Research Article

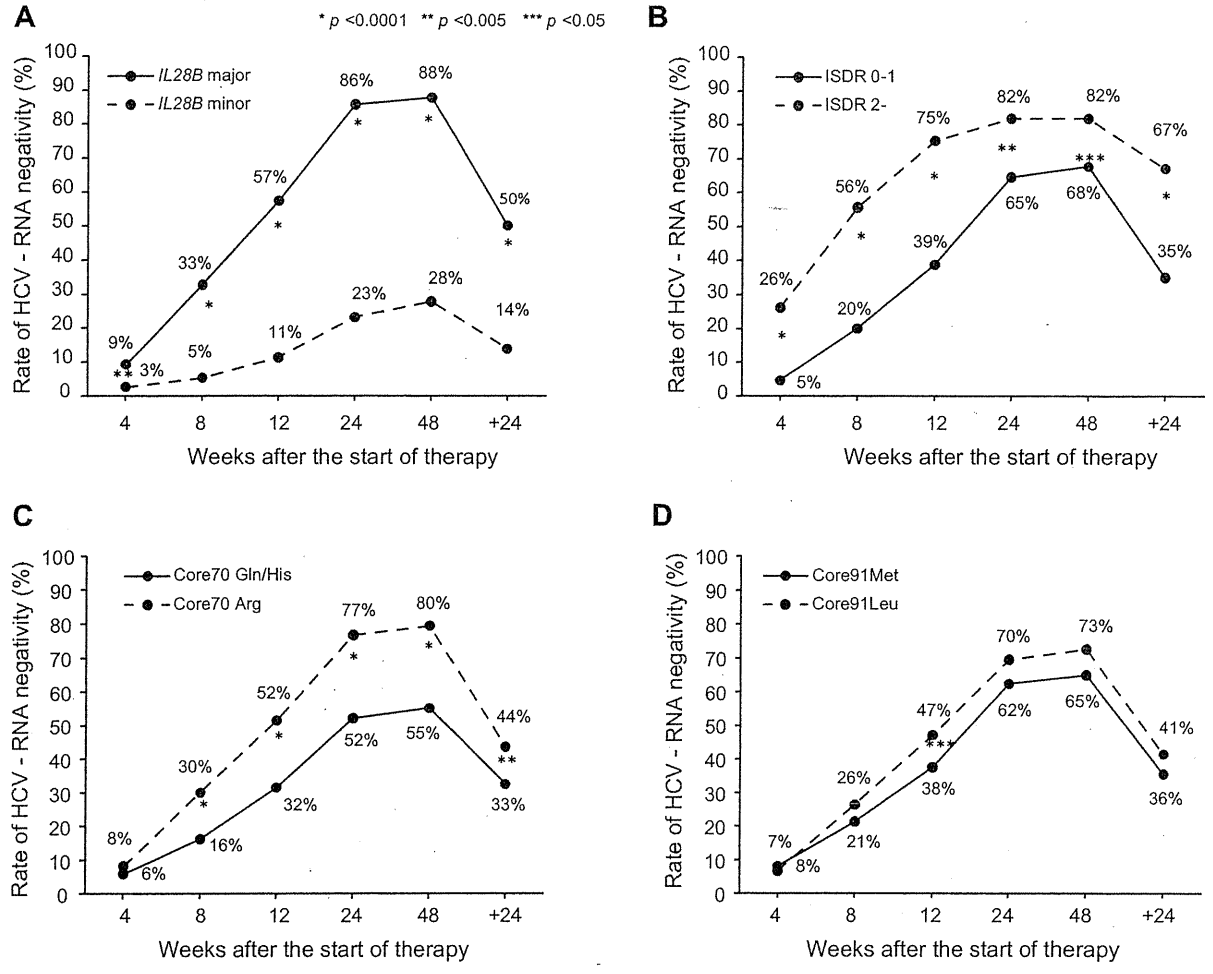

Fig. 2. Effect of *IL28B* mutations in the ISDR, Core70, and Core91 of HCV on time-dependent clearance of HCV. The rate of undetectable HCV-RNA was plotted for serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks) and for 24 weeks after the completion of therapy. Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele), (B) the number of mutations in the ISDR (0–1 mutation vs. 2 or more mutations), amino acid substitutions of (C) Core70 (Gln/His vs. Arg), and (D) Core91 (Met vs. Leu). The *p* values are from Fisher's exact test.

HCV-RNA ($p = 0.035$), Gln or His at Core70 ($p < 0.0001$), low platelet counts ($p = 0.009$), and advanced fibrosis ($p = 0.0002$) were associated with NVR. By multivariate analysis, the minor allele of *IL28B* (OR = 20.83, 95%CI = 11.63–37.04, $p < 0.0001$) was associated with NVR independent of other covariates (Table 2). Notably, mutations in the ISDR ($p = 0.707$) and at amino acid Core70 ($p = 0.207$) were not significant in multivariate analysis due to the positive correlation with the *IL28B* polymorphism ($p = 0.004$ for ISDR and $p < 0.0001$ for Core70, Fig. 4).

Genetic polymorphism of *IL28B* also was associated with SVR (OR = 7.41, 95% CI = 4.05–13.57, $p < 0.0001$) independent of other covariates, such as platelet counts, fibrosis, and serum levels of HCV-RNA. Mutation in the ISDR was an independent predictor of SVR (OR = 2.11, 95% CI = 1.06–4.18, $p = 0.033$) but the amino acid at Core70 was not (Table 3).

Factors associated with the IL28B polymorphism

Patients with the *IL28B* minor allele had significantly higher serum level of gamma-glutamyltransferase (GGT) and a higher frequency of hepatic steatosis (Table 4). When the association between the *IL28B* polymorphism and HCV sequences was analyzed, Gln or His at Core70, that is linked to resistance to PEG-IFN and RBV therapy [4,14,15], was significantly more frequent in patients with the minor *IL28B* allele than in those with the major allele (67% vs. 30%, $p < 0.0001$) (Fig. 4). Other HCV sequences with an IFN resistant phenotype also were more prevalent in patients with the minor *IL28B* allele than those with the major allele: Met at Core91 (46% vs. 37%, $p = 0.047$) and one or no mutations in the ISDR (94% vs. 85%, $p = 0.004$) (Fig. 4).

Data mining analysis

Data mining analysis was performed to build a model for the prediction of SVR and the result is shown in Fig. 5. The analysis selected four predictive variables, resulting in six subgroups of patients. Genetic polymorphism of *IL28B* was selected as the best predictor of SVR. Patients with the minor *IL28B* allele had a lower probability of SVR and a higher probability of NVR than those with the major *IL28B* allele (SVR: 14% vs. 50%, NVR: 72% vs.

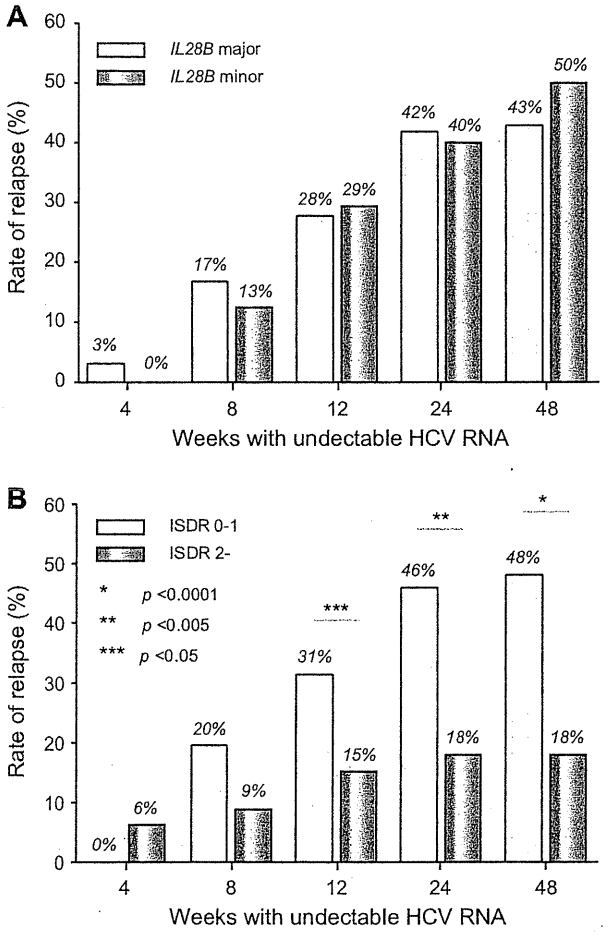

Fig. 3. Association between relapse and the *IL28B* allele or mutations in the ISDR. The rate of relapse was calculated for patients who had undetectable HCV-RNA at serial time points after the start of therapy (4, 8, 12, 24, and 48 weeks). Patients were stratified according to (A) the *IL28B* allele (minor allele vs. major allele) and (B) the number of mutations in the ISDR (0–1 mutation vs. 2 or more mutations). The *p* values are from Fisher's exact test.

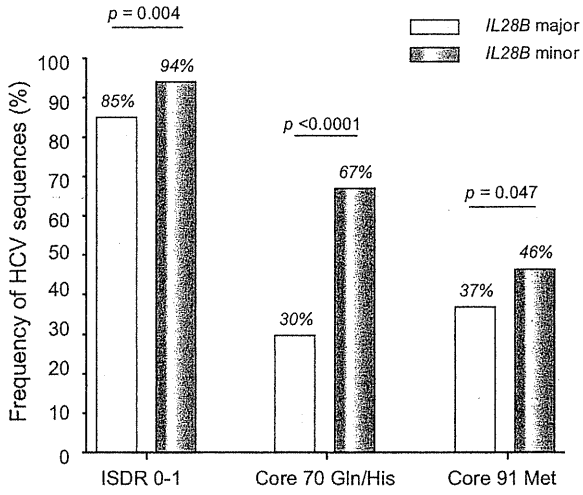

Fig. 4. Associations between the *IL28B* allele and HCV sequences. The prevalence of HCV sequences predicting a resistant phenotype to IFN was higher in patients with the minor *IL28B* allele than those with major allele. (A) 0 or 1 mutation in the ISDR of NS5A, (B) Gln or His at Core70, and (C) Met at Core91. *p* values are from Fisher's exact test.

12%). After stratification by the *IL28B* allele, patients with low platelet counts (<140 × 10^9/L) had a lower probability of SVR and higher probability of NVR than those with high platelet counts (⩾140 × 10^9/L): for the minor *IL28B* allele, SVR was 7% vs. 19%, and NVR was 84% vs. 62%, and for the major *IL28B* allele, SVR was 32% vs. 66% and NVR was 16% vs. 8%. Among patients with the major *IL28B* allele and low platelet counts, those with two or more mutations in the ISDR had a higher probability of SVR and lower probability of relapse than those with one or no mutations in the ISDR (SVR: 75% vs. 27%, and relapse: 8% vs. 57%). Among patients with the major *IL28B* allele and high platelet counts, those with a low HCV-RNA titer (<600,000 IU/ml) had a higher probability of SVR and lower probability of NVR and relapse than those with a high HCV-RNA titer (SVR: 90% vs. 61%, NVR: 0% vs. 10%, and relapse: 10% vs. 29%). The sensitivity and specificity of the decision tree were 78% and 70%, respectively. The area under the receiver operating characteristic (ROC) curve of the model was 0.782 (data not shown). The proportion of patients with advanced fibrosis (F3-4) was 39% (84/217) in patients with low platelet counts (<140 × 10^9/L) compared to 13% (37/279) in those with high platelet counts (⩾140 × 10^9/L).

