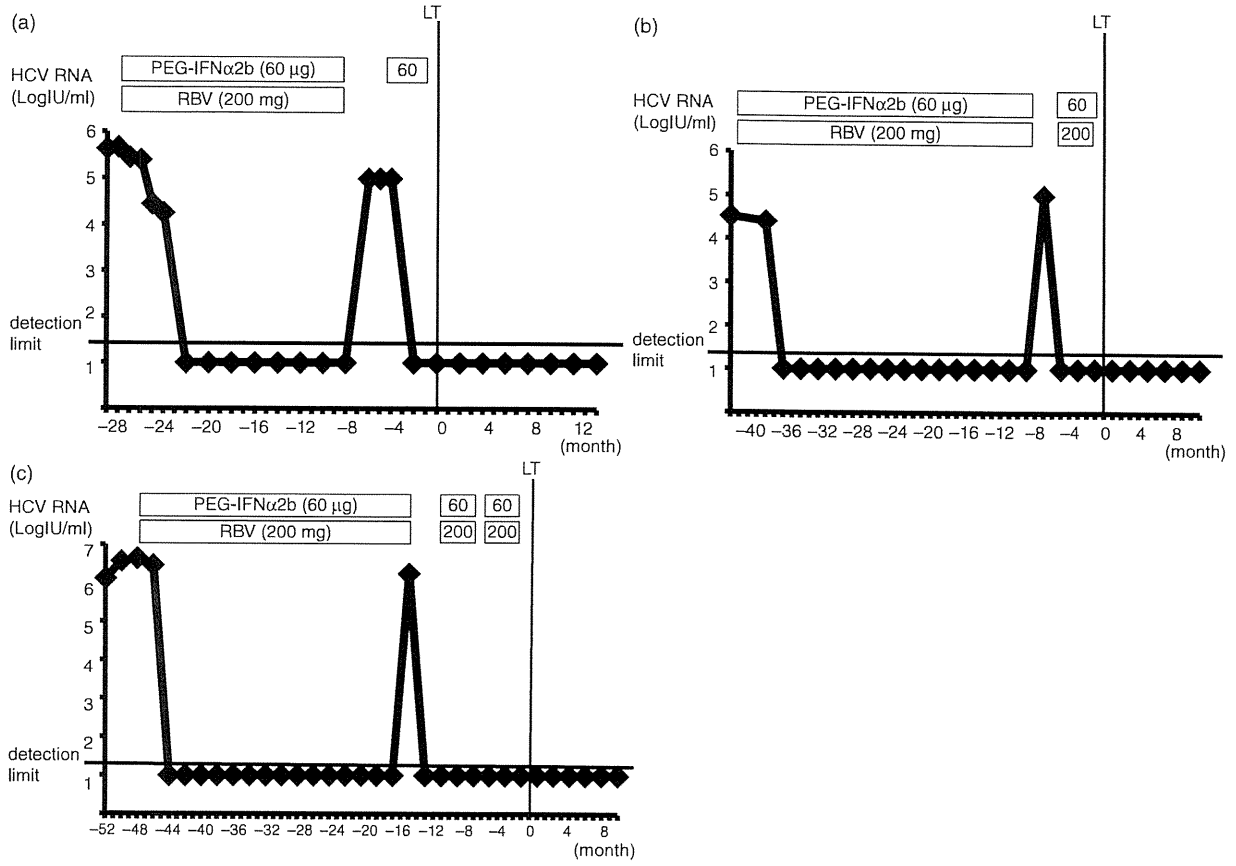

Figure 1 (a) Case 1. Changes in hepatitis C virus (HCV) RNA titer in response to treatment with the combination of pegylated interferon-α2b (PEG IFN) plus ribavirin (RBV). (b) Case 2. Changes in HCV RNA titer in response to treatment with the combination of PEG IFN plus RBV. (c) Case 3. Changes in HCV RNA titer in response to treatment with the combination of PEG IFN plus RBV.

achieved HCV eradication after LT. However, because of the lack of details about previous IFN therapy, these patients might have achieved SVR at stopping IFN therapy before LT. In support of this, another study that examined the presence of HCV RNA in liver explants showed no HCV RNA in 11 of 17 (64.7%) patients,[38] while six had detectable HCV RNA. Eradication of HCV after LT was reported in two of these six patients while re-infection after LT occurred in the other four. However, there was no detail information about VR duration and viral factors in these patients.

In the present study, although our three patients achieved virological response during IFN therapy, they did not achieve SVR due to HCV relapse after the withdrawal of IFN therapy. The VR in these patients continued until the time of LDLT, and HCV was eradiated after LDLT in all three. It was considered that HCV RNA titer was zero or markedly under the detection limit in serum, though HCV RNA persisted in the liver tissue. These findings suggest that HCV might have been eradicated from whole body at the time of LT. On the other hand, previous reports suggested that HCV replication also occurs in other organs such as the lymph node, bone marrow and brain.[38–40] Although HCV RNA titer was zero or markedly below the detection limit in serum, viral relapse could occur after LT if the above organs are positive for HCV RNA. Because HCV RNA was negative in these organs, it is highly likely that HCV was eradicated from the whole body at the time of LT in the three patients.

Recent studies have identified various host and virus factors as significant predictors of the response to IFN

treatment. With regard to the viral factors, the number of a.a. substitutions in the ISDR correlates with the SVR rate to IFN treatment in patients infected with HCV genotype 1b.[20,21] In a series of studies, Akuta *et al.*[23] reported that substitution of aa70 and/or aa91 in the HCV core region is an independent and significant predictor of virological response, including SVR and NVR, to the combination therapy. Other studies also reported the association between several SNP in the IL-28 locus and the effect of PEG IFN plus ribavirin combination therapy in patients with HCV genotype 1b.[31–34] In our patients, the numbers of a.a. substitutions in ISDR were 0–1 together with mutant-type a.a. at either aa70 or aa91 in the core region, which showed low susceptibility to IFN treatment. Furthermore, genotyping of IL-28 SNP (rs8099917) was TT genotype in two patients and GG genotype in one patient.

It is possible that these viral factors are not linked to the achievement of SVR with PEG IFN/RBV treatment for HCV infection before LT. In this regard, one previous study reported a viral response rate of 60–70% in patients with chronic hepatitis at the end of PEG IFN plus RBV treatment.[41–43] In other words, 60–70% of patients could attain viral response in spite of viral and host factors. Thus, these results suggest that it is better to treat the patients with PEG IFN plus RBV before LT, if possible. Should VR be achieved, continuation of IFN therapy to immediately before LT could result in HCV eradication after LT.

Of course, we do not recommend IFN therapy for all patients. Smallwood *et al.*[44] reported that IFN therapy before LT for HCV patients was associated with poor outcome after LT. However, among LT candidates, some patients may show viral response but not SVR. In addition, some patients can continue the IFN therapy even after the appearance of side-effects that are within the allowable limits. Further clinical trials of larger population samples are necessary to confirm the present findings.

In conclusion, the three cases presented here suggest that patients who show viral response to IFN therapy before LT can escape recurrence of HCV after LT due to the continuation of IFN therapy to just before LT.

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IL28B polymorphism may guide pegylated interferon plus ribavirin therapy even after curative treatment for hepatitis C virus-related hepatocellular carcinoma

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SUMMARY. The present study was designed to determine the predictive factors for the viral response to pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) administered after curative treatment for hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC). The study group was 78 patients treated between January 2005 and January 2009. The sustained viral response (SVR) rate was 25.8% (15/58) in patients infected with HCV-genotype 1 and 55.0% (11/20) in those with genotype 2. Among the 78 patients, 32 (41.0%) could not complete the treatment protocol, and this was because of HCC recurrence in 17 (53%) of them. Multivariate analysis identified partial early viral response (pEVR) as the only independent determinant of SVR [odds ratio (OR) 14.73, $P = 0.013$] for patients with genotype 1. Multivariate analysis identified male gender (OR 8.72, $P = 0.001$) and interleukin-28B (IL-28B) genotype (rs8099917) TT (OR 7.93, $P = 0.007$) as independent predictors of pEVR. Multivariate analysis also identified IL-28B genotype GG+TG (OR 14.1, $P = 0.021$) and α-fetoprotein >30 (OR 5.4, $P = 0.031$) as independent predictors of null response. Patients with SVR showed a better survival rate than those without SVR ($P = 0.034$). The second HCC recurrence rate tended to be lower in patients with SVR than in those without SVR ($P = 0.054$). With regard to the prognosis of patients with SVR, it is desirable to achieve SVR with interferon therapy even when administered after HCC treatment. IL-28B genotype is a potentially useful marker for the response to PEGIFN/RBV therapy administered after curative treatment of HCV-related HCC.

