycophenolate mofetil (MMF; Chugai Pharmaceutical, Tokyo, Japan). Tacrolimus was used in 44 recipients and cyclosporin in 57 recipients. Three recipients did not receive calcineurin inhibitor owing to postoperative poor disease course. A target trough of tacrolimus was set at 10 ng/mL for 3 months after LDLT, followed by 5–10 ng/mL thereafter. A target trough level of cyclosporin A was set at 250 ng/mL for 3 months after LDLT, followed by 150–200 ng/mL thereafter. Methylprednisolone was initiated on the day of LDLT, tapered and converted to prednisolone 7 days after LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDLT. MMF was used in 91 recipients and was started at 1000 mg/day on the day after LDLT, tapered and discontinued until 6 months after LDLT. A trough level was not measured for MMF.

All patients had monthly follow ups, and the median follow-up period was 1738 days, with 723 days and 2891 days as the 25th and 75th percentiles, respectively.

## Post-LDLT tumor recurrence and risk factors

Hepatocellular carcinoma recurrence after the LDLT was set as the primary end-point of this study. All patients underwent abdominal CT scan every 3 months, and chest CT scan and bone scintigraphy every 6 months within 5 years after LDLT. Tumor recurrence was defined as when any imaging studies revealed the recurrence of HCC. Recurrence-free survival was defined as the time period between LDLT and tumor recurrence.

Univariate and multivariate analyses were performed to identify the factors associated with recurrence-free survival after the LDLT.

## Statistical analysis

Recurrence-free survival rates were calculated by the Kaplan–Meier product-limited method. Data were expressed as means.

Cox regression analysis was applied to the multivariate analyses. Variables that were used for the analysis included recipient age, donor age, Model for End-Stage Liver Disease score, presence of hepatitis C virus, presence of diabetes mellitus, recipient sex, donor sex, GW : SLW ratio, the sum of the largest tumor size (in cm) and the number of tumors, pretransplant NLR, pretransplant AFP, pretransplant DCP, graft type, splenectomy, duration between first treatment for HCC and the LDLT, duration of last treatment for HCC and the LDLT, times of pretransplant treatment and type of calcineurin inhibitor. All statistical analyses were performed using JMP ver. 9.0 software (SAS, Cary, NC, USA). $P < 0.05$ was considered significant.

## Approval of institutional review board

The Institutional Review Board of Kyushu University Hospital approved this study protocol (no. 23–58).

## RESULTS

THE CHARACTERISTICS OF the recipients and donors from this study are shown in Table 1. Fifty-two of 104 patients (50.0%) exceeded the Milan criteria. Patients previously underwent at least one of the following treatments for primary or recurrent HCC: TACE ($n = 85$), RFA ($n = 54$), ethanol injection therapy ($n = 30$), MCT ($n = 17$), hepatic resection ($n = 11$) and hepatic arterial infusion chemotherapy ($n = 7$). Median times of treatment were 3.0 (1–11 times), median duration from first treatment to LDLT was 1199 days (61–4272 days) and median duration from last treatment to LDLT was 348 days (30–2140 days).

Receiver–operator curve (ROC) analysis for tumor recurrence after LDLT was used to detect the cut-off line of the sum of the largest tumor size (in cm) and number of tumors, and NLR. The area under the ROC (AUROC) of the sum of the largest tumor size (in cm) and number of tumors was 0.833. A cut-off value of the sum was set as 8.0, because ROC analysis revealed that a cut-off value of 8, which had 84.2% of the sensitivity and 80.0% of the specificity, was the most suitable value. Similarly, the AUROC of NLR was 0.700 and a cut-off value of NLR of 4 was set using the analysis.