Validation of the data mining analysis

The results of the data mining analysis were validated with 165 patients who differed from those used for model building. Each patient was allocated to one of the six subgroups for the validation using the flow-chart form of the decision tree. The rate of SVR and NVR in each subgroup was calculated. The rates of SVR and NVR for each subgroup of patients were closely correlated between the model building and the validation patients (r^2 = 0.99 and 0.98) (Fig. 6).

Discussion

The rate of NVR after 48 weeks of PEG-IFN/RBV therapy among patients infected with HCV of genotype 1 is around 20–30%. Previously, there have been no reliable baseline predictors of NVR or SVR. Because more potent therapies, such as protease and polymerase inhibitor of HCV [28,29] and nitazoxianide [30], are in clinical trials and may become available in the near future, a pre-treatment prediction of the likelihood of response may be helpful for patients and physicians, to support clinical decisions about whether to begin the current standard of care or whether to wait for emerging therapies. This study revealed that the *IL28B* polymorphism was the overwhelming predictor of NVR and is independent of host factors and viral sequences reported previously. The *IL28B* encodes a protein also known as IFN-lambda 3, which is thought to suppress the replication of various viruses including HCV [31,32]. The results of the current study and the findings of the GWAS studies [6–9] may provide the rationale for developing diagnostic testing or an IFN-lambda based therapy for chronic hepatitis C in the future.

Table 2. Factors associated with NVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.98	0.67-1.45	0.938	1.29	0.75-2.23	0.363
Age	1.01	0.97-1.01	0.223	0.99	0.97-1.02	0.679
ALT	1.00	1.00-1.00	0.867	1.00	0.99-1.00	0.580
GGT	1.004	1.00-1.01	0.029	1.00	1.00-1.00	0.715
Platelets	0.95	0.91-0.99	0.009	0.92	0.87-0.98	0.006
Fibrosis: F3-4	2.23	1.46-3.42	0.0002	1.97	1.09-3.57	0.025
HCV-RNA: ≥600,000 IU/ml	1.83	1.05-3.19	0.035	2.49	1.17-5.29	0.018
ISDR mutation: ≤1	2.14	1.08-4.22	0.030	0.96	0.78-1.18	0.707
Core 70 (Gln/His)	3.23	2.16-4.78	<0.0001	1.41	0.83-2.42	0.207
Core 91 (Met)	1.39	0.95-2.06	0.093	1.21	0.72-2.04	0.462
IL28B: Minor allele	19.24	11.87-31.18	<0.0001	20.83	11.63-37.04	<0.0001

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Gln, glutamine; His, histidine; Met, methionine; Minor allele, heterozygote or homozygote of minor allele.

Table 3. Factors associated with SVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.81	0.56-1.16	0.253	0.86	0.55-1.35	0.508
Age	0.97	0.95-0.99	0.0003	0.99	0.96-1.01	0.199
ALT	1.00	1.00-1.00	0.337	1.00	1.00-1.01	0.108
GGT	1.00	1.00-1.00	0.273	1.00	1.00-1.00	0.797
Platelets	1.12	1.01-116	<0.0001	1.13	1.08-1.19	<0.0001
Fibrosis: F0-2	2.64	1.65-4.22	<0.0001	1.87	1.07-3.28	0.029
HCV-RNA: <600,000 IU/ml	2.49	1.55-3.98	0.0001	2.75	1.55-4.90	0.001
ISDR mutation: 2≤	3.78	2.14-6.68	<0.0001	2.11	1.06-4.18	0.033
Core 70 (Arg)	1.61	1.11-2.28	0.012	0.84	0.52-1.35	0.470
Core 91 (Leu)	1.28	0.88-1.85	0.185	1.26	0.81-1.96	0.300
IL28B: Major allele	6.21	3.75-10.31	<0.0001	7.41	4.05-13.57	<0.0001

ALT, alanine aminotransferase; GGT, Gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Leu, leucine; Major allele, homozygote of major allele.

Among baseline factors, *IL28B* was the most significant predictor of NVR and SVR. Moreover, the *IL28B* allele type was also correlated with early virological response: the rate of RVR and cEVR was significantly high for the *IL28B* major allele compared to the *IL28B* minor allele: 9% vs. 3% for RVR and 57% vs. 11% for cEVR (Fig. 2). On the other hand, the relapse rate was not different between the *IL28B* genotypes within patients who achieved RVR or cEVR (Fig. 3). We believe that optimal therapy should be based on baseline features and a response-guided approach. Our findings suggest that the *IL28B* genotype is a useful baseline predictor of virological response which should be used for selecting the treatment regimen: whether to treat patients with PEG-IFN and RBV or to wait for more effective future therapy including direct acting antiviral drugs. On the other hand, baseline *IL28B* genotype might not be suitable for determining the treatment duration in patients who started PEG-IFN/RBV therapy and whose virological response is determined because the *IL28B* genotype is not useful for the prediction of relapse. The duration of therapy should be personalized based on the virological response. Future studies need to explore whether the combination of baseline *IL28B* genotype and response-guided approach further improves the optimization of treatment duration.

The SVR rate in patients having the *IL28B* minor allele was 14% in the present study while it was 23% in Caucasians and 9% in African Americans in a study by McCarthy et al. [33]. On the other hand, the SVR rate in patients having the *IL28B* minor allele was 28% in genotypes 1/4 compared to 80% in genotypes 2/3 in a study by Rauch et al. [9]. These data imply that the impact of the *IL28B* polymorphism on response to therapy may be different in terms of race, geographical areas, or HCV genotypes, and that our data need to be validated in future studies including different populations and geographical areas before generalization.

Table 4. Factors associated with IL28B genotype.

	IL28B major allele n = 345	*IL28B* minor allele n = 151	*p* value
Gender: male	166 (48%)	84 (56%)	0.143
Age (years)	57 ± 10	57 ± 10	0.585
ALT (IU/L)	79 ± 60	78 ± 62	0.842
Platelets (10^9/L)	153 ± 54	155 ± 52	0.761
GGT (IU/L)	51 ± 45	78 ± 91	0.001
Fibrosis: F3-4	76 (22%)	45 (30%)	0.063
Steatosis:			
>10%	16/88 (18%)	13/23 (57%)	0.024
>30%	6/88 (7%)	6/23 (26%)	0.017
HCV-RNA: >600,000 IU/ml	284 (82%)	125 (83%)	1.000

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

Four GWAS studies have shown the association between a genetic polymorphism near the *IL28B* gene and response to PEG-IFN plus RBV therapy. The SNPs that showed significant association with response were rs12979860 [8] and rs8099917 [6,7,9]. There is a strong linkage-disequilibrium (LD) between these two SNPs as well as several other SNPs near the *IL28B* gene in Japanese patients [34] but the degree of LD was weaker in Caucasians and Hispanics [8]. Thus, the combination of SNPs is not useful for predicting response in Japanese patients but may improve the predictive value in patients other than Japanese who have weaker LD between SNPs.

Other significant predictors of response independent of *IL28B* genotype were platelet counts, stage of fibrosis, and HCV RVA load. A previous study reported that platelet count is a predictor of response to therapy [35], and the lower platelet count was related with advanced liver fibrosis in the present study. The association between response to therapy and advanced fibrosis independent of the *IL28B* polymorphism is consistent with a recent study by Rauch et al. [9].

There is agreement that the viral genotype is significantly associated with the treatment outcome. Moreover, viral factors such as substitutions in the ISDR of the NS5A region [10] or in the amino acid sequence of the HCV core [4] have been studied in relation to the response to IFN treatment. The amino acid Gln or His at Core70 and Met at Core91 are repeatedly reported to be associated with resistance to therapy [4,14,15] in Japanese patients but these data wait to be validated in different populations or other geographical areas. In this study, we confirmed that patients with two or more mutations in the ISDR had a higher rate of undetectable HCV-RNA at each time point during therapy. In addition, the rate of relapse among patients who achieved cEVR was significantly lower in patients with two or more mutations in ISDR compared to those with only one or no mutations (15% vs. 31%, $p < 0.05$). Thus, the ISDR sequence may be used to predict a relapse among patients who achieved virological response during therapy, while the *IL28B* polymorphism may be used to predict the virological response before therapy. A higher number of mutations in the ISDR are reported to have close association with SVR in Japanese [11–13,15,36] or Asian [37,38] populations but data from Western countries have been controversial [39–42]. A meta-analysis of 1230 patients including 525 patients from Europe has shown that there was a positive correlation between the SVR and the number of mutations in the ISDR in Japanese as well as in European patients [43] but this correlation was more pronounced in Japanese patients. Thus, geographical factors may account for the different impact of ISDR on treatment response, which may be a potential limitation of our study.

To our surprise, these HCV sequences were associated with the *IL28B* genotype: HCV sequences with an IFN resistant phenotype were more prevalent in patients with the minor IL28B allele than those with the major allele. This was an unexpected finding, as we initially thought that host genetics and viral sequences were completely independent. A recent study reported that the *IL28B* polymorphism (rs12979860) was significantly associated with HCV genotype: the *IL28B* minor allele was more frequent in HCV genotype 1-infected patients compared to patients infected with HCV genotype 2 or 3 [33]. Again, patients with the *IL28B* minor allele (IFN resistant genotype) were infected with HCV sequences that are linked to an IFN resistant phenotype. The mechanism for this association is unclear, but may be related to an interaction between the *IL28B* genotype and HCV sequences in the development of chronic HCV infection as discussed by McCarthy et al., since the *IL28B* polymorphism was associated with the natural clearance of HCV [44]. Alternatively, the HCV sequence within the patient may be selected during the course of chronic infection [45,46]. These hypotheses should be explored through prospective studies of spontaneous HCV clearance or by testing the time-dependent changes in the HCV sequence during the course of chronic infection.