Keywords: curative treatment, hepatitis C virus, hepatocellular carcinoma, interleukin-28B, pegylated interferon-alpha plus ribavirin combination therapy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Chronic infection with hepatitis C virus (HCV) has been associated with hepatocarcinogenesis [1–3]. Recent advances in imaging and treatment modalities have brought about some improvement in the prognosis of patients with HCV-related HCC, but the overall outcome remains unsatisfactory; the 5-year survival rate is only 50–70%, even after curative treatment such as hepatic resection or local ablation [4]. The reasons for this unfavourable prognosis are considered to include high intrahepatic tumour recurrence rates and sustained hepatic damage, both resulting from HCV infection [5]. Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumour recurrence within 1 year is 20–40%, rising to about 80% by 5 years [4,6,7].

Intrahepatic recurrence of HCC may result from intrahepatic metastasis originating from the primary HCC or from ongoing multicentric carcinogenesis related to chronic HCV infection. The background HCV-related hepatic damage may

Abbreviations: AFP, α-fetoprotein; cEVR, complete early viral response; HCV, hepatitis C virus; IFN, interferon; IL-28B, interleukin-28B; NR, null response; PEGIFN, pegylated interferon; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response; RBV, ribavirin; SNP, single-nucleotide polymorphism; SVR, sustained viral response; TR, transient viral response.

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also compromise hepatic functional reserve and worsen clinical outcome. Thus, the prevention of HCC recurrence as well as preservation of liver function constitutes high priorities for the improvement of prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV [8,9] and thus IFN could be of value in minimizing hepatic necrosis, inflammation and fibrosis, as well as reducing the incidence of HCC. Several recent studies have reported that IFN therapy, applied even after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival [10–21].

The recent introduction of pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) has improved the treatment efficacy [22,23]. Recent studies have highlighted the relationship between various single-nucleotide polymorphisms (SNPs) in the IL-28 locus and the effect of PEGIFN/RBV in patients infected with HCV [24–29]. Further, the results of few recent studies suggest that PEGIFN/RBV could prevent HCC recurrence and improve survival even when used after curative treatment of HCV-related HCC [30,31]. To our knowledge, however, there are no studies on the factors that could predict a sustained viral response (SVR) to PEGIFN/RBV after treatment of HCC (e.g. IL-28B as a host factor). The present study was designed to determine the predictive factors of viral response to PEGIFN/RBV in patients with HCV treated for HCC.

MATERIAL AND METHODS

Patients

The study subjects were 78 patients treated with PEGIFN/RBV after curative intent treatment (hepatic resection or radiofrequency ablation) for HCV-related HCC between January 2005 and January 2009 in this retrospective cohort study. Tumour staging was defined based on the Liver Cancer Study Group of Japan/Tumour-Node-Metastasis staging system of the Liver Cancer Study Group of Japan (LCSGJ): stage I [fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion, n = 28 (36%)], stage II [two of the three intrahepatic conditions, n = 27 (35%)], stage III [one of the three intrahepatic conditions, n = 23 (29%)], stage IVa (none of the three intrahepatic conditions, with no distant metastases or any intrahepatic conditions with lymph node metastases) and stage IVb (any intrahepatic condition with distant metastases) [stage IV, n = 0 (0%)] [32]. The median duration was 7 months (range, 1–60) from curative intent treatment for HCC to the start of PEGIFN/RBV therapy.

Antiviral treatment protocol

Each patient received 1.5 μg/kg body weight (BW) pegylated interferon-alpha (PEGIFN) (Peg-Intron; Schering-Plough, Segrate, Italy) subcutaneously (s.c.) once weekly, together with ribavirin (RBV) (Rebetol; Schering-Plough). The RBV dose was adjusted according to BW to 600 mg for patients <60 kg BW, 800 mg for >60 but ≤80 kg BW and 1000 mg for >80 kg BW, based on the drug information for RBV supplied by the manufacturer. The above durations and dosages are those approved by the Japanese Ministry of Health, Labour and Welfare.

The daily dose of RBV was reduced by 200 mg when haemoglobin (Hb) fell below 10 g/dL, acute fall in Hb concentration followed by stabilization at more than 3 g/dL from baseline, or appearance of clinical symptoms of anaemia (e.g. fatigue, pallor, palpitation, dyspnoea on efforts and fatigue) associated with a decrease in Hb of >2 g/dL from baseline. Once the RBV dose was reduced, it was maintained at that level throughout the rest of study. The protocol also included withdrawal of RBV when Hb fell below 8.5 g/dL or when patients manifested more severe anaemia including orthostatic hypotension. After the end of the treatment, the patients were followed up for 24 more weeks without treatment. The treatment term was 48 weeks for patients infected with HCV genotype 1 and 24 weeks for those with genotype 2.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committees of all participating centres. Written informed consent was obtained from each patient. At each visit, information on possible side effects was obtained by questioning the patients in a structured manner about specific, commonly observed and expected side effects of the study medication, such as flu-like symptoms, fatigue, nausea, vomiting, diarrhoea, dizziness, depression and hair loss.

Single-nucleotide polymorphism genotyping and quality control

Because the two reported significant IL-28 SNPs (rs8099917 and rs12979860) are in strong linkage disequilibrium, we analysed only rs8099917 in this study. Some samples obtained from patients with HCV were determined using the Illumina HumanHap610-Quad Genotyping BeadChip, whereas the remaining samples were genotyped using the Invader assay, as described previously [33,34].

Analysis of nucleotide sequence of the core and NS5A region

The amino acid (aa) substitutions at aa 70 and aa 91 of the HCV core region and mutation at the interferon sensitivity-determining region (ISDR) in the nonstructural 5A (NS5A) region of HCV were analysed by the direct sequencing method as described previously by our group [35–37].

Assessment of viral response

Serum HCV RNA was determined at baseline, after 4, 8, 12, 16 and 20 weeks of treatment, at the end of treatment and

at the end of the 24-week drug-free follow-up period. HCV RNA was assessed by qualitative reverse transcription polymerase chain reaction (TaqMan RT-PCR). SVR represented a negative HCV RNA at 24-week follow-up without treatment after the end of active treatment. Transient viral response (TR) was defined as positive HCV RNA at 24-week follow-up after a negative HCV RNA at the end of active treatment. Complete early viral response (cEVR) was defined as negative HCV RNA at week 12 of active treatment. Partial early viral response (pEVR) was defined as HCV RNA ≥ 2 log10 drop from baseline at week 12 of active treatment. Null response (NR) was defined as HCV RNA that never dropped by ≥2 log10 from baseline at week 12 of active treatment.

Histopathological stage was assessed before treatment and determined based on the histological scoring system of Desmet *et al.* [38].

Assessment of hepatocellular carcinoma recurrence

The concentrations of serum tumour markers α-fetoprotein (AFP) and des-γ-carboxy prothrombin were measured once a month after hepatic resection or radiofrequency ablation. Follow-up US was performed every 3 months; and CT or MR imaging was performed every 6 months. IFN therapy was discontinued upon suspicion of HCC recurrence.

Statistical analysis

Nonparametric tests (chi-square test and Fisher's exact probability test) were used to compare the clinical and laboratory parameters of the two groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to early viral dynamics. The odds ratio and 95% confidence intervals (95% CI) were also calculated. All P values <0.05 using two-tailed tests were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors.

Cumulative survival and recurrence rates were calculated from the initial date of hepatic resection or radiofrequency ablation and assessed by the Kaplan–Meier life-table method, with differences evaluated by the log rank test. All statistical analyses were performed using PASW 18 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

Table 1 shows the baseline characteristics of the patients treated with PEGIFN/RBV after hepatic resection or radiofrequency ablation for HCC. The median age of the patients (55 men and 23 women) was 66 years. The median body mass index was 22.4 kg/m^2. The median pretreatment serum HCV RNA viral load was 6.0 log IU/mL. Most patients were infected with HCV genotype 1 (n = 58) followed by genotype 2 (n = 20). IL-28B genotype (rs8099917) was TT (n = 51), GG+TG (n = 25) and no date (n = 2).