The 1-, 3- and 5-year recurrence-free survival rates in enrolled recipients were 89.6%, 80.3% and 78.4%, respectively. Among the 104 recipients, 19 patients developed tumor recurrence after LDLT. A univariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more, had an NLR of 4 or more, AFP levels of more than 400 ng/mL, DCP levels of more than 300 mAU/mL and bilobar tumor distribution were risk factors for tumor recurrence after LDLT ($P < 0.0001$, $P = 0.0002$, $P < 0.0001$, $P < 0.0001$, and $P = 0.046$, respectively) (Table 2). Although the nodule size and number of nodules were risk factors of tumor recurrence by the univariate analysis, these factors statistically interfered with the sum of the largest tumor size (in cm) and number of tumors for performing multivariate analysis. The AUROC of the number of nodules was 0.790 and that of the largest nodule size was 0.753. Both data were less than that of the sum of the largest tumor size and number of tumors (0.833). Thus, we selected the sum of the largest tumor size and number of tumors for multivariate analysis. Multivariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more and an NLR of 4 or more were independent risk factors for tumor recurrence after LDLT in this study ($P = 0.0004$ and $P = 0.011$, respectively) (Table 3).

Table 4 shows the correlation between explant pathology and each risk factor. The frequency of microvascular invasion and poorly differentiated tumors increased among patients who had both independent risk factors of tumor recurrence.

The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an NLR of 4 or more were 100%, 81.8% and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0%, and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained (Fig. 1). The differences among the four groups were significantly different ($P < 0.0001$).

## DISCUSSION

THIS IS THE largest study to investigate LDLT with recurrent HCC.[4] It is crucial to clarify when patients with poor liver function and HCC should be listed as candidates for LDLT. We chose recurrence-free survival rate as the end-point in this study because preliminary analysis revealed that 27 deaths occurred in the enrolled recipients, of which 14 causes of death were not tumor-related.

To date, several studies have attempted to extend the Milan criteria to encompass HCC patients with potentially curable tumors.[7,14,19–22] The up-to-seven criteria may predict patient survival even after LDLT.[4,14] The ROC analysis for tumor recurrence after LDLT revealed that the sensitivity of the cut-off value of 7 was 89.4% and the specificity was 71.7%. It meant that a cut-off value of 7 was less suitable than that of 8 in this study. Although we previously proposed that the number of tumors did not affect tumor recurrence after LDLT,[4,7,16] the results obtained from the present study suggest that the number of tumors as well as largest tumor size should be taken into consideration to select HCC patients for LDLT.

The precise mechanism of how NLR affects tumor recurrence is still unclear. Infiltration of pro-inflammatory macrophages, cytokines and chemokines in the tumor microenvironment can boost tumor