How these host and viral factors can be integrated to predict the response to therapy in future clinical practice is an important question. Because various host and viral factors interact in the same patient, predictive analysis should consider these factors in combination. Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of SVR and NVR to PEG-IFN/RBV therapy. The classification of patients based on the genetic polymorphism of *IL28B*, mutation in the ISDR, serum levels of HCV-RNA, and platelet counts, identified subgroups of patients who have the lowest probabilities of NVR (0%) with the highest probabilities of SVR (90%) as well as those who have the highest probabilities of NVR (84%) with the lowest probability of SVR (7%). The reproducibility of the model was confirmed by the independent validation based on a second group of patients. Using this model, we can rapidly develop an

Research Article

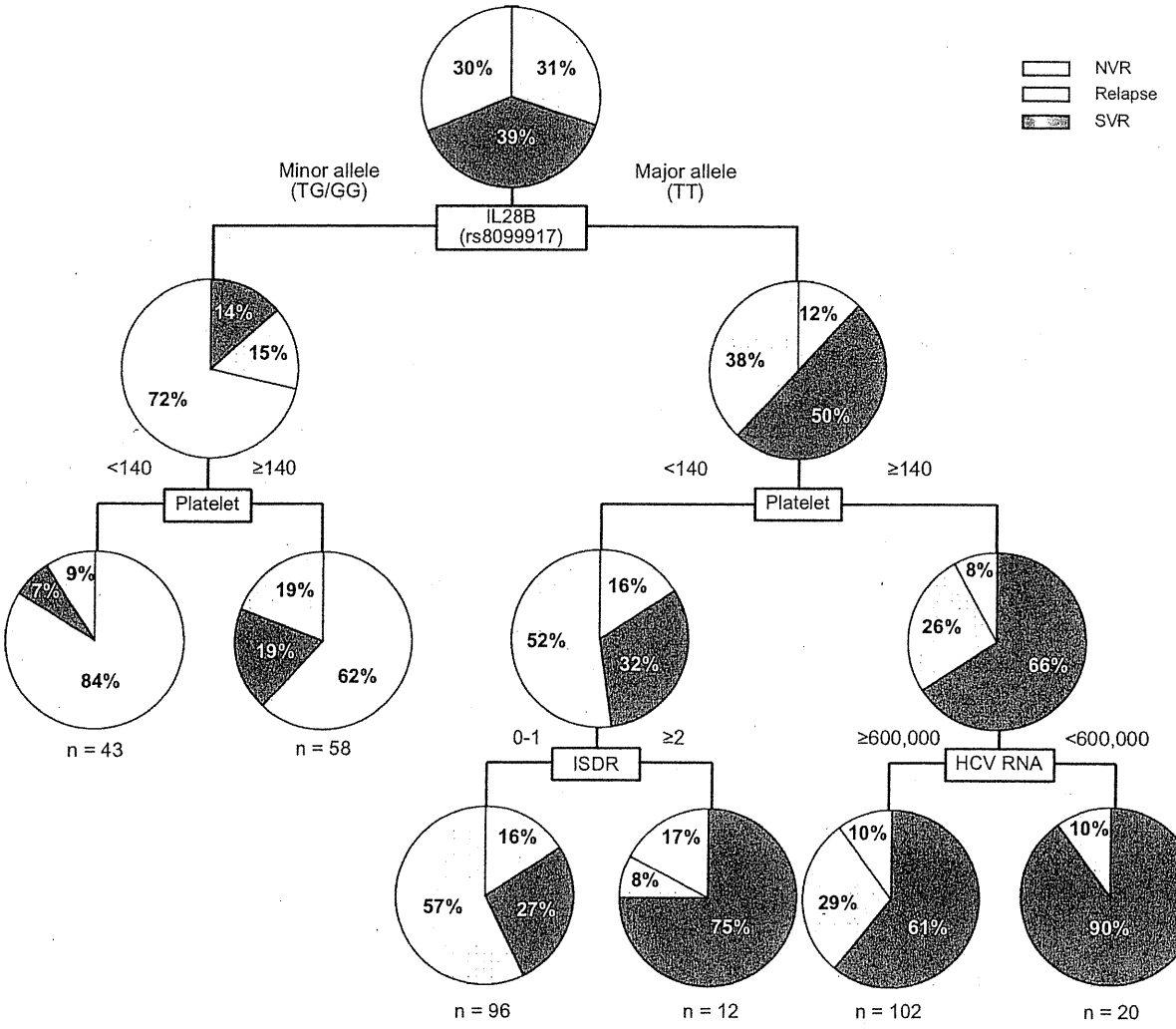

Fig. 5. Decision tree for the prediction of response to therapy. The boxes indicate the factors used for splitting. Pie charts indicate the rate of response for each group of patients after splitting. The rate of null virological response, relapse, and sustained virological response is shown.

estimate of the response before treatment, by simply allocating patients to subgroups by following the flow-chart form, which may facilitate clinical decision making. This is in contrast to the calculating formula, which was constructed by the traditional logistic regression model. This was not widely used in clinical practice as it is abstruse and inconvenient. These results support the evidence based approach of selecting the optimum treatment strategy for individual patients, such as treating patients with a low probability of NVR with current PEG-IFN/RBV combination therapy or advising those with a high probability of NVR to wait for more effective future therapies. Patients with a high probability of relapse may be treated for a longer duration to avoid a relapse. Decisions may be based on the possibility of a response against a potential risk of adverse events and the cost of the therapy, or disease progression while waiting for future therapy.

We have previously reported the predictive model of early virological response to PEG-IFN and RBV in chronic hepatitis C [26]. The top factor selected as significant was the grade of steatosis, followed by serum level of LDL cholesterol, age, GGT, and blood sugar. The mechanism of association between these factors and treatment response was not clear at that time. To our interest, a recent study by Li et al. [47] has shown that high serum level of LDL cholesterol was linked to the *IL28B* major allele (CC in rs12979860). High serum level of LDL cholesterol was associated with SVR but it was no longer significant when analyzed together with the *IL28B* genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels may reflect the underlining link of LDL cholesterol levels to *IL28B* genotype. Steatosis is reported to be correlated with low lipid levels [48] which suggest that *IL28B* genotypes may be also associated with steatosis. In fact, there were significant correlations between the *IL28B* genotype and the presence of steatosis in the present study (Table 4). In addition, the serum level of GGT, another predictive factor in our previous study, was signif-

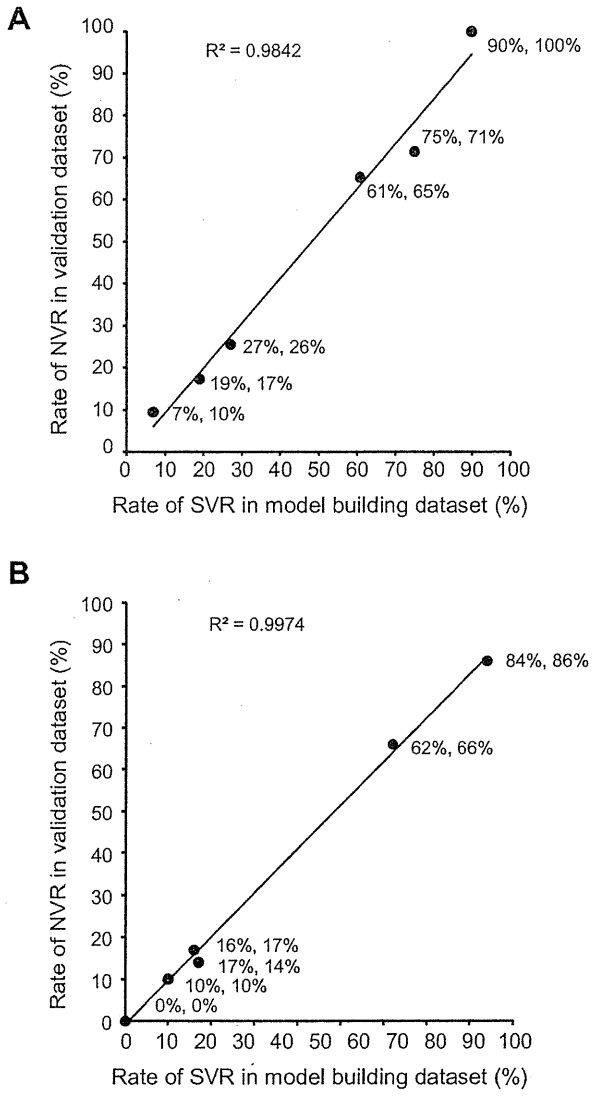

Fig. 6. Validation of the CART analysis. Each patient in the validation group was allocated to one of the six subgroups by following the flow-chart form of the decision tree. The rate of (A) sustained virological response (SVR) and (B) null virological response (NVR) in each subgroup was calculated and plotted. The *X*-axis represents the rate of SVR or NVR in the model building patients and the *Y*-axis represents those in the validation patients. The rate of SVR and NVR in each subgroup of patients is closely correlated between the model-building and the validation patients (correlation coefficient: $r^2 = 0.98–0.99$).

icantly associated with *IL28B* genotype in the present study (Table 4). The serum level of GGT was significantly associated with NVR when examined independently but was no longer significant when analyzed together with the *IL28B* genotype. These observations indicate that some of the factors that we have previously identified may be associated with virological response to therapy through the underlining link to the *IL28B* genotype.

In conclusion, the present study highlighted the impact of the *IL28B* polymorphism and mutation in the ISDR on the pre-treatment prediction of response to PEG-IFN/RBV therapy. A decision model including these host and viral factors has the potential to support selection of the optimum treatment strategy for individual patients, which may enable personalized treatment.

Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support

This study was supported by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan, (H19-kannen-013), (H20-kannen-006).

References

[1] Ray Kim W. Global epidemiology and burden of hepatitis C. Microbes Infect 2002;4 (12):1219–1225.
[2] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347 (13):975–982.
[3] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358 (9286):958–965.
[4] Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, et al. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48 (6):372–380.
[5] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38 (3):645–652.
[6] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;10:1105–1109.
[7] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;10:1100–1104.
[8] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461 (7262):399–401.
[9] Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138 (4):1338–1345.
[10] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96 (1):224–230.
[11] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334 (2):77–81.
[12] Kurosaki M, Enomoto N, Murakami T, Sakuma I, Asahina Y, Yamamoto C, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology 1997;25 (3):750–753.
[13] Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 2008;48 (6):1753–1760.
[14] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46 (3):403–410.

Research Article

[15] Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, et al. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009;44 (9):952–963.

[16] Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. Stat Med 1989;8 (5):539–550.

[17] LeBlanc M, Crowley J. A review of tree-based prognostic models. Cancer Treat Res 1995;75:113–124.

[18] Garzotto M, Beer TM, Hudson RG, Peters L, Hsieh YC, Barrera E, et al. Improved detection of prostate cancer using classification and regression tree analysis. J Clin Oncol 2005;23 (19):4322–4329.

[19] Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: implications on the future of cancer staging. Surgery 2002;132 (4):589–602.

[20] Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the german dermatological society. J Clin Oncol 2004;22 (18):3660–3667.

[21] Valera VA, Walter BA, Yokoyama N, Koyama Y, Iiai T, Okamoto H, et al. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. Ann Surg Oncol 2007;14 (1):34–40.

[22] Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. Clin Cancer Res 2005;11 (15):5440–5443.

[23] Thabane M, Simunovic M, Akhtar-Danesh N, Marshall JK. Development and validation of a risk score for post-infectious irritable bowel syndrome. Am J Gastroenterol 2009;104 (9):2267–2274.

[24] Wu BU, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. Gut 2008;57 (12):1698–1703.

[25] Fonarow GC, Adams Jr KF, Abraham WT, Yancy CW, Boscardin WJ. Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree analysis. Jama 2005;293 (5):572–580.

[26] Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, et al. A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. Hepatol Res 2010;40 (3):251–260.

[27] Nishida N, Tanabe T, Takasu M, Suyama A, Tokunaga K. Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. Anal Biochem 2007;364 (1):78–85.

[28] Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360 (18):1839–1850.

[29] McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360 (18):1827–1838.

[30] Rossignol JF, Elfert A, El-Gohary Y, Keeffe EB. Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. Gastroenterology 2009;136 (3):856–862.

[31] Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kotenko SV, MacDonald MR, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology 2006;131 (6):1887–1898.

[32] Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol 2005;79 (6):3851–3854.

[33] McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, et al. Replicated association between an IL28B Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin. Gastroenterology 2010;138:2307–2314.

[34] Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. A-interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. Hepatol Res 2010;40:449–460.

[35] Backus LI, Boothroyd DB, Phillips BR, Mole LA. Predictors of response of US veterans to treatment for the hepatitis C virus. Hepatology 2007;46 (1):37–47.

[36] Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, et al. Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol 2009;81 (4):640–649.