Table 1 The baseline characteristics of the all 78 patients treated with PEGIFN/RBV

	n = 78
Gender (male/female)	55/23
Age (years)*	66 (48–83)
Body mass index (kg/m^2)*	22.4 (15.6–40.1)
IL28B genotype (TT/GG+TG/ND)	51/25/2
White blood Cell (×10^3/μL)*	4.2 (2.4–7.5)
Haemoglobin (g/dL)*	13.3 (8.7–18.1)
Platelet count (×10^4/mm^3)*	11.1 (3.9–20.5)
T-bilirubin (mg/dL)*	0.7 (0.2–2.8)
Alanine aminotransferase (IU/L)*	44 (8–189)
Prothrombin time activity (%)*	87 (58–121)
Albumin (g/dL)*	4.0 (2.7–5.2)
γ-glutamyl transpeptidase (IU/L)*	45 (12–371)
HbA1c (%)*	5.3 (3.9–10.8)
Indocyanine green retention rate (%)*	15.4 (3.5–45.4)
Fibrosis stage (F1-3/F4/ND)	20/19/39
Genotype (1/2)	58/20
HCV viral load (Log IU/mL)*	6.0 (2.1–7.2)
Tumour stage (I/II/III/IV)†	28/27/23/0
α-Fetoprotein (ng/mL)*	11 (0.5–286)
Des-γ-carboxy prothrombin (mAU/mL)*	29 (10–4550)
Tumour size (mm)*	21 (7–110)
Number of tumour*	1 (1–4)
Hepatic resection/radiofrequency ablation	28/50

ND, not done; HCV, hepatitis C virus; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy. *Data are median and (range). †Tumour staging was defined based on the Liver Cancer Study Group of Japan/Tumor-Node-Metastasis staging system of the Liver Cancer Study Group of Japan.

Efficacy and tolerance of therapy and adverse events

Figure 1 shows the effects of PEGIFN/RBV treatment according to genotype. The SVR rate was 33.3% (26/78) for all patients. The PRGIFN/RBV treatment protocol could not be completed by 32 (41%) patients; 17 (53%) of the 32 developed HCC recurrence. In 58 patients with genotype 1, PEGIFN and RBV were discontinued in 29% (17/58) patients because of HCC recurrence and because of other reasons in another 9 (15.5%) [general fatigue (n = 3), cancer of the

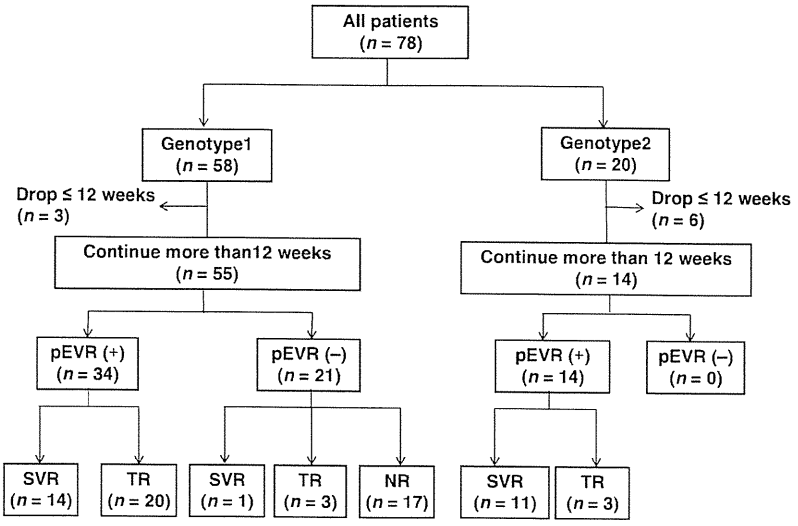

Fig. 1 Flow diagram showing the course of Peg-related interferon plus ribavirin therapy after curative treatment for hepatitis C virus (HCV)-related hepatocellular carcinoma. According to HCV genotype, 78 patients treated with pegylated interferon-alpha plus ribavirin combination therapy were divided into three groups, namely the sustained virological response, transient response and null response. *n*, number of patients.

throat (n = 1), vomiting (n = 1), itching (n = 1), pulmonary haemorrhage (n = 1), jumpiness (n = 1), sarcoidosis (n = 1)] within 48 weeks. Thus, PEGIFN and RBV treatment could be achieved in 55% (32/58) of the patients. Furthermore, 95% (55/58) of the patients continued the treatment for more than 12 weeks. Among 55 patients, 34 achieved pEVR, including 21 patients achieved cEVR, 14 achieved SVR and 20 showed TR. In the other 21 patients who did not achieve pEVR, one patient achieved SVR and three patients showed TR while 17 patients showed NR. Thus, the SVR rate was 25.8% (15/58) for patients infected with HCV genotype 1.

Among the 20 patients infected with genotype 2, 6 discontinued treatment because of side effects [general fatigue (n = 3), thrombocytopenia (n = 1), diabetes mellitus (n = 1), bleeding from oesophageal varices (n = 1)] within 12 weeks. The remaining 14 (70%) patients completed the treatment protocol. All 14 patients achieved pEVR, including 11 who showed SVR and three achieved TR. Thus, the SVR rate was 55.0% (11/20) for patients infected with genotype 2 (Fig. 1).

Relationship between IL-28B and viral response in patients infected with hepatitis C virus genotype 1

In patients infected with HCV genotype 1, number of patients with TT genotype of IL-28B was 44 (TT group) and GG+TG was 14 (GG+TG group). The SVR rate of the TT group [34.3% (n = 14/41)] was higher than that of the TG+GG group [7% (n = 1/14), P = 0.08, Fig. 2A]. The pEVR rate of TT group [73.1% (n = 30/41)] was also significantly higher than that of the TG+GG group [28.5% (n = 4/14), P = 0.009, Fig. 2B]. The NR rate of the TT group [19.5% (n = 8/41)] was significantly lower than that of the TG+GG group [64.2% (n = 9/14), P = 0.005, Fig. 2C].

Determinants of sustained viral response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine SVR using data of 55 patients infected with HCV genotype 1 who continued PEGIFN/RBV therapy for more than 12 weeks (Table 2). Univariate analysis identified five parameters that correlated with SVR: pEVR (P = 0.004), viral load (<6.0 g/dL; P = 0.008), completion of therapy (P = 0.06), IL-28B genotype (TT genotype; P = 0.08) and gender (man; P = 0.043). Multivariate analysis identified pEVR as the only significant and independent factor that influenced the SVR: (odds ratio, 14.73, 95%CI 1.7–123.2, P = 0.013).

Determinants of partial early viral response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine pEVR using data of 55 patients infected with HCV genotype 1 who continued PEGIFN/RBV treatment for >12 weeks. Univariate analysis identified three parameters that correlated with pEVR: IL-28B genotype (TT genotype; P = 0.009), gender (man; P = 0.005) and viral load (<6.0 g/dL; P = 0.068) (Table 3). Multivariate analysis identified two parameters that independently influenced the pEVR: gender (male; odds ratio 8.72, 95%CI 2.1–41.6, P = 0.001) and IL-28B genotype (TT genotype; odds ratio 7.93, 95%CI 1.7–36.0, P = 0.007, Table 4). Mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significantly different between the pEVR and non-pEVR groups among patients infected with HCV genotype 1b in our study.

Determinants of null response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine the NR in patients infected with HCV genotype 1 (n = 55). Univariate

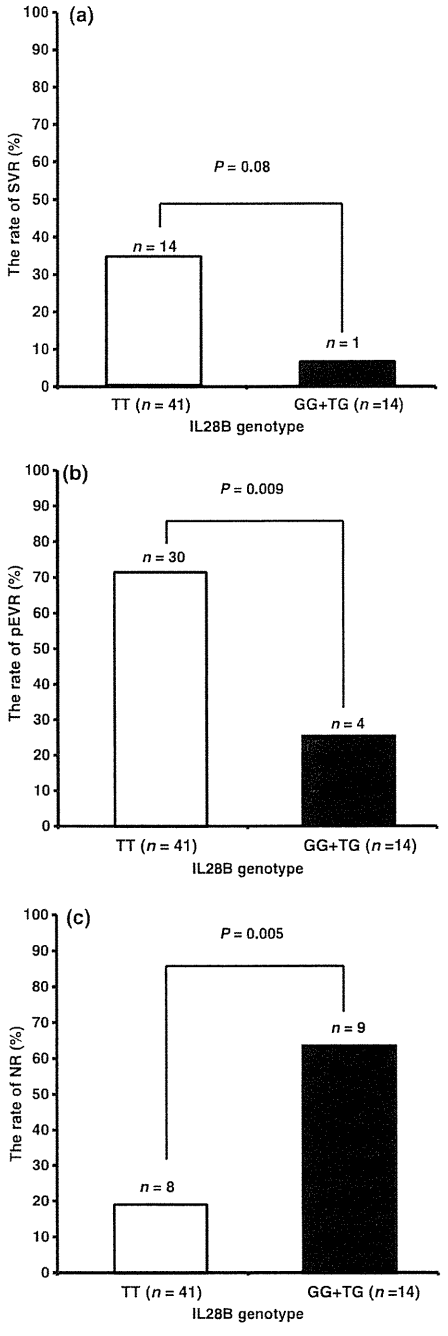

Fig. 2 Relationship between IL-28B and viral response in patients infected with hepatitis C virus genotype 1. (a) Sustained viral response rate, (b) Partial early viral response rate, (c) null response rate.

analysis identified three parameters that influenced NR: IL-28B genotype (genotype; GG+TG, $P = 0.005$), AFP (>30 ng/dL; $P = 0.054$) and gender (male; $P = 0.022$) (Table 5). Multivariate analysis identified two parameters that independently influenced the NR: IL-28B genotype (genotype GG+TG; odds ratio 7.8, 95%CI 1.81–34.4, $P = 0.006$) and AFP (>30; odds ratio 5.6, 95%CI1.40–22.8, $P = 0.015$) (Table 6). Mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significantly different between the NR and SVR+TR groups among patients infected with HCV genotype 1b.