**Table 2** Risk factors for tumor recurrence: univariate analysis

| Variables | *n* | Recurrence-free survival (%) | | | *P* |
|---|---|---|---|---|---|
| | | 1 year | 3 years | 5 years | |
| Recipient variables | | | | | |
| Sex | | | | | |
| Male | 63 | 84.5 | 82.7 | 79.5 | 0.81 |
| Female | 41 | 97.4 | 75.7 | 75.7 | |
| Age (years) | | | | | |
| >60 | 46 | 88.1 | 82.3 | 82.3 | 0.67 |
| ≤60 | 58 | 90.8 | 79.1 | 76.1 | |
| Etiology | | | | | |
| HCV | 75 | 88.8 | 79.6 | 77.2 | 0.64 |
| Others | 29 | 91.4 | 82.0 | 82.0 | |
| Pretransplant MELD | | | | | |
| <15 | 84 | 91.2 | 80.1 | 78.0 | 0.99 |
| ≥15 | 20 | 82.1 | 82.1 | 82.1 | |
| Diabetes mellitus | | | | | |
| Yes | 31 | 89.1 | 84.4 | 78.8 | 0.75 |
| No | 73 | 89.7 | 78.5 | 78.5 | |
| NLR | | | | | |
| ≥4 | 21 | 72.7 | 55.9 | 41.9 | 0.0002 |
| <4 | 83 | 93.5 | 86.2 | 86.2 | |
| Splenectomy | | | | | |
| Yes | 60 | 90.9 | 79.9 | 79.9 | 0.82 |
| No | 44 | 87.8 | 80.2 | 77.4 | |
| Calcineurin inhibitor | | | | | |
| TAC | 44 | 90.0 | 80.9 | 80.9 | 0.78 |
| CyA | 57 | 89.4 | 80.1 | 77.3 | |
| Donor variables | | | | | |
| Sex | | | | | |
| Male | 75 | 92.7 | 82.9 | 80.4 | 0.34 |
| Female | 29 | 82.1 | 74.1 | 74.1 | |
| Donor age (years) | | | | | |
| >40 | 25 | 95.2 | 89.6 | 89.6 | 0.19 |
| ≤40 | 79 | 88.0 | 77.6 | 75.3 | |
| Graft type | | | | | |
| Others | 67 | 90.2 | 75.4 | 72.5 | 0.13 |
| Right | 37 | 88.6 | 88.6 | 88.6 | |
| GW : SLW ratio | | | | | |
| <35 | 24 | 86.1 | 76.0 | 76.0 | 0.62 |
| ≥35 | 80 | 90.5 | 81.5 | 79.1 | |
| Tumor variables | | | | | |
| Nodule size (cm) | | | | | |
| ≥5 | 6 | 50.0 | 33.3 | 33.3 | 0.0004 |
| <5 | 98 | 92.2 | 83.5 | 81.4 | |
| No. of nodules | | | | | |
| ≥5 | 34 | 75.2 | 58.0 | 58.0 | 0.0002 |
| <5 | 70 | 96.8 | 91.6 | 88.7 | |
| Nodule size + number | | | | | |
| ≥8.0 | 33 | 67.9 | 46.4 | 46.4 | <0.0001 |
| <8.0 | 71 | 100 | 96.5 | 93.8 | |

**Table 2** *Continued*

| Variables | *n* | Recurrence-free survival (%) | | | *P* |
|---|---|---|---|---|---|
| | | 1 year | 3 years | 5 years | |
| DCP (mAU/mL)† | | | | | |
| >300 | 19 | 51.6 | 38.7 | 38.7 | <0.0001 |
| ≤300 | 84 | 97.3 | 89.5 | 87.1 | |
| AFP (ng/mL) | | | | | |
| >400 | 22 | 75.8 | 53.1 | 44.3 | <0.0001 |
| ≤400 | 82 | 93.3 | 87.5 | 87.5 | |
| Tumor distribution | | | | | |
| Bilobar | 65 | 85.3 | 74.7 | 72.1 | 0.046 |
| Unilobar | 39 | 97.0 | 90.4 | 90.4 | |
| Duration between the first treatment and the LDLT | | | | | |
| <1 year | 21 | 80.0 | 68.7 | 68.7 | 0.20 |
| ≥1 year | 83 | 92.1 | 83.3 | 80.7 | |
| Duration between the last treatment and the LDLT | | | | | |
| <1 year | 72 | 86.5 | 76.5 | 76.5 | 0.26 |
| ≥1 year | 32 | 96.6 | 89.1 | 82.3 | |
| Times of treatment | | | | | |
| ≥4 | 36 | 85.0 | 67.9 | 67.9 | 0.06 |
| <4 | 68 | 91.9 | 86.7 | 83.9 | |

†Data of one case was lacking because of warfarin intake.
AFP, α-fetoprotein; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HCV, hepatitis C virus; KU, Kyushu University; LDLT, living-donor liver transplantation; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus.

growth, invasion and metastases.[23,24] Recently, Motomura *et al.* reported that interleukin (IL)-17-producing T cells are thought to release CXC chemokines that recruit neutrophils, leading to elevated NLR, and promote the differentiation of tissue macrophages in peritumoral regions into tumor-associated macrophages (TAM).[14] Both IL-17-producing T cells and TAM may accelerate tumor progression and antitumor T-cell exhaustion. As shown in Table 4, pathological examination revealed poorly differentiated HCC and microvascular invasion in the explanted liver in seven of eight recipients who had both independent risk factors of tumor recurrence. The use of routine biopsy to identify tumor grading has been abandoned owing to concerns of tumor seeding, leading to an extensive search for suitable surrogate markers to predict tumor differentiation or vascular invasion. Halazun *et al.* showed that elevated NLR correlated with microvascular invasion and poorly differentiated tumors.[13] The results from our study are consistent with this previous report. The interpretation