[37] Hung CH, Lee CM, Lu SN, Lee JF, Wang JH, Tung HD, et al. Mutations in the NS5A and E2-PePHD region of hepatitis C virus type 1b and correlation with the response to combination therapy with interferon and ribavirin. J Viral Hepat 2003;10 (2):87–94.

[38] Yen YH, Hung CH, Hu TH, Chen CH, Wu CM, Wang JH, et al. Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209–2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. Aliment Pharmacol Ther 2008;27 (1):72–79.

[39] Zeuzem S, Lee JH, Roth WK. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 1997;25 (3):740–744.

[40] Squadrito G, Leone F, Sartori M, Nalpas B, Berthelot P, Raimondo G, et al. Mutations in the nonstructural 5A region of hepatitis C virus and response of chronic hepatitis C to interferon alfa. Gastroenterology 1997;113 (2):567–572.

[41] Sarrazin C, Berg T, Lee JH, Teuber G, Dietrich CF, Roth WK, et al. Improved correlation between multiple mutations within the NS5A region and virological response in European patients chronically infected with hepatitis C virus type 1b undergoing combination therapy. J Hepatol 1999;30 (6):1004–1013.

[42] Murphy MD, Rosen HR, Marousek GI, Chou S. Analysis of sequence configurations of the ISDR, PKR-binding domain, and V3 region as predictors of response to induction interferon-alpha and ribavirin therapy in chronic hepatitis C infection. Dig Dis Sci 2002;47 (6):1195–1205.

[43] Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, et al. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. Gut 2004;53 (9):1345–1351.

[44] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461 (7265):798–801.

[45] Kurosaki M, Enomoto N, Marumo F, Sato C. Evolution and selection of hepatitis C virus variants in patients with chronic hepatitis C. Virology 1994;205 (1):161–169.

[46] Enomoto N, Kurosaki M, Tanaka Y, Marumo F, Sato C. Fluctuation of hepatitis C virus quasispecies in persistent infection and interferon treatment revealed by single-strand conformation polymorphism analysis. J Gen Virol 1994;75 (Pt 6):1361–1369.

[47] Li JH, Lao XQ, Tillmann HL, Rowell J, Patel K, Thompson A, et al. Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. Hepatology 1904;51 (6):1904–1911.

[48] Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol 2001;34 (3):428–434.

Dig Dis Sci (2011) 56:2482–2490
DOI 10.1007/s10620-011-1622-1

ORIGINAL ARTICLE

Donor Hepatectomy for Living Donor Liver Transplantation: Learning Steps and Surgical Outcome

Shigeru Marubashi · Hiroaki Nagano · Hiroshi Wada · Shogo Kobayashi · Hidetoshi Eguchi · Yutaka Takeda · Masahiro Tanemura · Yuichiro Doki · Masaki Mori

Received: 8 December 2010 / Accepted: 6 February 2011 / Published online: 22 February 2011

Abstract
Background and Aim Complications associated with live liver donor surgery should be minimized. There is little information on the impact of team experience and learning on the surgical outcome. The aim of this study was to clarify the impact of team experience in a single center on the outcome of live donor hepatectomy.
Methods Graft livers consisted of 56 right lobes, 40 left lobes with/without caudate, 36 left lateral section (LLS), and 11 right posterior section (RPS). Surgeries were divided according to the time of execution: era I ($n = 50$), era II ($n = 50$) and era III ($n = 43$).
Results No postoperative mortality was recorded. Blood loss steadily decreased and operation time decreased after era II ($P < 0.0001$). The overall frequency of postoperative morbidities by the Clavien system was significantly less for LLS graft [$P = 0.009$, right lobe (42.9%) vs. LLS (13.9%)]. Multivariate risk factor analysis showed that donors in recent years were at low risk of morbidity and bile leakage ($P = 0.025$ and 0.010, respectively). There was less impact for team experience on the outcome in LLS graft than other types of grafts.
Conclusion Our analysis demonstrated several learning steps in live liver donor surgery and confirmed their positive impact on surgical outcome.

Keywords Living donor · Liver transplant · Hepatectomy · Postoperative morbidity · Surgical experience

S. Marubashi (✉) · H. Nagano · H. Wada · S. Kobayashi · H. Eguchi · Y. Takeda · M. Tanemura · Y. Doki · M. Mori
Department of Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita City 565-0871, Osaka, Japan
e-mail: smarubashi@gesurg.med.osaka-u.ac.jp

Abbreviations

BMI	Body mass index
DIC-CT	Drip-infusion cholangiography computed tomography
LDLT	Living donor liver transplantation
LLD	Live liver donor
MHV	Middle hepatic vein
MD-CT scan	Multi-detector row-computed tomography scan
POD	Postoperative day
PT-INR	Prothrombin time - international normalized ratio
SLV	Standard liver volume

Introduction

Since the first pediatric living donor liver transplantation (LDLT) in 1989 [1, 2], the procedure has been successfully developed and applied to adult-to-adult LDLT. Organ shortage due to limited availability of cadaveric donors in Japan as well as other Asian countries necessitates this trend, although the risk of donor hepatectomies in living donors should not be overlooked. In Japan, more than 5,000 living donor liver transplantations have been performed since December 2009 [3].

Donor hepatectomy can usually be well planned with intensive preoperative work-up including multidetector computed tomography (MDCT) and drip infusion cholangiography-computed tomography (DIC-CT), and the surgical techniques have been standardized [4]. In addition, the donor must be a healthy individual and liver function should be normal before the donation. Nevertheless, zero-mortality of living donor is not achievable because of the complexity of the treatment. Umeshita et al. [5] reported

that the living donor morbidity rate was 12.4% and increased to 19.0% among right lobe living donors. Hashikura et al. [3] subsequently reported lower donor morbidities with further experience in Japan. Nevertheless, one operative mortality has been reported in Japan [6] and several from other countries [7–9]. The latest morbidity rate in donor hepatectomy is 8.4% for all living donors and 9.4% for right lobe living donors [3].

While donor hepatectomy is a standardized surgery, it requires special care for both living donor and resected allograft. Therefore, donor hepatectomy should be performed by the most skilled and experienced hepatobiliary surgeons. We started donor hepatectomy in 1998. Since then, a total of 143 donor hepatectomies had been performed by November 2009 using a uniform policy. This included, for example, no use of inflow occlusion during donor hepatectomy, and no use of metallic clips inside the donor abdomen. There is also a general belief that the incidence of donor morbidities started to decrease with accumulated experience. The aim of the present study was to evaluate the importance of team experience on the outcome of LDLT by evaluating various operative parameters, morbidity graded by Clavien Dindo classification [10], and improvement with experience.

Patients and Methods

The study protocol was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine. A signed consent form was obtained from each donor before surgery.

Donors

We analyzed the results of 143 consecutive LDLT performed between 1998 and November 2009 at Osaka University. The donors comprised 98 males and 45 females, with a mean age of 38.6 ± 11.7 years (±SD). Furthermore, 94 recipients were adults (>18 years) and 47 recipients were children (≤18 years). One adult and one pediatric recipient each received a second LDLT due to graft failure. Donor graft was selected based on volumetric analysis and anatomical feasibility. Consequently, 56 right lobes, 40 left lobes, 11 right posterior sections, and 36 left lateral sections were selected and harvested.

Donor Evaluation

Donor evaluation was based on the criteria approved by the ethics review committee of Osaka University. All living liver donors were adults of ≤65 years of age. Donor candidates with systemic disease such as hypertension, diabetes mellitus, psychiatric disease, or were using medications for any systemic disease were strictly rejected. Preoperative evaluation consisted of complete history and physical examination, and laboratory tests (complete blood count, blood chemistry, coagulation factors, hepatitis B virus, hepatitis C virus, and serological profiles for other infectious diseases). Donors also underwent chest and abdominal radiography, four-phase MD-CT and DIC-CT with three-dimensional reconstruction. Liver volumetric analysis was conducted routinely using the Virtual Place software version 2.0 (AZE, Tokyo, Japan).

Graft Selection

The graft type was basically determined by the results of the volumetric study. The requirements for living donation were (1) an estimated volume of the remnant liver of more than 35% of donor whole liver volume and (2) an estimated donor graft liver volume of more than 40% of the recipient's standard liver volume (SLV). The anatomy of the donor liver (artery, portal vein, hepatic vein, and bile duct) was always taken into consideration when selecting the graft. Multiple and small arteries or portal veins for reconstruction are relative contraindication in selecting the graft [11].

The basic rule followed for graft selection in adult-to-adult LDLT is shown in Fig. 1. We first considered the left lobe without or with the caudate lobe. If it did not fulfill the criteria (1) and (2) above, we then selected the right lobe without the middle hepatic vein. If the right lobe also did not fulfill the criteria, we then considered the right posterior section after referring to the findings of various imaging studies.

Donor Surgery

All donor surgery was planned and simulated on the MDCT and DIC-CT scans prior to surgery. However, DIC-CT was not performed preoperatively in eight emergency cases with fulminant hepatic failure. In elective surgery, autologous blood (800 ml) was collected routinely under standard protocol 2 weeks prior to surgery.

During the study period (1998–2009), donor surgery was performed in our department by four expert staff surgeons of equal expertise in hepatic-biliary surgery. Each surgery was conducted by two surgeons, the primary surgeon assisted by another surgeon. In 1998, the donor surgery was conducted by the first primary surgeon assisted by another surgeon, until the former moved for other duties, at which time the assistant surgeon became the primary surgeon and conducted the surgery with another hepatic-biliary assistant surgeon. This change of surgeons/roles continued with time to finally include four surgeons within the study period. Thus, all surgeons had equal share in acting as the primary surgeon and assistant surgeon.

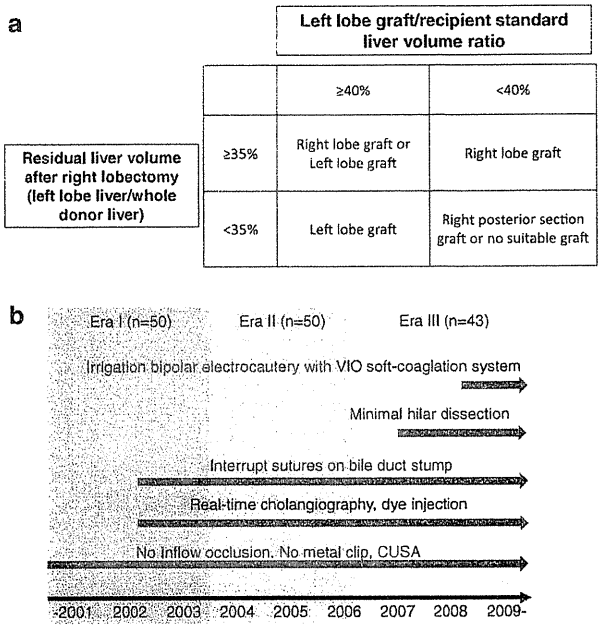

Fig. 1 a Criteria used for graft selection in adult-to-adult LDLT in our institution based on volumetric analysis. The criteria for living donation were (1) an estimated volume of the remnant liver of more than 35% of donor whole liver volume, and (2) an estimated donor graft liver volume of more than 40% of the recipient's standard liver volume (SLV). b Technical evolution of donor hepatectomy according to the three Eras

Modifications to the donor surgery protocol were discussed and agreed by the team and executed thereafter by all surgeons as illustrated on Fig. 1b. Donor surgery was conducted initially under general and epidural anesthesia, then modified in 2008 to general anesthesia to avoid possible neural injury associated with epidural anesthesia.