Survival rates

The overall survival rate was significant different between patients of the SVR and non-SVR groups ($P = 0.034$). The survival rate of the SVR groups was 100% at 1 year, 100% at 3 years and 100% at 5 years. In contrast, the rates of the non-SVR group were 100%, 96% and 74%, respectively (Fig. 3).

Comparison of the first and second recurrence rates of hepatocellular carcinoma

Finally, we compared the overall cumulative rates of the first and second recurrence of HCC between the SVR and non-SVR groups (Fig. 4). The 1-, 3- and 5-year rates of the first recurrence of HCC in the SVR and non-SVR group were not different (0% *vs* 6.7%, 38.1% *vs* 37% and 48% *vs* 68%, respectively, Fig. 4A, $P = 0.41$). The 1-, 3- and 5-year rates of the second recurrence in the SVR and non-SVR groups were 0% *vs* 0%, 41% *vs* 64% and 48% *vs* 78%, respectively (Fig. 4(B), $P = 0.054$). These results demonstrated that patients of the SVR group tended to have a better chance of escaping a second HCC recurrence compared with those of the non-SVR group.

DISCUSSION

Several recent studies have reported that IFN therapy can prevent HCC recurrence and improve survival, especially in patients with SVR, even when administered after curative treatment for HCV-related HCC [10–21,31,39]. While there are a few reports of the use of PEGIFN/RBV after curative treatment for HCV-related HCC [30,31], none have discussed the SVR rate and the factors that determine the viral response to PEGIFN/RBV in such patients. In the present study, we reported the viral response and determinants (specially SNPs) of viral response with PEGIFN/RBV after treatment of HCC.

In our study, the SVR rate was 33.3% (26/78) for all patients, while that for patients with genotype 1 was 25.8% (15/58) and genotype 2 was 55.0% (11/20). These SVR rates are lower than that of patients with chronic hepatitis. The lower rate in the present study was probably because of the low number of patients who completed the therapy. The reason for the latter was the relatively high rate of HCC recurrence [53% (17/32)].

One of the major reasons of the low SVR rate was probably of discontinuation of therapy because of HCC recurrence.

Table 2 Univariate analysis of factors associated with SVR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	SVR (n = 15)	TR+NR (n = 40)	P
Gender (male/female)	1/14	25/15	0.043
Age (years)*	65 (54–74)	65 (53–83)	0.94
Body mass index (kg/m^2)*	21.2 (18.4–28.5)	23.0 (18.7–40.1)	0.174
White blood Cell (×10^3/μL)*	5050 (4390–6130)	4280 (2470–6660)	0.8
Haemoglobin (g/dL)*	13.7 (11.2–14.8)	13.4 (9.3–18.1)	0.96
Platelet count (×10^4/mm^3)*	12.5 (3.9–19.6)	10.0 (4.7–20.8)	0.138
T-bilirubin (mg/dL)*	0.7 (0.4–1.8)	0.7 (0.2–1.7)	0.58
Alanine aminotransferase (IU/L)*	33 (12–189)	45 (17–166)	0.25
Prothrombin time activity (%)*	88 (80–106)	86 (64–121)	0.49
Albumin (g/dL)*	4.1 (3.7–4.9)	4.0 (2.7–4.9)	0.52
Fibrosis stage (F1-3/F4/ND)	2/2/11	10/15/15	1.0
γ-glutamyl transpeptidase (IU/L)	43 (12–87)	46 (15–294)	1.2
HbA1c (%)	5.1 (4.2–10.2)	5.4 (3.9–10.8)	0.41
Indocyanine green retention rate (%)	17.7 (7.5–37.8)	17.4 (3.5–45.4)	0.92
HCV viral load (Log IU/mL)	5.59 (4.3–7.1)	6.23 (1.2–7.2)	0.08
HCV Core70(mutant/wild)	8/7	23/17	1.0
HCV Core91 (mutant/wild)	5/10	21/19	0.23
HCV ISDR (0–1/>2)	9/6	26/14	0.75
α-Fetoprotein (ng/mL)*	6.9 (5–286.8)	19.7 (5–63240)	0.11
IL28B genotype (TT/GG+TG)	14/1	27/13	0.08
pEVR (yes/no)	14/1	20/20	0.004
Dose of PEGIFN at administration (μg/kg)*	80 (40–100)	80 (50–120)	0.74
Dose of RBV at administration(mg)*	600 (200–800)	600 (200–1000)	0.26
Therapy were completed (yes/no)	12/3	20/20	0.06

ND, not done; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NR, null response; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response; SVR, sustained viral response; TR, Transient viral response. *Data are median and (range).

Alternatively, the low rate could be because of a high proportion of patients with advanced liver fibrosis. In fact, 19 (11.5%) patients were classified as F4 stage, and the median of platelet count was 11.1 × 10^4/mm^3. These reasons may explain the low IFN therapy continuation rate (55.2%) and the low SVR rate.

We analysed the factors that affect SVR in 55 patients infected with HCV genotype 1 who were able to continue therapy for more than 12 weeks. Multivariate analysis identified a single parameter that independently influenced the SVR: pEVR. Among the 55 patients, 34 (61.8%) achieved pEVR. Among the pEVR group, 14 (41.1%) patients achieved SVR. Recent studies reported the importance of the response guide-based therapy in the treatment of chronic hepatitis; i.e. 70–80% of patients of the cEVR group achieved SVR [40–43].

On the other hand, gender (male) and IL-28B genotype (TT) were identified as significant and independent predictors of pEVR. These factors are probably also significant and independent predictors of SVR in patients with chronic hepatitis C.

Thus, male patients with IL-28B genotype TT were more likely to achieve pEVR even when PEGIFN/RBV treatment was introduced after curative treatment for HCV-related HCC.

Evidence suggests that the SVR rate could be improved by IFN therapy (long-term low-dose IFN of 72 weeks instead of 48 weeks). In fact, Pearlman *et al.* [43] reported that the SVR rate was superior in patients treated for 72 *vs* 48 weeks (38% *vs* 18%, respectively; P = 0.026) in the pEVR groups. Furthermore, the SVR rate could be improved by combination therapy for HCC and HCV. For example, to achieve SVR, it might be better to restart PEGIFN/RBV therapy immediately after curative treatment of HCC.

On the other hand, multivariate analysis identified IL-28B genotype (GG+TG) as an independent parameter that influenced the NR. In this group, it is better to select low-dose intermittent IFN therapy than PEGIFN/RBV based on the SVR. In fact, it is reported that low-dose intermittent IFN therapy after hepatectomy for HCC improved liver function of patients with HCV-related HCC, and the preservation of hepatic function increased the chance of successful treatment against recurrence [10]. In contrast, mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significant and independent predictors of pEVR and NR.