**Table 3** Risk factors for tumor recurrence: multivariate analysis

| Variables | Odds ratio | 95% CI | *P* |
|---|---|---|---|
| Nodule size + number ≥8.0 | 15.2 | 3.34–68.9 | 0.0004 |
| NLR ≥4 | 4.02 | 1.38–11.6 | 0.011 |
| DCP >300 mAU/mL | 3.09 | 0.87–11.0 | 0.082 |
| AFP >400 ng/mL | 1.23 | 0.37–4.08 | 0.73 |
| Bilobar distribution | 1.12 | 0.24–5.21 | 0.88 |

AFP, α-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; NLR, neutrophil-to-lymphocyte ratio.

**Table 4** Correlation between explant pathology and risk factors

| Variables | No risk factor ($n = 58$) | NLR ≥4 ($n = 13$) | Tumor size and number of tumors ≥8 ($n = 25$) | Both risk factors ($n = 8$) | *P* |
|---|---|---|---|---|---|
| Microvascular invasion | 12 (20.7%) | 4 (30.8%) | 16 (64.0%) | 7 (87.5%) | <0.0001 |
| Poorly differentiated tumor | 12 (20.7%) | 3 (23.1%) | 12 (48.0%) | 7 (87.5%) | 0.0005 |

NLR, neutrophil-lymphocyte ratio.

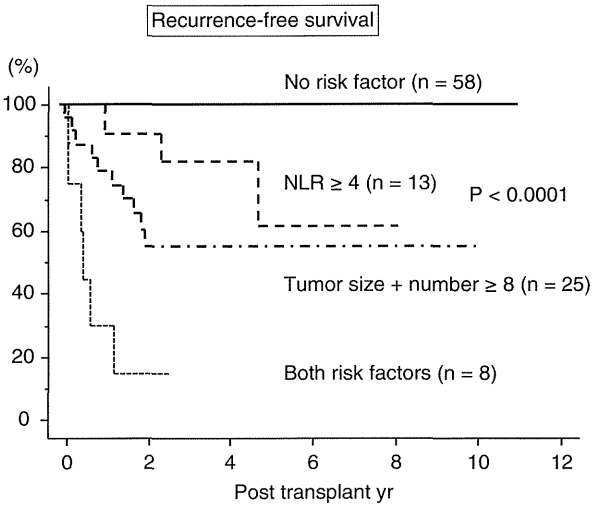


**Figure 1** Recurrence-free recipient survival after living-donor liver transplantations for hepatocellular carcinoma. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an neutrophil-to-lymphocyte ratio (NLR) of 4 or more were 100%, 81.8%, and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0% and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained. The differences among the four groups were significantly different ($P < 0.0001$). yr, years.

of NLR in patients with end-stage liver disease, often complicated with hypersplenism and pancytopenia, seems to require caution. Furthermore, patients with end-stage liver disease often develop specific bacterial peritonitis or other bacterial infections because of impaired immune system. There may be limitation for the evaluation of NLR in such patients.

Seventy-eight of 104 patients underwent pretransplant treatment more than twice in this study. Moreover, the times of pretransplant treatment, the interval between the first treatment and LDLT, and the interval between the last pretransplant treatment and LDLT did not affect the outcome of LDLT. Next, we focused on how to predict patients with a high risk of tumor recurrence after LDLT. For the univariate and multivariate analysis, we chose variables that had been obtained before transplantation. The 5-year recurrence-free survival rate after the LDLT was 100% for recipients who did not have both risk factors of tumor recurrence. Therefore, according to our results, HCC can be treated with any treatment modality whenever the patient's liver function is tolerable to such treatments. However, patients who have the sum of the largest tumor size (in cm) and the number of tumors of 8 or more and have an NLR of 4 or more should be excluded from LDLT. Further study is needed on whether LDLT can be performed for patients who have a single independent risk factor or not, because the 5-year recurrence-free survival rate for patients who had the sum of the largest tumor size (in cm) and the number of tumors of 8 or more was 55.4%, and for patients who had an NLR of 4 or more was 61.4%. A recent report recommended giving psychosocial considerations careful attention for both donor and recipient in LDLT.[25]