In all 143 cases, the technique of parenchymal dissection was applied, using an ultrasonic dissector (CUSA, Tyco Healthcare, Tokyo), without inflow occlusion. No metallic clip was used during dissection of the parenchyma to avoid any interference with the evaluation of abdominal CT scan after surgery. The stump of the bile duct was closed in monofilament running sutures in the early surgeries but subsequently changed in 2002 to interrupted sutures because of the high incidence of bile leakage after donor surgery. Real-time cholangiography of the bile duct was introduced in 2002, and dye injection via the cystic duct at the end of dissection [12] commenced in 2002. Most recently, the dissection of hilar structures was minimized; limiting the dissection to the cut line around the portal vein, artery, and bile duct from 2007, and the energy device used for parenchymal hemostasis was changed from conventional monopolar electrocautery to irrigation bipolar electrocautery with VIO soft-coagulation system [13] from 2008 (Fig. 1b).

After securing the hepatic artery and portal vein at the cut point, the hemi-liver was mobilized. The cholangiogram was repeated twice, when necessary, for accurate recognition of bile duct anatomy, before liver resection and at the time of cutting the bile duct after parenchymal resection. The liver anatomy was confirmed constantly during parenchymal resection by ultrasonography. In grafting the right lobe without the MHV, tributaries of the MHV larger than 5 mm in diameter were carefully saved for later anastomosis by auto-vein graft. A Penrose drain tube was used to lift the parenchyma, a procedure helpful in dissecting the tissue close to the inferior vena cava [14]. In hepatectomy involving the left and the caudate lobes, drainage veins with diameters ≥5 mm were preserved in the caudate to be later used in reconstruction in the recipient surgery.

After intravenous administration of 1,500 units heparin sodium, the bile duct, hepatic artery, portal vein, and hepatic veins were cut and the graft liver was removed and flushed with the University of Wisconsin colloid-based preserving solution. The bile duct stump was closed with 4-0 absorbable monofilament in running sutures until supplanted with interrupted sutures using 6-0 absorbable monofilament after 2004. After complete hemostasis, 10-ml indigo carmine solution was injected via the cystic duct tube into the biliary system. When dye leakage was identified, additional monofilament sutures were placed and the dye injection was repeated to confirm the leakage was fixed. Furthermore, Seprafilm® (Kaken Pharm. Co., Tokyo) was used to prevent adhesion of the stomach to the cut-surface of the left lobectomy or left lateral sectionectomy. One or two drains were placed at the Winslow's foramen or cut surface of the liver. Operative time of donor surgery represented technically working time and excluded any waiting/holding time before the start and during recipient surgery.

Postoperative Management and Care

After donor surgery, the donors were moved to the general ward and vital signs were monitored for 2 days. Oral intake usually started on postoperative day 1. Drains were removed at postoperative days (POD) 3–5 according to the volume and condition of the drainage. Bile leakage represented the presence of bile leak from the drainage tube when inspected on POD8 or direct identification of bile during exploratory laparotomy conducted before POD8.

Postoperative Morbidities and Evaluation of Donor Surgery

Postoperative morbidities were recorded according to the grading system used by Clavien et al. [10]. Differences in the clinical background of living donors, operation time,

blood loss during surgery, graft types and postoperative morbidities according to graft type and throughout the postoperative course were compared. The time course was divided into three bins of eras: era I, case nos. 1–50 (1998–2003); era II, case nos. 51–100 (2004–2006); and era III, case nos. 100–143 (2006–2009).

Statistical Analysis

Continuous data were expressed as mean ± SD. Differences between groups were analyzed for statistical differences by the Student's t test or Mann–Whitney U test. Categorical data were presented as percentages, and differences between proportions were compared using the chi-square test. Univariate and multivariate analyses of risk factors for postoperative morbidities and bile leakage were performed using logistic regression. A P value less than 0.05 was considered significant.

Results

Clinical Findings

There was no postoperative mortality among the 143 living donors. No allogenic transfusion was used during the peri- and postoperative course and all donors are alive and in healthy condition. The background characteristics of the liver donors including age, gender, body mass index of the four graft groups were similar with respect to the type of graft (Table 1). The graft liver weight was significantly larger for the right lobe (677 ± 102 g) than other graft types ($P < 0.0001$).

Experience and Operation Time

The operation time tended to decrease with the increase in case number (Fig. 2a); it was almost constant in eras I and II, then decreased significantly in era III for the right ($P < 0.0001$ era II vs. III) and left lobe grafts ($P = 0.0005$ era II vs. III) (Fig. 2b). On the other hand, there was no difference in operation time between era I and era II for all graft types or between era II and era III for the left lateral section and right posterior section grafts (Fig. 2b).

Experience and Intraoperative Blood Loss

Blood loss during surgery also decreased steadily with further gains in experience (Fig. 3a). Blood loss was the most markedly reduced in right lobe graft surgery between era I and era II ($P = 0.009$). Blood loss tended to decrease with gain in experience, with the exception of the right posterior section graft, where blood loss tended to increase slightly in recent cases, although the difference was not significant (Fig. 3b).

Effect of Donation on Liver Function Tests

The results of liver function tests performed postoperatively are shown in Fig. 4a–d. Serum bilirubin reached a peak level at day 1 and tended to be higher in donors with right lobectomy than other types of grafts, especially when compared with donors of the left lateral graft, and remained slightly elevated throughout the postoperative period ($P < 0.0001$, POD1) (Fig. 4a). Changes in prothrombin time (PT-INR) showed a similar pattern; the level was higher in donors of the right lobe graft than in donors of other grafts ($P = 0.0004$, right lobe vs. left lobe;

Table 1 Donors' characteristics

Characteristics	Right lobe $n = 56$	Left lobe with/without caudate $n = 40$	Right posterior section $n = 11$	Left lateral section $n = 36$
Age (years)	38.6 ± 13.8	40.3 ± 11.6	42.2 ± 13.3	34.9 ± 6.6
Gender (male/female)	37/19	32/8	8/3	21/15
Body weight (kg)	62.6 ± 9.8	66.6 ± 10.2	67.3 ± 8.9	60.8 ± 11.1
Body height (cm)	166.6 ± 9.6	168.8 ± 8.3	167.8 ± 7.5	164.6 ± 8.8
Body mass index (kg/m^2)	22.6 ± 2.8	23.3 ± 2.9	23.8 ± 2.1	22.3 ± 3.0
Graft weight (g)	677 ± 102	473 ± 82	499 ± 82	255.2 ± 45.3
Graft weight/recipient weight ratio (GWRW)	1.02 ± 0.22	0.79 ± 0.24	0.86 ± 0.18	2.99 ± 1.03
Operative time (min)	435 ± 85	419 ± 64	454 ± 57	346 ± 65
Blood loss (ml)	765 ± 657	584 ± 403	889 ± 534	584 ± 403
Autologous blood transfusion (%)	0	0	0	0
Duration of hospitalization (days)	24.8 ± 18.2	21.5 ± 22.9	22.6 ± 11.8	15.3 ± 4.9

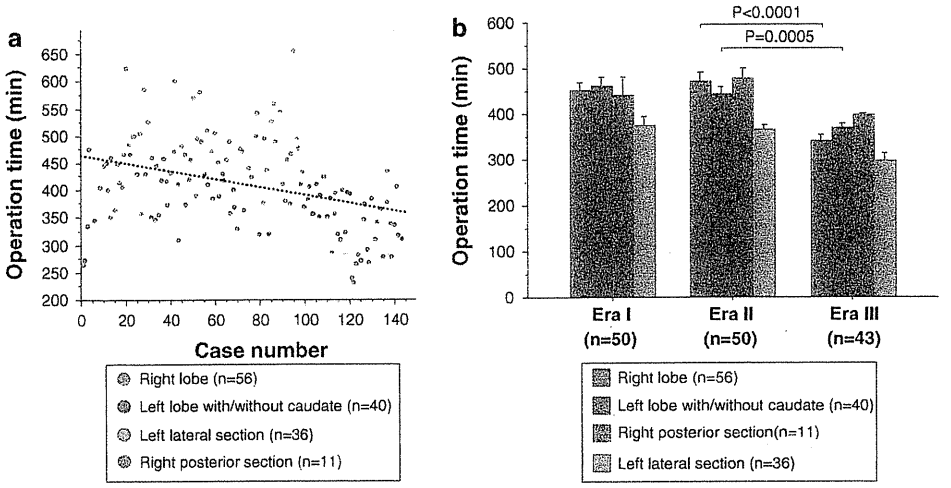

Fig. 2 Changes in operation time with gained experience. **a** Operation time decreased with increased case numbers of living liver donors [$y = -0.727 \times$ (case number) + 463.7, $r^2 = 0.134$]. **b** Operation time according to the time of surgery (era I: 1998–2003, era II: 2004–2006, era III: 2006–2009). Improvements were noted from era II to era III in right lobe graft ($P < 0.0001$), and in left lobe with/without caudate ($P = 0.0005$). Data are mean ± standard deviation (SD)

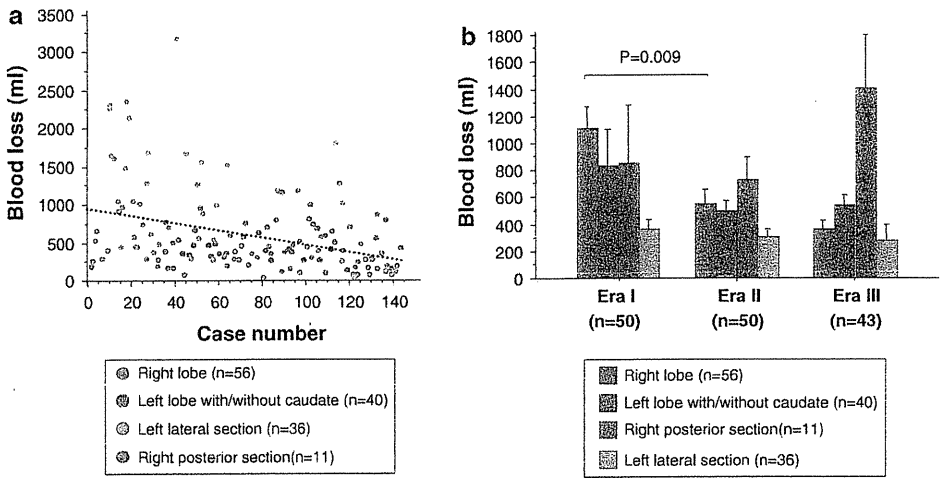

Fig. 3 Changes in blood loss during surgery with gained experience. **a** Blood loss during surgery decreased with increased case numbers of living liver donors [$y = -4.748 \times$ (case number) + 954.1, $r^2 = 0.135$]. **b** Blood loss during surgery according to the time of surgery (era I: 1998–2003, era II: 2004–2006, era III: 2006–2009). A significant decrease in blood loss was noted from era I to era II in right lobe graft ($P = 0.009$). Data are mean ± standard deviation (SD)

$P < 0.0001$, right lobe vs. left lateral section; $P = 0.013$, right lobe vs. right posterior section, POD1) (Fig. 4b). Interestingly, the level of aspartate aminotransferase (AST) was elevated in donors of the left lateral section and right posterior section grafts than those of right and left lobe grafts ($P = 0.024$, left lateral section vs. left lobe; $P = 0.001$, left lateral section vs. right lobe; $P = 0.005$, right posterior section vs. left lobe; $P = 0.0001$, right posterior section vs. right lobe, POD1) (Fig. 4c). Similar findings were noted in alanine aminotransferase (ALT) (Fig. 4d). The results of liver function tests were not different among the three Eras for each graft type (data not shown).