Table 3 Univariate analysis of factors associated with pEVR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	pEVR (n = 34)	non-pEVR (n = 21)	P
Gender (male/female)	29/5	10/11	0.005
Age (years)*	67 (54–83)	63 (53–72)	0.977
Body mass index (kg/m^2)*	23.6 (18.7–40.1)	22.2 (18.4–30.0)	0.151
White blood Cell (×10^3/μL)*	5150 (4390–6660)	3610 (2470–4930)	0.8
Haemoglobin (g/dL)*	13.8 (10.2–18.1)	12.3 (9.3–17.4)	0.745
Platelet count (×10^4/mm^3)*	11.1 (3.9–20.8)	10.0 (4.7–18.2)	0.126
T-bilirubin (mg/dL)*	0.7 (0.2–1.8)	0.7 (0.5–1.7)	0.53
Alanine aminotransferase (IU/L)*	44 (17–189)	37 (12–134)	0.319
Prothrombin time activity (%)	88 (68–114)	85 (64–121)	0.41
Albumin (g/dL)*	4.0 (3.4–4.9)	4.0 (2.7–4.9)	0.405
Fibrosis stage (F1-3/F4/ND)	8/8/18	9/4/8	0.43
γ-glutamyl transpeptidase (IU/L)	52 (12–219)	26 (15–294)	0.172
HbA1c (%)	5.5 (4.2–8.8)	5.0 (3.9–10.8)	0.49
Indocyanine green retention rate (%)	17.4 (3.5–37.8)	18.7 (7.6–45.4)	0.92
HCV viral load (Log IU/mL)	6.04 (4.3–7.2)	6.23 (1.2–6.7)	0.068
HCV Core70(mutant/wild)	19/15	13/8	0.78
HCV Core91 (mutant/wild)	13/21	13/8	0.17
HCV ISDR (0–1/>2)	19/15	14/7	0.24
α-Fetoprotein (ng/mL)*	9.1 (5.0–909.2)	42.0 (5.0–63240)	0.116
IL28B genotype (TT/GG+TG)	30/4	11/10	0.009
Dose of PEGIFN at administration (μg/kg)*	80 (40–120)	80 (50–100)	0.689
Dose of RBV at administration (mg)*	600 (200–1000)	600 (200–800)	0.20
Therapy were completed (yes/no)	21/13	11/10	0.4

HCV, hepatitis C virus; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response. *Data are median and (range).

Table 4 Multivariate analysis of factors associated with pEVR

Factor	Category	Odds ratio (95%CI)	P
Gender	Female	1	0.001
	Male	8.72 (2.1–41.6)	
IL28B genotype	GG+TG	1	0.007
	TT	7.93 (1.7–36.0)	

pEVR, partial early viral response.

Achieving SVR by PEGIFN/RBV treatment, even when administered after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival. Although achieving SVR had no impact on the occurrence of HCC at the initial site, patients of the SVR group tended to show a lower rate of second HCC recurrence in this and another study [31]. It was reported that IFN therapy had no impact on the occurrence of HCC shortly after IFN therapy was started. It was speculated that IFN therapy does not suppress latent HCC. In our study, although the first recurrence rate of HCC was similar between patients with and without SVR, the second HCC recurrence rate tended to be lower in patients with SVR than in those without SVR (P = 0.054). Therefore, efforts should be directed to achieve SVR by PEGIFN/RBV therapy after curative treatment of HCV-related HCC, whenever possible. Importantly, the SVR rate for PEGIFN/RBV combination therapy was better than that for IFN monotherapy. On the other hand, the high rate of incomplete PEGIFN/RBV therapy (44.8%) was one of the causes of the high HCC recurrence rate and the advanced liver fibrosis. Our study identified factors that affect the viral response to PEGIFN/RBV therapy, and the identification of these factors should help in the selection of patients who will best benefit from such therapy.

On the other hand, the SVR rate was 55.0% (11/20) in patients infected with HCV genotype 2. Although the sample size was small, 78.5% (11/14) patients who showed pEVR achieved SVR. Therefore, continuation of treatment is likely to result in achievement of SVR even when PEGIFN/RBV treatment is started after curative treatment for HCV-related HCC. Efforts should be made to achieve SVR by PEGIFN/RBV therapy in patients infected with HCV genotype 2 after curative treatment for HCV-related HCC. Recently, the relationship between IL-28B and the effect of PEGIFN/RBV

Table 5 Univariate analysis of factors associated with NR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	NR (n = 17)	TR+SVR (n = 38)	P value
Gender (male/female)	8/9	31/7	0.022
Age (years)*	66 (53–83)	67 (48–80)	0.75
Body mass index (kg/m^2)*	22.2 (19.3–30.0)	21.2 (15.6–28.5)	0.86
White blood Cell (×10^3/μL)*	5050 (4390–6130)	4280 (2470–6660)	0.6
Haemoglobin (g/dL)*	12.6 (9.3–17.4)	13.7 (8.7–15)	0.66
Platelet count (×10^4/mm^3)*	10.1 (4.7–18.2)	12.1 (3.9–19.6)	0.43
T-bilirubin (mg/dL)*	0.7 (0.4–1.7)	0.8 (0.4–2.3)	0.45
Alanine aminotransferase (IU/L)*	45 (19–134)	45 (12–189)	0.75
Prothrombin time activity (%)*	86 (64–121)	88 (69–112)	0.79
Albumin (g/dL)*	3.8 (2.7–4.9)	4 (3.4–5.2)	0.106
Fibrosing stage(F1-3/F4/ND)	3/8/6	9/9/20	0.21
γ-glutamyl transpeptidase (IU/L)*	52 (12–219)	26 (15–294)	0.113
HbA1c (%)*	5.3 (4–10.8)	5.2 (4.2–8.8)	0.99
Indocyanine green retention rate (%)	18.7 (7.6–45.4)	15.4 (8–29.2)	0.21
HCV viral load (Log IU/mL)*	6.28 (2.1–6.7)	6.18 (1.2–6.7)	0.25
HCV Core70 (mutant/wild)	11/6	20/18	0.55
HCV Core91 (mutant/wild)	10/7	16/22	0.38
HCV ISDR (0–1/>2)	12/5	21/17	0.23
α-Fetoprotein (ng/mL)*	45.3 (5–63240)	10 (0.5–909.2)	0.054
IL28B genotype (TT/GG+TG)	8/9	33/5	0.005
Dose of PEGIFN at administration (μg/kg)*	80 (40–120)	80 (50–100)	0.34
Dose of RBV at administration (mg)*	600 (200–1000)	600 (200–800)	0.77
Therapy were completed (yes/no)	9/8	23/15	0.76

HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NR, null response; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; SVR, sustained viral response; TR, Transient viral response. *Data are median and (range).

Table 6 Multivariate analysis of factors associated with NR

Factor	Category	Odds rate (95%CI)	P value
IL28B genotype	TT	1	0.006
	GG+TG	7.8 (1.81–34.4)	
AFP	<30	1	0.015
	>30	5.6 (1.40–22.8)	

AFP, α-fetoprotein; NR, null response.

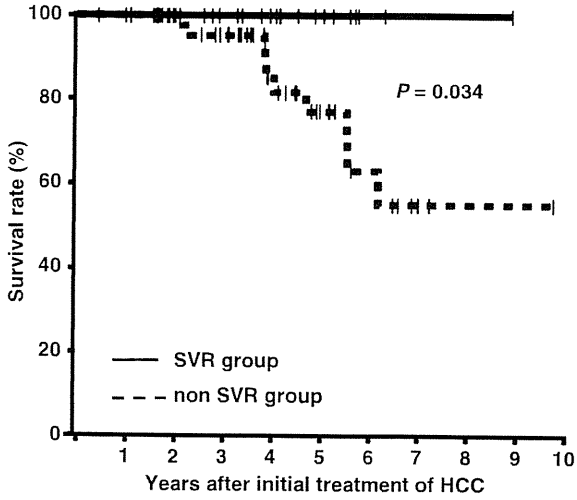

Fig. 3 Comparison of cumulative survival rates in the sustained viral response (SVR) and non-SVR groups. The cumulative survival rate was significantly higher in the SVR group than in the non-SVR group (P = 0.034).

therapy in patients with HCV genotype 2 was reported in two independent studies [28,29]. Further studies of larger sample size are needed to confirm the relationship between IL-28B genotype and the viral response to PEGIFN/RBV after treatment of HCV-related HCC in patients infected with HCV genotype 2.