In conclusion, the type or duration of treatment for HCC did not affect the outcome of LDLT, but LDLT should not be performed for patients who have the sum of the largest tumor size (in cm) and number of tumors of 8 or more and with an NLR of 4 or more after any treatments for HCC to prevent tumor recurrence.

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## REFERENCES

1 Yoshizumi T, Taketomi A, Uchiyama H *et al.* Graft size, donor age, and patient status are the indicators of early graft function after living donor liver transplantation. *Liver Transpl* 2008; **14:** 1007–13.
2 Renz J, Kin C, Saggi B, Emond J. Outcomes of living donor liver transplantation. In: Busuttil R, Klintmalm G, eds. *Transplantation of the Liver*, 2nd edn. Philadelphia, PA: Elsevier Saunders, 2005; 713–24.
3 Gordon-Weeks AN, Snaith A, Petrinic T, Friend PJ, Burls A, Silva MA. Systematic review of outcome of downstaging hepatocellular cancer before liver transplantation in patients outside the Milan criteria. *Br J Surg* 2011; **98:** 1201–8.
4 Yoshizumi T, Shirabe K, Soejima Y *et al.* Living donor liver transplantation in patients who have received pretransplant treatment for hepatocellular carcinoma. *Transplantation* 2011; **91:** e61–2.
5 Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235:** 373–82.

6 Hashikura Y, Makuuchi M, Kawasaki S *et al.* Successful living-related partial liver transplantation to an adult patient. *Lancet* 1994; **343:** 1233–4.
7 Taketomi A, Sanefuji K, Soejima Y *et al.* Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009; **87:** 531–7.
8 Yoshizumi T, Shirabe K, Soejima Y *et al.* Living donor liver transplantation in patients older than 60 years. *Transplantation* 2010; **90:** 433–7.
9 Yoshizumi T, Shirabe K, Taketomi A *et al.* Risk factors that increase mortality after living donor liver transplantation. *Transplantation* 2012; **93:** 93–8.
10 Walsh SR, Cook EJ, Goulder F, Justin TA, Keeling NJ. Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer. *J Surg Oncol* 2005; **91:** 181–4.
11 Halazun KJ, Aldoori A, Malik HZ *et al.* Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. *Eur J Surg Oncol* 2008; **34:** 55–60.
12 Gomez D, Farid S, Malik HZ *et al.* Preoperative neutrophil-to-lymphocyte ratio as a prognostic predictor after curative resection for hepatocellular carcinoma. *World J Surg* 2008; **32:** 1757–62.
13 Halazun KJ, Hardy MA, Rana AA *et al.* Negative impact of neutrophil-lymphocyte ratio on outcome after liver transplantation for hepatocellular carcinoma. *Ann Surg* 2009; **250:** 141–51.
14 Motomura T, Shirabe K, Mano Y *et al.* Neutrophil-lymphocyte ratio reflects hepatocellular carcinoma recurrence after liver transplantation via inflammatory microenvironment. *J Hepatol* DOI: http://dx.doi.org/10.1016/j.jhep.2012.08.017.
15 Mazzaferro V, Llovet JM, Miceli R *et al.* Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10:** 35–43.
16 Soejima Y, Taketomi A, Yoshizumi T *et al.* Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. *Transplantation* 2007; **83:** 893–9.
17 Urata K, Kawasaki S, Matsunami H *et al.* Calculation of child and adult standard liver volume for liver transplantation. *Hepatology* 1995; **21:** 1317–21.
18 Yoshizumi T, Taketomi A, Soejima Y *et al.* The beneficial role of simultaneous splenectomy in living donor liver transplantation in patients with small-for-size graft. *Transpl Int* 2008; **21:** 833–42.
19 Shirabe K, Taketomi A, Morita K *et al.* Comparative evaluation of expanded criteria for patients with hepatocellular carcinoma beyond the Milan criteria undergoing living-related donor liver transplantation. *Clin Transplant* 2011; **25:** E491–8.
20 Yao FY, Ferrell L, Bass NM *et al.* Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33:** 1394–403.
21 Toso C, Trotter J, Wei A *et al.* Total tumor volume predicts risk of recurrence following liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2008; **14:** 1107–15.
22 Zheng SS, Xu X, Wu J *et al.* Liver transplantation for hepatocellular carcinoma: Hangzhou experiences. *Transplantation* 2008; **85:** 1726–32.
23 Bertuzzo VR, Cescon M, Ravaioli M *et al.* Analysis of factors affecting recurrence of hepatocellular carcinoma after liver transplantation with a special focus on inflammation markers. *Transplantation* 2011; **91:** 1279–85.
24 Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454:** 436–44.
25 Clavien PA, Lesurtel M, Bossuyt PM, Gores GJ, Langer B, Perrier A, OLT for HCC Consensus Group. Recommendations for liver transplantation for hepatocellular carcinoma: an international consensus conference report. *Lancet Oncol* 2012; **13:** e11–22.