Complications Associated with Donor Surgery

The incidence of postoperative morbidities including Clavien grade I was 30.8% ($n = 44$) for all donors, 42.9% ($n = 24$) for right lobe, 27.5% ($n = 11$) for left lobe, 36.4% ($n = 4$) for right posterior section, and 13.9% ($n = 5$) for donors of the left lateral section. There was no significant difference in the incidence of morbidities according to graft type, except that they were significantly higher in right lobe graft donors than in left lateral section graft donors ($P = 0.009$). Morbidities with Clavien grade over II was noted in 28 donors (19.6%), including Clavien grade IIIa in 24 donors (16.8%) and grade IIIb in two donors (1.4%). Morbidities with Clavien grade over II according to the graft type are shown in Fig. 5a. Bile leak was noted in 13 (9.1%) donors, and was the most frequent morbidity among Clavien grade IIIa and IIIb complications. The frequency of morbidities steadily decreased with time (Eras I, II and III), including the incidence of bile leak (Table 2, Fig. 5b, c).

Postoperative complications in two donors (Grade 3b) were due to bile leak ($n = 1$) and portal vein thrombosis

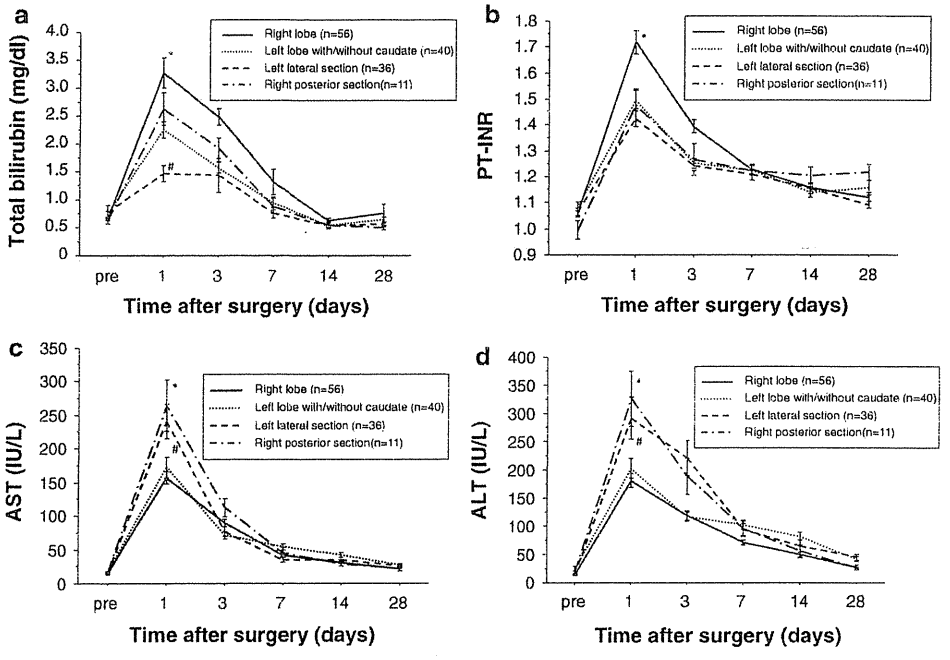

Fig. 4 Changes in liver function tests after donor surgery according to type of liver graft. **a** Serum total bilirubin levels before and after surgery. $^{*}P < 0.0001$ (right lobe vs. left lateral section), $P = 0.003$ (right lobe vs. left lobe). $^{\#}P = 0.0003$ (left lateral section vs. right posterior section), $P = 0.0002$ (left lateral section vs. left lobe). **b** PT-INR before and after operation. $^{*}P < 0.0001$ (right lobe vs. left lateral section), $P = 0.0004$ (right lobe vs. left lobe), $P = 0.013$ (right lobe vs. right posterior section). **c** Serum aspartate aminotransferase (AST) levels before and after surgery. $^{*}P = 0.0005$ (left lateral section vs. right lobe), $P = 0.022$ (left lateral section vs. left lobe). $^{\#}P = 0.0001$ (right posterior section vs. right lobe), $P = 0.012$ (right posterior section vs. left lobe). **d** Serum alanine amino transferase (ALT) levels before and after surgery. $^{*}P = 0.001$ (left lateral section vs. right lobe), $P = 0.024$ (left lateral section vs. left lobe). $^{\#}P = 0.0001$ (right posterior section vs. right lobe), $P = 0.005$ (right posterior section vs. left lobe). Data are mean ± standard deviation (SD)

($n = 1$). Both patients required emergency laparotomy at POD1 and the problems were fixed without any further complications. These two donors were discharged on POD20 and POD31.

Uni- and Multi-Variate Analyses of Factors Associated with Postoperative Morbidity

Univariate logistic regression analysis showed that early era ($P = 0.0007$), graft type (right lobe vs. left lateral section, $P = 0.005$), amount of blood loss ($P = 0.011$), and longer operation time ($P = 0.005$) were risk factors for postoperative morbidity, while age, gender, weight, and BMI were not associated with postoperative morbidity (Table 3). Multivariate logistic regression analysis of these factors showed that only early era was an independent risk factor ($P = 0.025$) for postoperative morbidity (Table 3).

Comparative analysis of donors with bile leak ($n = 13$) and those without ($n = 130$) showed that more recent cases had lower risk of bile leak after surgery ($P = 0.010$), while age, gender, weight, BMI, graft type, operation duration, blood loss, and graft weight were not different between the two groups.

Discussion

Live donor morbidity and mortality are basically inevitable. The reports of deaths of live donors associated with donor surgery in several institutions both in Japan and western countries [6–9] prompted extensive discussion of the ethics and merits of live donation [15, 16]. Nevertheless, LDLT is still needed for selected patients in certain circumstances especially in Japan, where cadaveric organ transplantation is still very limited; although, an increase of cadaveric donation is expected in the future due to the recent approval (July 2009) of the revised bill of Organ Transplant Law by the Japanese Government.

The principle of our practice is to go first through extensive preoperative work-up for donor candidates, so as not to miss any contraindication for live donation, and then to give the best practice for the donor before, during, and after surgery. We have gained vast experience and knowledge about donor surgery and care, and witnessed a progressive improvement in the surgical outcome and postoperative clinical course. In this regard, only a few other studies described improvement of outcome of donor surgery [17], and to our knowledge, there is no study to

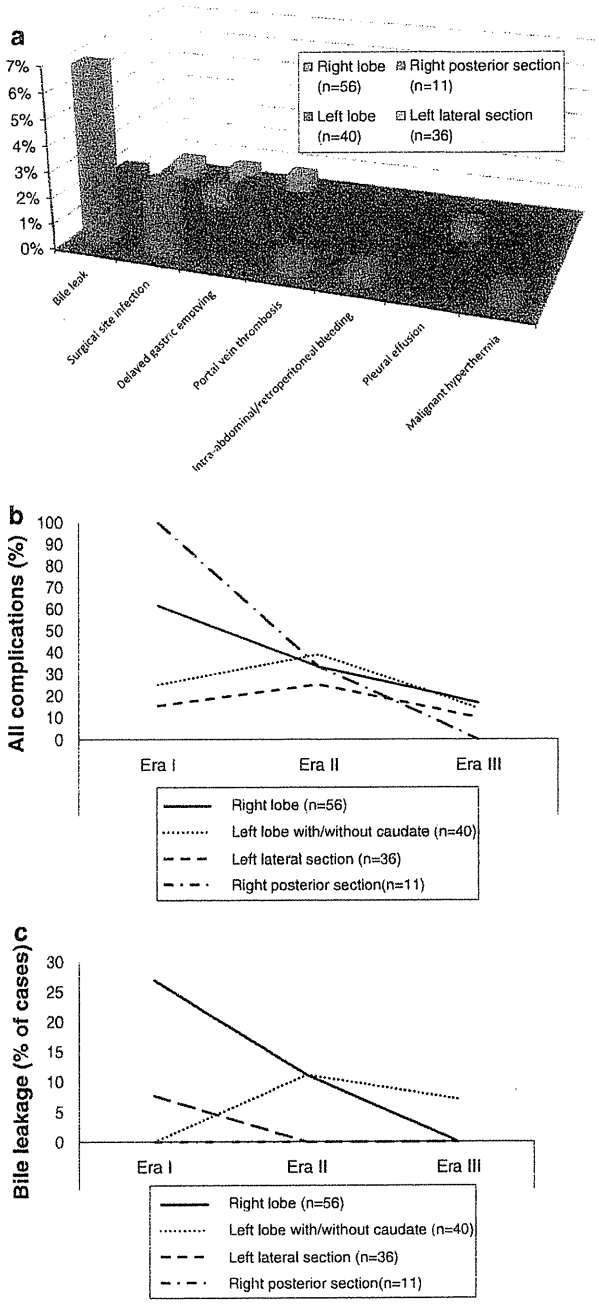

Fig. 5 Frequency of complications after living liver donor surgery. **a** Morbidities with Clavien grade over II according to the graft type. **b** Percentage of all complications after donor surgery according to the time of surgery. **c** Percentage of bile leakage after donor surgery according to the time of surgery (era I: 1998–2003, era II: 2004–2006, era III: 2006–2009)

date that has compared the donor surgical outcome according to the type of graft (right lobe, left lobe, right lateral section, and left lateral section) in LDLT.

Assessment of postoperative liver function serves to identify the potential risk of graft failure and other postoperative complications. In the present study, the results of liver function tests showed increased levels of serum bilirubin and PT-INR after right lobe surgery, and a larger increase in transaminases in left lateral section and right posterior section surgeries. These results indicate that selection of grafts other than the right lobe could spare the donor any postoperative rise in serum bilirubin, while parenchymal injury, represented by high levels of serum transaminases, was more severe in donors of the left lateral section or right posterior section graft. The high transaminase in donors of the left lateral section is probably due to ischemia of the left medial section followed by tissue atrophy, since the inflow to this area is sacrificed following preservation of inflow to the left lateral graft. On the other hand, after removal of the right posterior section, the right anterior sector becomes congested due to reduced flow in the right hepatic vein, resulting in rises in serum transaminases. Thus, it is important to recognize changes in these laboratory data since they reflect various physiopathological phenomena.

One of the important findings of this study was the progressive improvement in the operative outcome, as reflected by operation time, blood loss, and morbidity rate. Interestingly, intraoperative blood loss diminished significantly in the second 50 cases (era II), though operation time did not change. However, operation time improved after era II. Exceptions to the progressive improvement of surgical outcome were the stable and short operation time, low blood loss and morbidity rate in left lateral sectionectomy; these parameters were almost stable from Eras I to III.