Our results suggest that IL-28B genotype could be potentially used as a marker for the viral response to PEGIFN/RBV therapy. Furthermore, PEGIFN/RBV therapy should be recommended after curative treatment for HCV-related HCC for patients who are likely to achieve pEVR [those with IL-28B genotype (TT)]. In addition, the SVR rate might improve by IFN therapy and combination therapy HCC and HCV. On the other hand, it might be better to

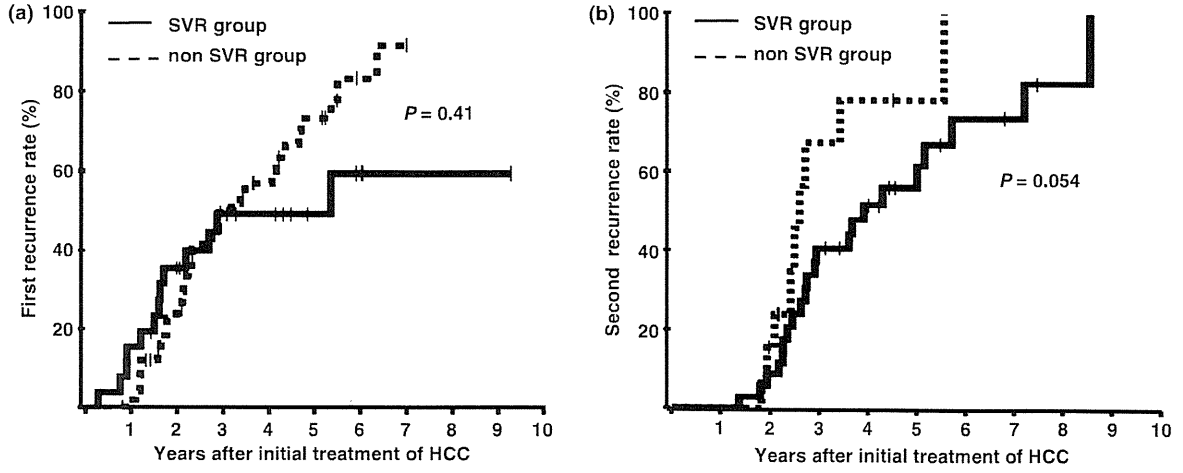

Fig. 4 Cumulative recurrence rates after curative treatment of hepatocellular carcinoma. (a) Rates of first recurrence for the sustained viral response (SVR) and non-SVR groups (P = 0.41). (b) Rates of second recurrence for the SVR and non-SVR groups. The second recurrence rate for the SVR group tended to be lower than that for the non-SVR group (P = 0.054).

administer low-dose intermittent IFN therapy for patients considered to show NR [those with IL-28B genotype (GG+TG)]. This therapy might result in the improvement of liver function and prevention of HCC recurrence, even if not to obtain SVR.

In conclusion, with regard to the prognosis of patients who undergo curative treatment for HCC, it is desirable to achieve SVR with interferon therapy even after treatment of HCC. IL-28B genotype could potentially be a suitable marker for the response to PEGIFN/RBV combination therapy after treatment of HCV-related HCC.

DISCLOSURES

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Technical refinements of bile duct division in living donor liver surgery

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Abstract
Background/purpose In spite of the great risk involved, the donor bile duct division procedure has not been thoroughly addressed in the literature. The purpose of this study is to show the appropriate approach to bile duct division in living donor hepatectomy.
Methods Of 87 living donor liver surgeries, we performed bile duct division by marking the cutting point using a small vascular clip under ordinary cholangiography in the first 37 patients, while the current procedure was used in 50 patients by encircling the cutting point using a radiopaque marker filament under real-time C-arm cholangiography.
Results Regarding the procurement of the 51 right lobe grafts, the incidence of multiple bile ducts in the graft was significantly reduced by our novel procedure [20/28 (71%) vs. 7/23 (30%), $P < 0.01$, Fisher's test]. Overall, there were no biliary strictures after surgery in any of the donors, with a median follow-up period of 43 months (range 8–136).
Conclusions Our procedure of bile duct division in living liver donor surgery enabled us to avoid the biliary stricture while cutting the bile duct of the donor with great accuracy.

Keywords Liver transplantation · Living donor · Bile duct

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Introduction

Living donor liver transplantation (LDLT) has been established as an effective modality for the treatment of various end-stage liver diseases. However, compared to deceased donor liver transplantation, more technical and ethical dilemmas exist, primarily because it is difficult to strike a balance between donor safety and recipient benefit. Regarding biliary reconstruction in this context, the recipient requires a large, single bile duct orifice in order to reduce the risk of post-surgical biliary complications [1, 2]. Thus, while it is desirable to cut the bile duct as close as possible to the hepatic hilum during donor surgery, this leads to significant concerns about biliary stricture in the donor. Donor safety should be the top priority in LDLT, and therefore the bile duct must be cut with great caution and according to the most appropriate procedure. In spite of the great risk involved, the donor bile duct division procedure has not been thoroughly addressed in the literature. The aim of this study was therefore to describe our technical refinements to the procedure of bile duct division in living donor liver surgery.

Patients and methods

Patients

Eighty-seven living donor hepatectomies for primary liver transplantation were performed at our institution from August 1997 to April 2008. The living donors consisted of 44 males and 43 females with a median age of 39 (range 19–67). The following types of grafts were procured: 9 left lateral segments, 2 left lobes without the middle hepatic vein (MHV), 8 extended left lobes with the MHV, 12

extended left lobes with the caudate lobe, 51 right lobes without the MHV, and 5 right posterior segments. These cases were divided as follows into two groups according to the treatment period: group A (August 1997 to December 2004, $n = 37$) and group B (January 2005 to April 2008, $n = 50$) (Table 1). Preoperative evaluation of the biliary anatomy was performed by magnetic resonance cholangiopancreatography in both groups. In group A, we adapted a right lobe graft only for adult-to-adult LDLT, but thereafter we used various types of grafts (e.g., left liver grafts and right posterior segment grafts), primarily to enhance donor safety. Moreover, we altered the bile duct division procedure. Initially, we carried out ordinary cholangiography after the cholecystectomy via a catheter that had been placed at the cystic duct, and the bile duct was cut prior to parenchymal transection by marking the cutting point using a small vascular clip (Fig. 1). However, we frequently encountered patients with multiple bile ducts during right lobe graft procurement, such that we adapted the previous procedure to arrive at the current approach to bile duct division, as described below. This procedure was adapted in group B.

Surgical procedure of bile duct division

During hilar dissection, the gall bladder was dissected away from the liver, and the hepatic artery and portal branch were fully exposed and isolated from the hilar plate. Particular attention was paid to retaining the surrounding tissue of the hilar plate without exposing the bile duct; in order to avoid heat injury, electric cautery should not be used at this step. At the final step of the subsequent parenchymal transection, the hilar plate was fully exposed and encircled with a radiopaque marker filament obtained from surgical gauze (Fig. 2a). Real-time cholangiography using C-arm fluoroscopy was then performed via the catheter, which was placed in the cystic duct (Fig. 2b). To verify the optimal point for cutting the bile duct, the radiopaque filament was retracted (Fig. 2c), and the C-arm was rounded to adjust the apparatus to the accurate angle. After confirmation of the accurate cutting point, parenchymal transection was further advanced using a liver hanging-maneuver technique [3] with preservation of the hilar plate. We then interposed the surgery after completion of the liver parenchymal transection. When the surgeon for

Table 1 Characteristics of donor and recipient

	Group A ($n = 37$)	Group B ($n = 50$)	P value
Donor			
Age	39 (21–67)	37 (19–64)	NS
Gender	Male 16, female 21	Male 28, female 22	NS
Graft			
RL	28	23	NS
LL + CL	0	12	<0.01
LL	0	10	<0.01
LLS	9	0	<0.01
RPS	0	5	<0.01
Multiple ducts in RL graft	20/28 (71%)	7/23 (30%)	<0.01
Biliary stricture	0	0	NS
Recipient			
Age	41 (0–65)	57 (11–68)	NS
Gender	Male 20, female 13	Male 32, female 18	NS
Disease			
BA	8	1	<0.01
Viral cirrhosis	11	33	<0.01
With HCC	5	27	
W/o HCC	6	6	
FHF	7	2	<0.01
Others	11	13	NS
Biliary reconstruction			
HJ	13	7	<0.05
DD	24	43	
Biliary stricture	9	8	NS
Follow-up period	79 months (52–136)	24 months (8–48)	<0.01

Statistical analyses were performed with Fisher's test

RL right lobe, *LL* left lobe, *CL* caudate lobe, *LLS* left lateral segment, *RPS* right posterior segment, *BA* biliary atresia, *HCC* hepatocellular carcinoma, *FHF* fulminant hepatic failure, *HJ* hepaticojejunostomy, *DD* duct to duct

the recipient required the graft, the hilar plate, including the hepatic duct, was precisely divided with scissors, and the stump of the remnant bile duct was closed with continuous, 6-0 absorbable monofilament sutures (PDS II, Ethicon, Somerville, NJ). Cholangiography with C-arm fluoroscopy was then performed once again in order to check for biliary leakage and strictures in the remnant bile duct (Fig. 2d). The liver graft was then removed after the hepatic artery, portal vein, and hepatic vein were divided.

Biliary reconstruction in recipients

Biliary reconstruction in the recipients was performed by Roux-en-Y hepaticojejunostomy or duct-to-duct anastomosis, with or without biliary stenting, and with interrupted, 6-0 absorbable monofilament sutures (PDS II, Ethicon, Somerville, NJ) (Table 1). Duct-to-duct anastomosis was the first line, and hepaticojejunostomy was performed only in cases of biliary atresia and primary sclerosing cholangitis, and in cases in which the quality of the recipient bile duct was not good for various reasons, such as biliary ischemia. In case of multiple bile ducts in the graft, duct plasty was performed whenever possible [4], and duct-to-duct anastomosis was still the first line for biliary reconstruction. Concerning decreasing the incidence of biliary atresia, the incidence of hepaticojejunostomy was significantly lower in group B (Table 1).