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***Original Article***

# Early extensive viremia, but not rs8099917 genotype, is the only predictor for cholestatic hepatitis C after living-donor liver transplantation

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*Aim:* Cholestatic hepatitis C is one of the most serious but still unaddressed disorders after liver transplantation.

*Methods:* In this study, we analyzed 49 patients who underwent living-donor liver transplantation (LDLT) to treat hepatitis C virus (HCV) infection.

*Results:* Five patients developed cholestatic hepatitis C, with total bilirubin of 15.2 ± 3.1 mg/dL at diagnosis 6.2 ± 1.0 weeks after LDLT. Univariate analysis showed that larger graft to standard liver volume ratio, higher HCV RNA titer at 2 weeks, earlier peak HCV RNA titer and cytomegalovirus infection were the significant risk factors. The development of cholestatic hepatitis C was not significantly associated with interleukin-28B genotype (rs8099917); four out of five affected patients had the T/T genotype. Multivariate analysis showed that higher HCV RNA titer at 2 weeks was the only significant factor ($P = 0.026$) for the development of cholestatic hepatitis C. Receiver–operator curve analysis showed that that HCV RNA titer of more than 7.2 $\log_{10}$IU/mL was the optimal cut-off for characterizing cholestatic hepatitis C. All of the patients were serum HCV RNA negative after treatment with pegylated interferon and ribavirin and all the patients are alive.

*Conclusion:* Early extensive viremia, but not the rs8099917 genotype, was the only predictor for cholestatic hepatitis C after LDLT.

**Key words:** cholestatic hepatitis, hepatitis C, interleukin 28B, liver transplantation, living donor, splenectomy

## INTRODUCTION

ALTHOUGH END-STAGE LIVER disease secondary to hepatitis C virus (HCV) is the leading indication for liver transplantation (LT), re-infection of HCV is a widespread, unaddressed and serious event.[1] It has been reported that approximately one-quarter of patients develops cirrhosis within 10 years after LT for HCV; therefore, graft outcomes after LT for HCV are inferior to those for other indications.[2]

Nevertheless, recurrent hepatitis C after LT is represented by a spectrum of disorders, including mild to severe inflammation with various degrees of fibrosis progression over several years.[1,2] Of note, HCV re-infection can result in very aggressive hepatitis in a small number of patients, and is usually characterized by rapid progression of cholestasis with fibrosis resulting in graft failure and death.[3,4] This outcome has been termed post-transplant cholestatic hepatitis C and its risk factors include higher donor age, HCV genotype 1, extremely high viral titers and bolus steroid administration for acute rejection.[3,4] More recently, two reports

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