In our hands, postoperative morbidity improved progressively with experience. Bile leakage was the most frequent complication in this series. We have so far introduced several techniques to handle bile duct leakage, including real-time cholangiography during donor surgery, the technique used to close the bile duct stump, dye injection via the cystic duct, and minimizing the dissection of hilar structures. Several surgical techniques are available for closure of the bile duct stump. We changed the method from running sutures with 4-0 absorbable monofilament to interrupted sutures with 6-0 absorbable monofilament. Ligation of the bile duct stump is one of the choices, but it is not recommended because the bile duct in the graft becomes too short to anastomose duct-to-duct biliary reconstruction on the recipient side. It is possible that one or more of these techniques contributed to the improvement in surgical outcome, although in general, the most significant parameter associated with the reduced rate of bile leakage was the era of surgery, i.e., the experience of the surgical team.

With regard to the surgical outcome of donor surgery, there was a substantial learning curve to achieve qualified surgery for right and left lobe graft, while there was little improvement in right posterior section graft and left lateral

Table 2 Morbidities encountered in living donors according to graft type

Grade/ incidence	Right lobe ($n = 56$)			Left lobe with/without caudate ($n = 40$)			Right posterior section ($n = 11$)			Left lateral section ($n = 36$)		
	Era I	Era II	Era III	Era I	Era II	Era III	Era I	Era II	Era III	Era I	Era II	Era III
n	26	18	12	8	18	14	3	6	2	13	8	15
I	4	3	1	2	2	–	2	–	–	1	1	–
II	1	–	–	–	1	–	–	–	–	–	–	–
IIIa	11	2	1		4	1	1	1	–	1	1	1
IIIb	–	1	–	–	–	1	–	–	–	–	–	–
IV, V	–	–	–	–	–	–	–	–	–	–	–	–
Incidence	61.5%	33.3%	16.7%	25%	38.9%	14.3%	100%	33.3%	0%	15.4%	25%	6.7%
Total incidence	42.9%			27.5%			36.4%			13.9%		

Table 3 Risk factors for postoperative complications

Risk factors	Donors without complications ($n = 99$)	Donors with complications ($n = 44$)	*P* (Logistic regression)	OR	95% CI	*P* (Multivariate, logistic regression)
Age (years)	38.1 ± 12.0	39.0 ± 11.5	0.654	1.007	(0.978, 1.037)	
Gender						
M/F	64/35	10/34	0.137	1.859	(0.821, 4.202)	
Weight (kg)	63.0 ± 10.2	65.3 ± 10.5	0.234	1.022	(0.986, 1.060)	
Body mass index (kg/m^2)	22.6 ± 2.6	23.3 ± 3.2	0.238	1.080	(0.949, 1.234)	
Era						
I	27	23				
II	34	16	0.153	0.552	(0.245, 1.247)	0.609
III	38	5	0.0007	0.155	(0.052, 0.457)	0.025
Operation time (min)	397 ± 83.0	441 ± 69.9	0.005	1.007	(1.002, 1.012)	0.845
Blood loss (g)	525 ± 500.3	794 ± 569.1	0.011	1.001	(1.000, 1.002)	0.373
Graft type						
Left lateral section	31	5				
Left lobe with/without caudate	29	11	0.153	2.353	(0.728, 7.58)	0.243
Right lobe	32	24	0.005	4.65	(1.57, 13.7)	0.079
Right posterior section	7	4	0.11	3.546	(0.751, 16.7)	0.330

OR odds ratio, *CI* confidence interval

sectionectomy. Clinical outcome of the left lateral section was good from the beginning, while that of the right posterior section could be improved with more experience in this type of graft. Therefore, we recommend that surgical teams with limited experience (<50 cases) should start conducting donor hepatectomy with left lateral sectionectomy, then shift to any type of donor surgery/graft after gaining sufficient experience (>100 donor surgeries).

Of course, all efforts should be employed to reduce complications in the donors. After gaining experience between 1998 and 2009, we anticipate better management and improved outcome in living liver donation surgery. In conclusion, our self-analysis study of a single center experience demonstrated a clear and progressive learning curve, which was instrumental in improvement of living donor liver surgery.

Conflict of interest The authors declare no conflict of interest.

References

1. Raia S, Nery JR, Mies S. Liver transplantation from live donors. *Lancet*. 1989;ii:497.
2. Strong RW, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. *N Engl J Med*. 1990;322:1505–1507.
3. Hashikura Y, Ichida T, Umeshita K, et al. Donor complications associated with living donor liver transplantation in Japan. *Transplantation*. 2009;88:110–114.

4. Marubashi S, Dono K, Nagano H, et al. Biliary reconstruction in living donor liver transplantation: technical invention and risk factor analysis for anastomotic stricture. *Transplantation*. 2009; 88:1123–1130.
5. Umeshita K, Fujiwara K, Kiyosawa K, et al. Operative morbidity of living liver donors in japan. *Lancet*. 2003;362:687–690.
6. Akabayashi A, Slingsby BT, Fujita M. The first donor death after living-related liver transplantation in japan. *Transplantation*. 2004;77:634.
7. Broering DC, Wilms C, Bok P, et al. Evolution of donor morbidity in living related liver transplantation: a single-center analysis of 165 cases. *Ann Surg*. 2004;240:1013–1024, discussion 1024–1016.
8. Trotter JF, Adam R, Lo CM, Kenison J. Documented deaths of hepatic lobe donors for living donor liver transplantation. *Liver Transpl*. 2006;12:1485–1488.
9. Ghobrial RM, Freise CE, Trotter JF, et al. Donor morbidity after living donation for liver transplantation. *Gastroenterology*. 2008;135:468–476.
10. Dindo D, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6,336 patients and results of a survey. *Ann Surg*. 2004;240: 205–213.
11. Marubashi S, Dono K, Sakon M, et al. Portal venous reconstruction in a living liver donor with an anomalous hepatic arterial and portal venous anatomy. *J Gastrointest Surg*. 2005;9: 365–368.
12. Suehiro T, Shimada M, Kishikawa K, et al. In situ dye injection bile leakage test of the graft in living donor liver transplantation. *Transplantation*. 2005;80:1398–1401.
13. Hirokawa F, Hayashi M, Miyamoto Y, et al. A novel method using the vio soft-coagulation system for liver resection. *Surgery*. 2010. [Epub ahead of print]
14. Belghiti J, Guevara OA, Noun R, Saldinger PF, Kianmanesh R. Liver hanging maneuver: a safe approach to right hepatectomy without liver mobilization. *J Am Coll Surg*. 2001;193:109–111.
15. Pruett TL, Tibell A, Alabdulkareem A, et al. The ethics statement of the Vancouver forum on the live lung, liver, pancreas, and intestine donor. *Transplantation*. 2006;81:1386–1387.
16. Klintmalm GB. Primum non nocere. *Am J Transplant*. 2008;8: 275–276.
17. Chan SC, Fan ST, Lo CM, Liu CL, Wong J. Toward current standards of donor right hepatectomy for adult-to-adult live donor liver transplantation through the experience of 200 cases. *Ann Surg*. 2007;245:110–117.

Ann Surg Oncol (2011) 18:2200–2209
DOI 10.1245/s10434-011-1577-7

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Clinical Significance of Alpha-Fetoprotein mRNA in Peripheral Blood in Liver Resection for Hepatocellular Carcinoma

Shigeru Marubashi, MD, Hiroaki Nagano, MD, Hiroshi Wada, MD, Shogo Kobayashi, MD, Hidetoshi Eguchi, MD, Yutaka Takeda, MD, Masahiro Tanemura, MD, Koji Umeshita, MD, Yuichiro Doki, MD, and Masaki Mori, MD

Department of Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

ABSTRACT

Purpose. Detection of AFP mRNA in peripheral blood is considered a useful predictor of HCC recurrence after resection. However, its interpretation and clinical significance remains to be determined. This study was designed to evaluate the clinical significance of detecting AFP mRNA positive cells in peripheral blood.

Methods. A total of 153 patients without macroscopic vascular invasion, who underwent liver resection, were prospectively enrolled in this study. The pattern of HCC recurrence was confirmed by image studies and divided into four types: (1) no recurrence (control group, $n = 68$); (2) intrahepatic single recurrence (SR group, $n = 28$); (3) intrahepatic multiple recurrences (MR group, $n = 38$); and (4) extrahepatic HCC recurrence (EX group, $n = 19$).

Results. HCC recurrence was identified in 85 (55.6%) patients during a follow-up of 8.6 ± 6.7 (range, 0.7–36) months. Multivariate analysis identified preoperative AFP mRNA (HR = 2.54; $P = 0.006$) as an independent risk factor for HCC recurrence. Preoperative AFP mRNA expression was a significant predictor of HCC recurrence in the MR/EX group ($P = 0.029$) but not in the SR group ($P = 0.467$).

Conclusions. Detection of AFP mRNA expression in peripheral blood before surgery for HCC is a useful predictor of multiple or extrahepatic HCC recurrences.

First Received: 20 January 2010;
Published Online: 8 February 2011

S. Marubashi, MD
e-mail: smarubashi@gesurg.med.osaka-u.ac.jp

Hepatocellular carcinoma (HCC) is the fifth commonest malignant disease and is highly associated with viral hepatitis in up to 90% of cases. Similar to other malignant tumors, HCC has the potential of recurrence with local and distant metastasis. Liver resection has been established as the first-line treatment for HCC, although the high incidence of postoperative recurrence of HCC remains a serious problem. HCC recurrence after liver resection is recognized to have unique characteristics and is divided into three patterns of recurrence: (1) intrahepatic metastasis; (2) multicentric HCC; and (3) extrahepatic metastasis. The diagnosis of these patterns of recurrence requires close follow-up with image studies after liver resection as well as histopathological evaluation of the tumor recurrence, if available.[1]

Circulating tumor cells (CTC) in the peripheral blood or disseminated tumor cells (DTC) in the bone marrow are reported to be the cause of tumor recurrence in various malignant tumors.[2] In liver transplantation for HCC, the fact that the most common site of tumor recurrence is the transplanted allograft provides strong support for this notion and the central role of CTC and DTC in tumor recurrence.[3,4]

The mRNA level of alpha-fetoprotein (AFP) in peripheral blood is a candidate marker of CTC. We reported previously the efficacy of detecting AFP-expressing cells by quantitative RT-PCR in patients who had undergone liver resection or liver transplantation for HCC.[5,6] Despite numbers of publications on this prognostic marker of HCC recurrence, it has not been studied in reference with the patterns of HCC recurrence.

This study was designed to determine the prognostic value of detecting AFP mRNA-positive cells in peripheral blood in patients with HCC who underwent curative resection, in predicting HCC recurrence after surgery, and to clarify the correlation between AFP mRNA expression in peripheral blood and the three patterns of HCC recurrence.

PATIENTS AND METHODS

The study protocol was approved by the Human Subjects Review Committee of Osaka University. All study subjects provided written, informed consent.

Patients

Among 295 consecutive patients who underwent liver resection for HCC between December 2001 and October 2008 in our hospital, 188 patients who underwent curative resection were free of macroscopic portal or venous invasion and consented to this prospective study. Peripheral blood samples (16 ml) were obtained from each participant for analysis of AFP mRNA at the following time points: within 3 days before surgery, and postoperatively immediately after surgery. Of the 188 patients, 37 were excluded because of short follow-up period without HCC recurrence (<12 months), and thus data of 153 patients were subjected to the analysis of risk factors.