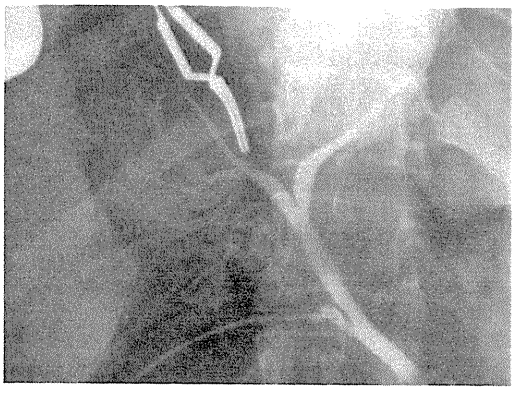

Fig. 1 Our original procedure for bile duct division. The bile duct is divided under ordinary cholangiography by placing a small vascular clip around the hepatic duct

Results

Incidence of multiple bile ducts in grafts

The characteristics of the biliary anatomy increase the probability of multiple bile ducts in right lobe grafts over that in left lobe grafts [5]. The incidence of multiple ducts in right lobe grafts was compared between groups. In the procurement of 51 right lobe grafts, the incidence of multiple bile ducts was significantly reduced by the current procedure [20/28 (71%) in group A vs. 7/23 (30%) in group B, $P < 0.01$, Fisher's test]. In these cases, anatomic variation was similar in both groups, i.e., according to the classification system of Varotti et al. [5] (Fig. 3): type 1 [right anterior and right posterior hepatic ducts (HD) join together to form the right HD: 19/28 (67.9%) in group A

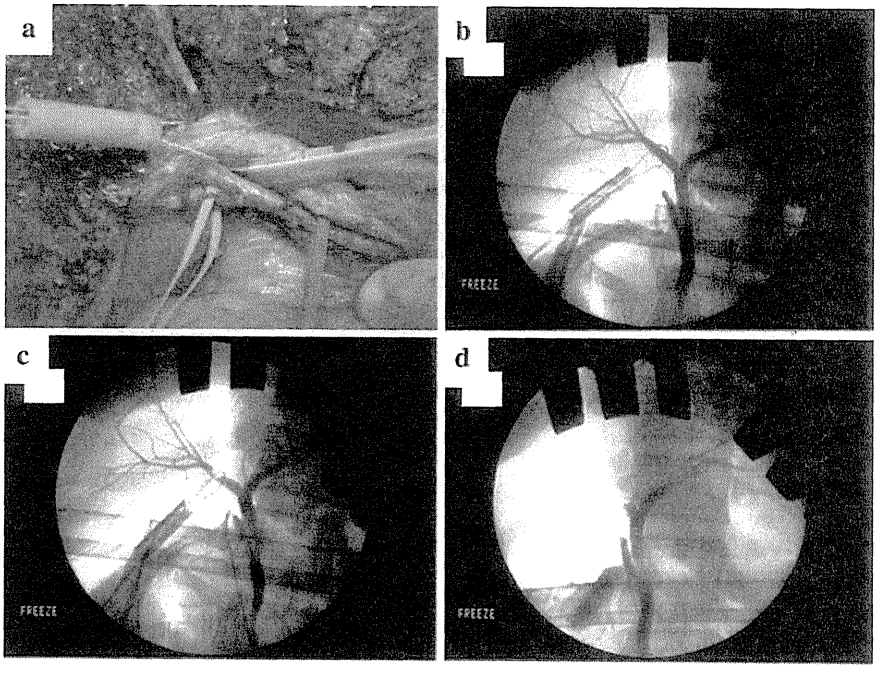

Fig. 2 Bile duct division in the procurement of a right lobe graft. The right hilar plate is encircled with a radiopaque marker filament, and sufficient surrounding tissue is preserved without exposure of the bile duct (**a**). C-arm cholangiography revealed that the radiopaque marker filament was placed at an adequate point at the right hepatic duct (**b**). The radiopaque marker filament was pulled in order to verify the cutting point (**c**). C-arm cholangiography after bile duct division revealed that the bile duct was cut at the optimal point, without inducing a stricture in the remnant left hepatic duct (**d**)

vs. 17/23 (73.9%) in group B]; type 2 [the right HD is absent and the right anterior HD and right posterior HD join directly to the confluence with the left HD to form the common HD: 2/28 (7.1%) in group A vs. 3/23 (13.0%) in group B]; type 3 [the right anterior HD or the right posterior HD open directly into the left HD: 6/28 (21.4%) in group A vs. 3/23 (13.0%) in group B]; type 4 [the right anterior HD or the right posterior HD open directly into the common HD: 1/28 (3.6%) in group A vs. 0/23 (0%) in group B)].

Bile duct division in complex cases

In cases involving multiple bile ducts in the graft, one hilar plate was encircled, including all of the hepatic ducts (Fig. 4). In these cases as well, the use of a radiopaque marker filament as the reference for the optimal cutting point was feasible and allowed preservation of the surrounding tissue. In cases involving a right posterior segment graft, it can generally be relatively difficult to determine the optimal cutting point, because the targeted point tends to be more distal and supports smaller bile duct(s); however, in all of our five donor cases involving this type of graft, we easily identified the optimal cutting point without any difficulty (Fig. 5). In some cases involving a complex variation in the bile duct branching pattern (i.e., right posterior hepatic duct independently branching from the left hepatic duct), we were able to avoid bile duct injury in the donor by clearly making the cutting point with pulling the radiopaque marker filament (Fig. 6).

Biliary complications in donors

A total of 16 complications (18.4%) were seen in the donors (6 bile leakages, 3 wound infections, 2 pleural effusions, 2 cases of gastric stasis, 1 portal vein thrombosis, 1 case of postoperative bleeding, and 1 paralytic ileus). All

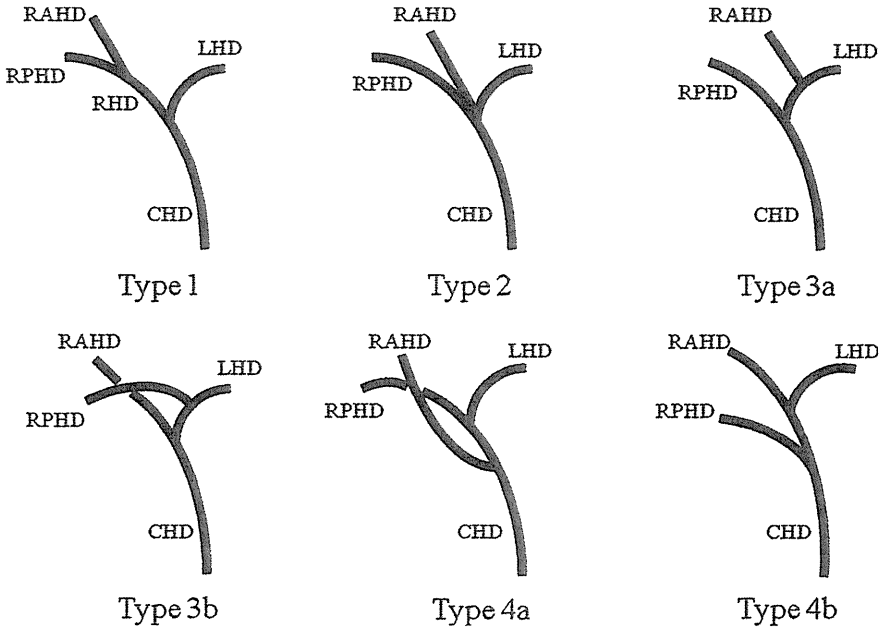

Fig. 3 Classification of the biliary tree anatomy by Varotti et al. [5]. *CHD* common hepatic duct, *LHD* left hepatic duct, *RAHD* right anterior hepatic duct, *RPHD* right posterior hepatic duct

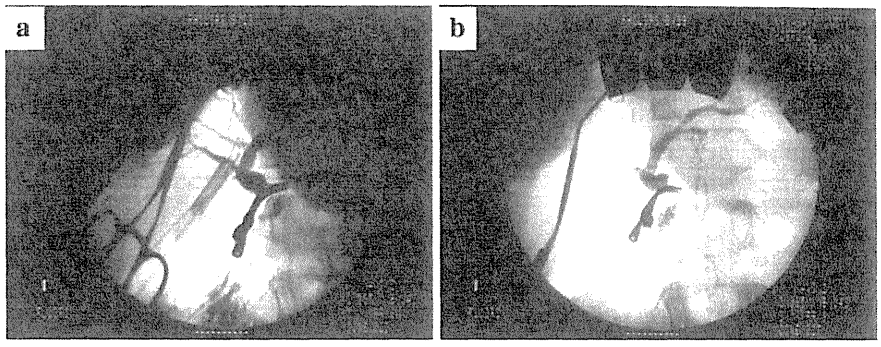

Fig. 4 Bile duct division in the procurement of a right-lobe graft with multiple ducts. The right hilar plate, including both the right anterior branch and the right posterior branch, is encircled by a radiopaque marker filament (**a**). There was no stricture in the remnant left hepatic duct after division (**b**)

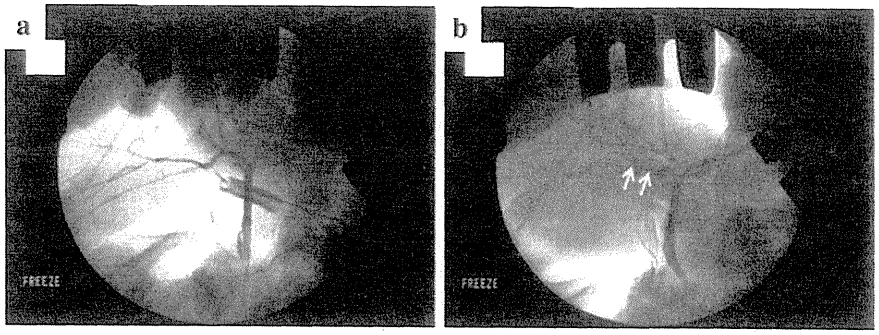

Fig. 5 Bile duct division in the procurement of a right posterior segment graft. A radiopaque marker filament was placed at an adequate point in the right posterior hepatic duct (**a**). After division of the right posterior hepatic duct, there was no stricture in the remnant right anterior hepatic duct (*arrows*, **b**)

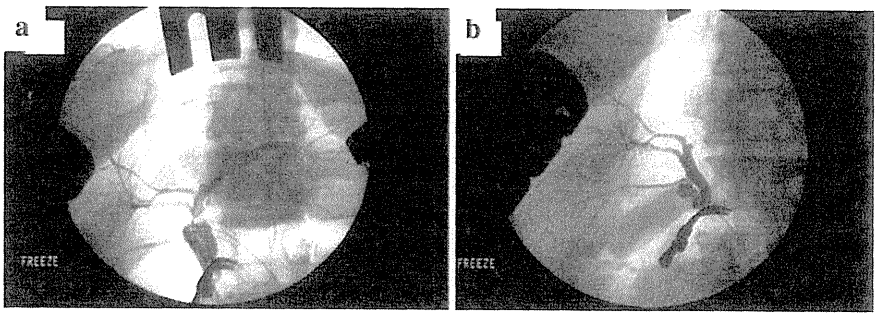

Fig. 6 Bile duct division in the procurement of a left lobe graft, in which the right anterior hepatic duct branched from the left hepatic duct (**a**). The bile duct was divided at an adequate point without subsequent stricture in the remnant right anterior hepatic duct (**b**)

bile leakage cases were treated by percutaneous drainage, and all spontaneously resolved without requiring surgical intervention. There was no significant difference in the incidence of bile leakage between the groups [4/37 (10.8%) in group A vs. 2/50 (4.0%) in group B]. No biliary strictures were observed in any of the donors in either of the groups. All donors are alive and currently doing well, carrying out normal daily activities after a median follow-up period of 43 months (range 8–136).

Biliary complications in recipients

The incidence of biliary stricture requiring endoscopic or surgical treatment of recipients was compared between adult cases in both groups (>18 years). It would not be appropriate to formally compare the incidence of biliary complications in recipients according to the groups as defined here, because the follow-up period for group B was significantly shorter than that for group A. However, it should be noted that the incidence of biliary stricture was non-significantly lower in group B than group A [i.e., 9/37 (24.3%) in group A vs. 8/50 (16.0%) in group B].

Discussion

Biliary stricture is one of the most significant complications in liver transplant recipients. The etiology of this complication is multifactorial, and especially in LDLT, the presence of tiny, multiple ducts can contribute to a higher incidence of biliary stricture than that encountered in deceased donor liver transplantation. Although the relationship between the presence of multiple ducts and the incidence/severity of biliary complication remains controversial [6, 7], several studies have indicated that the presence of multiple bile ducts in a graft is a risk factor for biliary complication [1, 2]. Additionally, biliary ischemia is an important, well-documented factor that affects biliary stricture [8, 9]. Considering these factors, surgical innovations are required not only in recipient surgeries, but also in donor surgeries. In order to maintain the blood supply, it is desirable to harvest a large bile duct orifice in the graft, together with a sufficient amount of surrounding tissue. However, there are still several technical and ethical dilemmas associated with this procedure. In particular, in order to obtain a graft containing a single large orifice, it is necessary to cut the bile duct as close as possible to the hepatic hilum, which can lead to biliary stricture in the residual bile duct of the donor. Additionally, in the attempt to cut the bile duct at a precise point, it becomes necessary to expose the bile duct by also dissecting back the surrounding tissue, which can lead to biliary ischemia. The technical innovations described in this study yielded a resolution to these problems. By encircling the hilar plate using a radiopaque marker filament, the cutting point was easily identified, and the surrounding tissue of the hilar plate could be maintained. Under real-time C-arm cholangiography while pulling the filament, the cutting point

was clearly visualized in a three-dimensional image. This procedure using a radiopaque marker filament and C-arm cholangiography was originally introduced by Chen in Taiwan [10]. We then modified the procedure by pulling the filament in order to render the cutting point more clear, as described above. Initially, we adopted this approach for the procurement of right lobe grafts because of the high incidence of multiple bile ducts encountered in these grafts [11]. After the adoption of this novel technique, the incidence of encountering multiple ducts in grafts was significantly reduced without increasing the rate of biliary stricture in donors. Biliary stricture is one of the most significant complications in living donors. According to a survey conducted by the Japanese Liver Transplantation Society, 11% of 1852 donors had biliary leaks and strictures, the majority of which occurred after right lobe hepatectomy; a total of 10 donors underwent surgical revision for biliary complications [12]. To date, we have not observed any biliary complications requiring surgical treatment among our donors. Six cases (7.7%) exhibited minor bile leakage, which in each case was successfully treated by percutaneous drainage alone, i.e., no subsequent biliary strictures developed in any of these donors. With the increasing rate of adult LDLT, several types of graft have been introduced (e.g., left lobe with or without the caudate lobe, right posterior segment graft). In all cases presented here, our novel approach was found to be very effective at enhancing the accuracy of setting the cut point for resection of the bile duct.

One disadvantage of this technique could be possible bile duct injury during encirclement of the hilar plate. Thus, very careful attention should be taken at this step to notice any resistance and thus to avoid forced penetration of the Kelly clamp into the fibrous tissue. Furthermore, it remains important to obtain clear preoperative visualization of the biliary anatomy, thereby to avoid missing any significantly aberrant branching pattern around the hilum. Here, we used magnetic resonance cholangiopancreatography for the preoperative evaluation of the biliary anatomy, and no significant bile duct injuries occurred. In general, the key point of the present procedure is that the hilar plate (i.e., not the bile duct) should be fully exposed before it is marked with the radiopaque marker filament. Thus, the hilar anatomy should be clearly visualized, including assessment of the relationship between the hepatic artery, the portal vein, and the hilar plate. As long as the tissue of hilar plate is preserved, no significant biliary ischemia is likely to occur. Once the encirclement of the hilar plate was completed, this procedure was found to be useful even in cases involving complex bile duct branching patterns. Intraoperative cholangiography just prior to the encirclement of the hilar plate may be helpful in yielding the most secure bile duct division possible.

Whereas donor safety should be the top priority in LDLT, the recipient's outcome is almost equally important in terms of rewarding the donor for his or her devotion to the patient. Our current procedure contributed to a reduction in the incidence of biliary stricture in recipients as well. Randomized and controlled studies would be ideal, but until we have access to such studies, we believe that harvesting a single orifice with sufficient surrounding tissue of bile ducts is a feasible means of performing the most straightforward surgery possible, and this novel approach is expected to contribute to successful outcomes. Moreover, simple anastomosis using a single orifice might facilitate the treatment of any remaining cases involving biliary stricture.

In conclusion, the present procedure of dividing the bile duct during living-donor liver surgery using a radiopaque marker filament and C-arm cholangiography is feasible for avoiding biliary stricture in the donor while maintaining graft quality with sufficient surrounding tissue and reducing the chance of encountering multiple bile duct orifices.

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