The patient demographic and operative data, tumor characteristics, preoperative serum AFP levels, serum levels of protein induced by vitamin K antagonist II (PIVKA-II), and computed tomographic (CT) scans of the abdomen and chest after surgery were collected prospectively. The standard postoperative follow-up consisted of abdominal dynamic CT scan or magnetic resonance imaging (MRI) every 3–4 months with serum AFP, PIVKA-II, and chest X-ray or chest CT scan every 3–6 months. Bone scintigraphy or brain MRI was performed whenever metastasis was suspected.

Patients with HCC > 5 cm in preoperative image studies received transcatheter arterial chemoembolization (TACE) therapy 1–2 months before liver resection. No adjuvant chemotherapy, TACE, or other anticancer treatment was provided to the study patients until HCC recurrence was confirmed.

HCC recurrence confirmed by image studies was divided based on the patterns of the recurrence into: (1) no recurrence (control group); (2) intrahepatic single recurrence after liver resection (SR group); (3) multiple intrahepatic recurrences (MR group); and (4) extrahepatic HCC recurrence (EX group).

Real-Time Quantitative RT-PCR for AFP mRNA in Peripheral Blood

Peripheral blood (16 ml) samples were obtained prospectively from each patient within 3 days before surgery (preoperative AFP mRNA) and again immediately after surgery (postoperative AFP mRNA). The method used for the detection of AFP mRNA in peripheral blood was described previously.[7,8] Briefly, blood samples were collected in a VACUTAINER CPT™ cell preparation tubes with sodium citrate (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 17,000×*g* for 20 min. The separated mononuclear cells were placed into a 15-ml centrifugation tube, suspended with 10 ml of phosphate buffered saline (PBS), and centrifuged at 2,000 rpm for 10 min. After washing with PBS again, the cells were suspended with TRIzol Reagent (Molecular Research Center, Cincinnati, OH), and stored at −80°C until RNA isolation. AFP mRNA was quantified with the LightCycler™ analysis software (Roche Diagnostics, Mannheim, Germany) using the protocol provided by the manufacturer. The level of AFP mRNA in the blood was expressed relative to that of the mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The lower limit of detection of the AFP mRNA by this method was 1.0×10^{-8}, and any value above this level was designated as positive, as described previously.[5,6]

Statistical Analysis

Continuous data were expressed as mean ± standard deviation, and group data sets were compared using the Mann–Whitney *U* test or Kruskal–Wallis test. Categorical data are presented as percentages, and differences between proportions were compared using the chi-square test. The cumulative risk of HCC recurrence and the 95% confidence intervals (CI) were computed by Kaplan–Meier analysis. Univariate and multivariate risk-factor assessments were performed using the Kaplan–Meier method (log-rank test) and Cox's proportional hazards model. Variables that correlated with the risk of HCC recurrence in the univariate analysis ($P < 0.1$) were entered into the multivariate analysis. $P < 0.05$ was considered significant.

RESULTS

The 153 patients with HCC comprised 116 men and 37 women. The underlying liver disease was HCV ($n = 90$, 58.8%), HBV ($n = 33$, 21.6%), Laennec's ($n = 4$, 2.6%), and no apparent background liver disease ($n = 32$, 20.9%). The mean follow-up duration was 13.4 ± 10.8 (range, 0.4–54.2) months. Of the 153 patients, 68 (44.4%) were recurrence-free after a follow-up period of 22.6 ± 11.3 (range, 12–54.2) months, whereas 85 patients (55.6%) developed HCC recurrence within a follow-up period of 8.6 ± 6.7 (range, 0.7–36) months. The proportion of patients showing each type of recurrence pattern was 44.4% ($n = 68$) for the control group (no recurrence), 16.3% ($n = 28$) for the SR group (intrahepatic single recurrence after liver resection), 24.8% ($n = 38$) for the MR group (multiple intrahepatic recurrences after liver

resection), and 12.4% ($n = 19$) for the EX group (extrahepatic HCC recurrence), which included pulmonary metastasis ($n = 10$, 53%), lymph node metastasis ($n = 3$, 16%), diaphragm metastasis ($n = 3$, 16%), bone metastasis ($n = 2$, 11%), and adrenal gland metastasis ($n = 1$, 5%).

Table 1 shows the demographic and clinical features of the four groups. Age, gender, and background liver disease were similar among the four groups. Tumor size tended to be smaller in the control group and largest in the MR group ($P = 0.018$ between control vs. MR groups). Tumor number was single in 54 of 68 (79.4%)

TABLE 1 Characteristics of patients and hepatocellular carcinoma

	Control group ($n = 68$)	SR group ($n = 28$)	MR group ($n = 38$)	EX group ($n = 19$)	P
Age (years)	65.2 ± 9.9	67.1 ± 9.9	66.6 ± 7.6	63.9 ± 7.8	0.515
Gender (male/female)	46/22	22/6	31/7	17/2	0.157
Primary diagnosis					
HCV	41 (60.3)	16 (57.1)	25 (65.8)	8 (42.1)	0.213
HBV	16 (23.5)	5 (17.9)	5 (13.1)	7 (36.8)	
Laennec's	2 (2.9)	1 (0.4)	0 (0)	1 (5.3)	
Non-B, non-C	14 (20.6)	9 (32.1)	9 (23.6)	5 (26.3)	
Tumor characteristics					
Size (cm)	3.74 ± 2.47	4.14 ± 2.22	5.18 ± 3.63	4.78 ± 3.75	0.055
Number	128 ± 0.67	1.57 ± 1	1.97 ± 1.46	1.8 ± 1.24	0.093
Microscopic vascular invasion (%)	25.4	26	50	26.3	0.06
Histological differentiation (Edmondson classification)					
1	1 (1.8)	1 (3.7)	0 (0)	0 (0)	0.119
2	19 (33.3)	15 (55.6)	12 (31.6)	9 (47.3)	
3	34 (59.6)	10 (37)	25 (65.8)	6 (31.6)	
4	3 (5.3)	1 (3.7)	1 (2.6)	3 (15.8)	
Preoperative TACE (%)	45.5	46.4	47.4	68.4	0.353
Hepatectomy (HR)[a]					
0	34 (50)	17 (60.7)	20 (52.6)	9 (47.4)	0.9
S	8 (11.8)	1 (3.6)	4 (10.5)	3 (15.8)	
1	16 (23.5)	6 (21.4)	7 (18.4)	6 (31.6)	
2	9 (13.2)	4 (14.3)	7 (18.4)	1 (5.3)	
3	1 (1.5)	0 (0)	0 (0)	0 (0)	
Blood loss (ml)	842 ± 1280	647 ± 595	1460 ± 2683	721 ± 454	0.075
Transfusion	6/68 (8.8)	6/28 (21.4)	6/38 (15.8)	0	0.102
Transfused RC-M.A.P. (ml)	133 ± 610	89 ± 253	302 ± 1098	0	0.769
TNM stage[a]					
1	4 (5.9)	4 (14.3)	2 (5.3)	1 (5.3)	0.096
2	50 (73.5)	13 (46.4)	19 (50)	10 (52.6)	
3	12 (17.6)	8 (28.6)	12 (31.6)	5 (26.3)	
4a	2 (2.9)	3 (10.7)	3 (7.9)	3 (15.8)	
4b	0 (0)	0 (0)	2 (5.3)	0 (0)	
AFP (median; range)	17.5 (2–206249)	36.5 (3–31310)	52 (4–179200)	38 (4–947500)	0.314
PIVKA	105 (28–61330)	300 (9–32539)	334 (20–122976)	252 (23–304000)	0.356
AFP mRNA (%)					
Preoperative	4.4	10.7	15.8	10.5	0.264
Postoperative	20.6	42.9	36.8	31.6	0.095
Preoperative and postoperative	4.4	0	5.3	5.3	0.466

Data are mean ± standard deviation or number of patients with percentages in parentheses unless otherwise indicated

RC-M.A.P. Red cell concentrates mannitol adenine phosphate, *AFP* alpha-fetoprotein, *PIVKA* protein induced by vitamin K antagonist, *TACE* transcatheter arterial chemoembolization, *SR* single recurrence, *MR* multiple recurrence, *EX* extrahepatic recurrence

[a] According to the Liver Cancer Study Group of Japan (LCSGJ)

patients of the control group and in 18 of 28 (64.3%) patients of the SR group, whereas a solitary tumor was less frequently seen in 21 of 38 (55%) patients of the MR group and 11 of 19 (58%) patients of the EX group. The number of tumors was the lowest in the control group compared with the MR ($P = 0.007$) and EX ($P = 0.035$) groups. Tumor differentiation according to Edmondson classification, HAI score in background liver, and the extent of liver resection were not different among the four groups. The estimated blood loss and transfused red cell concentrates mannitol adenine phosphate were not significantly different among the groups. AFP and PIVKA-II were not different among the four groups.

The AFP mRNA/GAPDH mRNA ratio in peripheral blood ranged from undetectable and 1.04E−4. AFP mRNA was detected in 14 (9.2%) patients before surgery, whereas 46 (30.1%) patients were positive postoperatively. Six (3.9%) patients were positive for AFP mRNA both preoperatively and postoperatively. A larger proportion of patients of the MR group were AFP mRNA-positive preoperatively and less in the control group than the SR and EX groups, whereas a larger proportion of patients of the SR, MR, and EX groups were AFP-mRNA-positive postoperatively than the control group. The status of AFP mRNA (positive/negative) did not correlate with tumor characteristics, such as microscopic vascular invasion, blood loss, blood transfusion, TNM stage, and PIVKA-II,

TABLE 2 Relationship between preoperative AFP mRNA and various clinical parameters

	Preoperative AFP mRNA		*P*
	Positive ($n = 14$)	Negative ($n = 139$)	
Age (years)	70.1 ± 6.8	65.3 ± 9.2	0.057
Gender (male/female)	11/3	105/34	0.064
Primary diagnosis (%)			
HCV	42.9	60.4	0.203
HBV	14.3	22.3	0.487
Non-B, non-C	42.9	21.6	0.187
Tumor characteristics			
Size (cm)	5.2 ± 3.5	4.2 ± 2.9	0.146
Number	2.57 ± 1.83	1.47 ± 0.92	0.070
Microscopic vascular invasion (%)	30.8	32.4	0.906
Histological differentiation (Edmondson classification)			
1	0 (0)	2 (1.4)	0.947
2	5 (35.7)	50 (36)	
3	8 (57.1)	67 (48.2)	
4	1 (7.1)	7 (5)	
Preoperative TACE (%)	57.1	48.9	0.557
Hepatectomy (HR)[a]			
s	0	16	0.094
0	11	69	
1	0	35	
2	3	18	
3	0	1	
Blood loss (median; range) (ml)	480 (20–16600)	550 (30–2400)	0.724
Blood transfusion (RC-M.A.P.) incidence (amount (ml))	14% (780 ± 736)	10.1% (1539 ± 1781)	0.571
TNM stage[a]			
1	1	10	0.527
2	6	86	
3	6	31	
4a	1	10	
4b	0	2	
AFP (median; range)	396 (4–947500)	32 (2–206249)	0.039
PIVKA	115 (31–304000)	174 (9–122976)	0.917

Data are mean ± standard deviation or number of patients with percentages in parentheses unless otherwise indicated

RC-M.A.P. Red cell concentrates mannitol adenine phosphate, *AFP* alpha-fetoprotein, *PIVKA* protein induced by vitamin K antagonist, *TACE* transcatheter arterial chemoembolization

[a] According to the Liver Cancer Study Group of Japan (LCSGJ)